European Biophysics Journal (2023) 52 (Suppl 1):S1–S220
https://doi.org/10.1007/s00249-023-01668-7

EBSA
Biophysics in Europe

14th EBSA congress

July 31 – August 4, 2023, Stockholm, Sweden

SFBBM
Svenska föreningen för biokemi, biofysik och molekylärbiologi
Swedish society for biochemistry, biophysics and molecular biology

SWEDISH NATIONAL COMMITTEE FOR MOLECULAR BIOSCIENCES
THE ROYAL SWEDISH ACADEMY OF SCIENCES

This supplement was not sponsored by outside commercial interests. It was funded entirely by the publisher.

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14th EBSA European Biophysics Congress Stockholm (Sweden), July 31 – August 4, 2023

Organized by

Swedish Society for Biochemistry, Biophysics and Molecular Biology (SFBBM)

in collaboration with

Royal Swedish Academy of Sciences National Committee for Molecular Biosciences

The Protein Society

and

European Biophysical Societies' Association (EBSA)

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Welcome to Stockholm and the 14th EBSA Congress!

Dear Colleagues,

On behalf of the Swedish Society for Biochemistry, Biophysics and Molecular Biology (SFBBM), and of the European Biophysical Societies Association (EBSA), it is our pleasure, as Meeting Chairs, to welcome you to the 14th EBSA congress in Stockholm.

An EBSA congress in Sweden is timely. Forty years ago, the formal organization of EBSA was drafted by a group of dedicated scientists from the Belgian, British, Danish, German, Italian, Netherlands, Swedish and Swiss Biophysical Societies. This outstanding group of scientists included a future Nobel prize laureate (prof Kurt Wütrich) and the Swedish professor Anders Ehrenberg, who was to become the first president of EBSA at its first inaugural General Assembly during the IUPAB congress in 1984.

Questions at the front of their minds are still urgent to us today:

- How do we collaborate more efficiently?
- How do we publish in biophysics easily and accessibly?
- How can we promote and support biophysical societies in Europe?
- How can we encourage young scientists to join the biophysics field?

Actively engaging with these essential questions quickly gave results. Already by the time of the congress, new society applications were submitted from Israel and France, and with observers from Bulgaria, Hungary, Norway, Poland, Romania, Spain, USSR and Yugoslavia, most of whom successfully joined EBSA soon afterwards. Workshops and meetings were arranged, encouraging students to enter this exciting field of science. The European Biophysics Journal, which is the Journal of EBSA, was also founded in early 1984 and has after its full transfer to EBSA in 2000 provided a stable platform for an expansion of activities, including the development of the congresses.

In Biophysics, we investigate properties of time, force, structure and motion in biomolecular assemblies and processes – from single molecules to cells and tissues - and relate these to biological function. Biophysics has evolved dramatically since 1984, and many Nobel prizes have been awarded to biophysicists and previous EBSA Plenary Lecturers. The EBSA-2023 will be held at the Aula Magna at Stockholm University, which is also known for hosting the annual Nobel Lectures in Physics and Chemistry. We welcome to this meeting Nobel Laureate David Julius, and we are convinced that some of you participating in this meeting will be similarly honored one day.

In 2023, after a series of great congresses and innumerable biophysics training events throughout Europe, the SFBBM has now taken up the baton to arrange the 14th bi-annual EBSA congress. The great traditions that EBSA holds cannot be taken for granted. More than ever, we need to safe-guard structures that ensure scientific innovation and independence, and reliable channels for publications, training and communication. Biophysics is an intrinsically interdisciplinary area where we need to meet across disciplines to discuss scientific opportunities and challenges. To this end, EBSA supports activities such as the current congress that offer necessary creative meeting grounds, where we can jointly explore how experimental, theoretical and computational approaches in biophysics can be synergistically tailored to improve our understanding of biological function. By this work, we contribute to building essential scientific bridges between physics, biology and medicine.

Setting up an EBSA congress is not a task that you do alone. The Board and members of SFBBM have been working closely together with the Royal Swedish Academy of Sciences and its National Committee for Molecular Biosciences to make this congress a reality. We have been extensively supported by a fabulous Scientific Committee and Scientific Advisory Board, and we acknowledge extensive support by EBSA Executive Committee members and previous Congress Chairs, as well as the Protein Society who have arranged four of the sessions during the current meeting. In this meeting, we are honored to also collaborate with the Biophysical Society, IUPAB and SciLifeLab who together with EBSA, SFBBM and the Protein Society have each supported a Plenary speaker in this meeting. EBSA has contributed 55 full Student Bursaries for graduate and undergraduate students, and IUPAB has added another 6 scholarships to bring in students from further away. Added to this, we are delighted to be able to award over 30 poster prizes, primarily sponsored by EBSA and the Biophysical Society. Furthermore, this meeting would not have been possible without the extensive contributions made by the Session Chairs. They have designed attractive Session themes, suggested Keynotes and Invited Speakers, and screened hundreds of abstracts to select short Oral presentations. For the Conference Proceedings, we gratefully thank our Guest editors from the SFBBM Board. We also thank the professional congress organizing team from MKON, headed by Åsa Eklund, and acknowledge the extensive support from Konferenslokaler at Stockholm University.

We are now much looking forward to a week of intense immersion in the latest and most novel fields of biophysics! This, indeed, will be a party of Science, with a “smörgåsbord” containing all the key ingredients of biophysics with many exciting flavors! Together at last, after several years with COVID limiting our ability to meet IRL, we will share, discuss, learn, and jointly enjoy thrilling breakthroughs in our wide and exciting field of biophysics!

With our very best wishes for a great congress to all!

The EBSA-2023 Congress Chairs

Maria Sunnerhagen, Erik Lindahl, Lena Mäler, Pål Stenmark and Jerker Widengren

Welcome from the President of EBSA

Dear colleagues, dear friends

On behalf of the EBSA Executive Committee, I am pleased to warmly welcome you to the 14th European Biophysics Congress in Stockholm, Sweden. After the successful Biophysics Congress held in hybrid mode in Vienna in 2021 under the challenging conditions of the COVID pandemic, it is now a great relief to return to "normal" life, to meet colleagues in the relaxing environment of the beautiful campus of Stockholm University, to enjoy the scientific lectures in the Aula Magna, feeling connected to the major scientific events such as the annual awarding of the Nobel Prizes.

The biennial EBSA Congress is a significant event of the European biophysics community and has been held regularly in different European cities since 1984. EBSA aims to bring together nearly 1000 scientists from many different research fields such as (bio)physics, (bio)chemistry, biology, engineering, pharmacology, mathematics, computer science, and materials science. To give more visibility to biophysics, EBSA provides extensive support for activities related to biophysics. In addition to supporting participation in EBSA congresses, EBSA provides financial support for local biophysics-related meetings and grants for working visits by students and young scientists to another laboratory in an EBSA country.

This year, EBSA is pleased to support 55 young talented scientists with bursaries to attend the Congress. I am grateful for the growing collaboration with other biophysical societies, such as the Protein Society, the Biophysical Society of America and IUPAB. As a result, we have plenaries, sessions and awards sponsored by these societies in Stockholm.

I thank the members of the EBSA Executive Committee (EC) for their tireless efforts and time devoted to the activities of the Association. Special thanks go to EBSA Secretary John Seddon and EBSA EC Member Anthony Watts (currently President-elect of IUPAB) for their helpful advice and continued valuable input.

I invite you to attend the General Assembly during this Congress, even if you are not the voting representative of your country, to get an insight into the work of the EBSA EC and to participate in the discussion on ways to increase the visibility of biophysics.

I also would like to draw your attention to the European Biophysics Journal, which is the "scientific voice" of EBSA. I thank Robert Gilbert, the Managing Editor, and the Editorial Board for their work. The European Biophysics Journal is also a major source of revenue for EBSA through Springer, which, among many other activities, allows us to sponsor our biannual meeting. We look forward to getting acquainted with the new initiatives Robert Gilbert recently prepared to further enhance the journal's reputation and visibility.

One of the scientific highlights of the Congress is the award ceremony. As always, the EBSA EC received several outstanding nominations, demonstrating the high quality of biophysical research in Europe. The Avanti Polar Lipids Prize goes to Dimitrios Stamou from the University of Copenhagen. His research focuses on the biophysics of membranes and membrane proteins. Raya Sorkin from Tel Aviv University receives the Young Investigator Award for her achievements in studying membranes using a unique combination of single-molecule mechanical techniques: optical tweezers, confocal fluorescence microscopy, and atomic force microscopy. We look forward to the plenary lectures given by the winners.

On behalf of the EBSA Executive Committee and all the participants, I would like to express my thanks and appreciation to the Organizing and Scientific Committees and especially to Maria Sunnerhagen, Erik Lindahl, Lena Mäler, Pål Stenmark and Jerker Widengren. For at least three years, they and their teams have been working hard and creatively to make this Congress a special event in biophysics this year.

Let's have an exciting congress in Stockholm this summer!

The tradition of biennial EBSA congresses will continue in June 2025 in Rome, Italy.
We hope to see you there as well!

Elena Pohl
EBSA President

Congresses of the

EUROPEAN BIOPHYSICAL SOCIETIES' ASSOCIATION

1st	EUROPEAN BIOPHYSICS CONGRESS, 1971, BADEN, AUSTRIA
2nd	CONGRESS, 1997, ORLEANS, FRANCE
3rd	CONGRESS, 2000, MUNICH, GERMANY
4th	CONGRESS, 2003, ALICANTE, SPAIN
5th	EBSA / 15th IUPAB / SFB CONGRESS, 2005, MONTPELLIER, FRANCE
6th	CONGRESS, 2007, LONDON, UNITED KINGDOM
7th	CONGRESS, 2009, GENOA, ITALY
8th	CONGRESS, 2011, BUDAPEST, HUNGARY
9th	CONGRESS, 2013, LISBON, PORTUGAL
10th	CONGRESS, 2015, DRESDEN, GERMANY
11th	EBSA / 19th IUPAB / CONGRESS, 2017, EDINBURGH, UNITED KINGDOM
12th	EBSA / 10th ICBP-IUPAP / CONGRESS, 2019, MADRID, SPAIN
13th	CONGRESS, 2021, VIENNA, AUSTRIA

—

14th EBSA CONGRESS in Stockholm, Sweden!

(see http://ebsa2023.org/ for details)

The EBSA Executive Council at a committee meeting in Rome, Italy, 2023.
Members present are (from left to right): Anthony Wilkinson, York, England; Robert Gilbert, Oxford, England; Laszlo Matyus, Debrecen, Hungary; Maria Sunnerhagen, Linköping, Sweden; Anthony Watts, Oxford, England; Elena Pohl, Vienna, Austria; John Seddon, London, England; Helmut Grubmüller, Göttingen, Germany; Primoz Ziherl, Ljubljana, Slovenia; Jesús Pérez-Gil, Madrid, Spain; Dejan Žikić, Belgrade, Serbia; Nuno Santos, Lisbon, Portugal; Mauro Dalla Serra, Trento, Italy. Not present are: Jacqueline Cherfils, Paris, France; Sarah Köster, Göttingen, Germany; Pierre-Emmanuel Milhiet, Montpellier, France; Arwen Pearson, Hamburg, Germany; Ilpo Vattulainen, Helsinki, Finland.

Supporting academic societies and infrastructures

EBSA-2023 acknowledges support from the following non-commercial academic organisations:

Satellite Meetings

EBSA 2023 Summer Biophysics School
Stockholm University campus, July 29-31, 2023

Organisers:
Isabel Alves, University of Bordeaux, France
Lena Mäler, Stockholm University, Sweden
Anthony Watts, University of Oxford, Great Britain

The EBSA-2023 Summer Biophysics School provides great opportunity to network with participants ahead of the main congress, in a relaxed and informal environment in the Royal National City Park of Stockholm. This pre-meeting to the EBSA congress provides opportunities for younger scientists to become more familiar with the fundamentals of a range of methods and approaches, that will enable a deeper understanding and appreciation of the research talks at the main congress. Invited and Plenary speakers from the congress participate at this workshop, as well as regular teachers at EBSA Biophysics courses and workshops. A senior editor from Elsevier will give an insight to publishing both for the scientist and as a career path, and younger scientists are invited to practise their EBSA-2023 presentations in front of the workshop participants and tutors.

Support: EBSA, IUPAB.

Satellite Meeting on Bioenergetics and Biological Thermodynamics
Stockholm University campus, July 31st, 2023

Organisers:
Karim Fahmy, Helmholtz-Zentrum Dresden-Rossendorf, Technische Universität Dresden, Germany
Jana Oertel, Helmholtz-Zentrum Dresden-Rossendorf, Germany
Daumantas Matulis, Vilnius University, Lithuania

The Satellite meeting brings together scientists who are interested in discussing current developments in the field of “Bioenergetics and Biological Thermodynamics” and who like to interact in a workshop-like manner as an “add-on” to the session on the same topic within the EBSA main conference. Speakers from the main session will contribute to the Satellite Meeting as well. We will have a “Science-Coffee”, rather than a poster session, to provide opportunity for direct communication.

Support: EBSA, Deutsche Gesellshaft für Biophysik e. V. (DGfB)

Fluorescence-based Fluctuation and Single-molecule spectroscopy
Albanova University Center, Stockholm, July 29th to July 31st, 2023

Organisers:
Victoria Birkedal, Aarhus University, Denmark
Jerker Widengren, KTH-Royal Institute of Technology, Stockholm, Sweden.

Fluctuation and single molecule-based fluorescence methods give key views of how biomolecules carry out their functions in living cells. They allow uncovering the conformations, dynamics, interactions and spatial distributions of biomolecules. This meeting will focus on latest applications to biology and technological developments within single molecule fluorescence and fluorescence fluctuation spectroscopy and imaging, including super-resolution fluorescence imaging. The Satellite meeting aims at bringing together senior and young scientists who are interested in discussing recent developments within single-molecule fluorescence and fluorescence-based fluctuation spectroscopy.

Support: EBSA, STINT (The Swedish Foundation for International Cooperation in Research and Higher Education) and Wenner-Gren Foundations, Hübner photonics and PicoQuant.

NMR Methods for Biomolecules – a Deeper Dive
Stockholm University Campus, July 31st, 2023

Organisers:
Mikael Akke, Lund University, Sweden
Göran Karlsson, The Swedish NMR Center, University of Gothenburg, Sweden

The Satellite meeting is aimed for graduate students, postdocs, and other scientists interested in learning more about current developments in the field of biomolecular NMR. Here you get the opportunity to interact with the speakers in a tutorial/workshop-like manner as an "add-on" to the lectures presented by the same speakers in their sessions within the EBSA main conference.

Support: EBSA and the SwedNMR (Swedish Research infrastructure Coumcil).

Advances in mRNA translation and protein synthesis
Uppsala Biomedical Centre, July 30th to July 31st, 2023

Organisers:
Maria Selmer, Suparna Sanyal and Magnus Johansson, Uppsala University
Alexey Amunts, SciLifeLab

The Satellite meeting is aimed for graduate students, postdocs, and other scientists interested in in-depth discussions of recent advances in mechanistic and structural aspects of mRNA translation and protein synthesis, incorporating results from a wide range of biophysical methods.

Support: EBSA and SciLifeLab

Plenary Lectures

Molly Stevens
Imperial College London,
UK

Monday, July 31, 2023
Designing and translating new biomaterials to heal the body and detect diseases earlier
- EBSA honorary speaker

David Julius
Nobel Prize 2021
University of California San Francisco (UCSF),
USA

Monday, July 31, 2023
Structural insights into TRP channel gating: visualizing a polymodal signal integrator
- Supported by Protein Society

Lewis Kay
University of Toronto,
Canada

Tuesday, August 1, 2023
Structural biology after AlphaFold - what is left? NMR has the answer
- Supported by Royal Swedish Academy of Sciences National Committee for Molecular Biosciences

Karen Fleming
Johns Hopkins University Baltimore,
USA

Wednesday, August 2, 2023
Chaperoning Unfolded Outer Membrane Proteins
- Supported by Biophysical Society

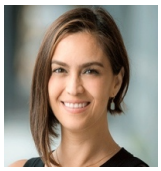
Hao Wu
Harvard Medical School Boston,
USA

Thursday, August 3, 2023
Structural and mechanistic elucidation of the inflammasome pathway
- SFBBM honorary speaker

Thorsten Wohland
National University of Singapore,
Singapore

Friday, August 4, 2023
Fluorescence Correlation Spectroscopy as a tool in developmental biology: Morphogen dynamics and interactions in live zebrafish embryos
- Supported by IUPAB

Keynote Lectures



Elizabeth Villa
University of California San Diego (UCSD)
USA

Tuesday, August 1, 2023
Opening Windows into the Cell: Bringing structure to cell biology using cryo-electron tomography

Claus AM Seidel
Heinrich Heine Universität Düsseldorf
Germany

Tuesday, August 1, 2023
Integrative dynamic structural biology with multi-modal fluorescence spectroscopy and nanoscopy: From single molecules to live cells

Simon Scheuring
Cornell University
USA

Wednesday, August 2, 2023
High-speed atomic force microscopy captures conformational transitions and the kinetics of rare, transient conformational states

Harald Schwalbe
Goethe University Frankfurt
Germany

Wednesday, August 2, 2023
NMR studies to support RNA targeting by small molecules

Ville Kaila
Stockholm University, DBB
Sweden

Thursday, August 3, 2023
Deciphering Long-Range Charge Transport Mechanisms of Biological Energy Transduction

Julie Forman-Kay
Hospital for Sick Children, University of Toronto
Canada

Thursday, August 3, 2023
Regulating biochemical processes by modulating disordered protein phase separation

Syma Khalid
University of Oxford
UK

Friday, August 4, 2023
Computational microbiology: a focus on bacterial cell envelopes

Hendrik Dietz
Technical University of Munich
Germany

Friday, August 4, 2023
Virus traps and other molecular machines of the future

Invited Speakers

Chiara Autilio, San Carlo Hospital, Potenza, Italy
Julie Biteen, University of Michigan, USA
Enrica Bordignon, Department of Physical Chemistry, University of Geneva, Switzerland
Jonathan Brewer, University of Southern Denmark, Denmark
Ada Cavalcanti-Adam, Max Planck Institute for Medical Research & Heidelberg University
Pedro de Pablo, Universidad Autonóma de Madrid, Spain
Charlotte Deane, University of Oxford, UK
Hamid Dehghani, University of Birmingham, UK
Nynke Dekker, Delft University of Technology, Netherlands
Kristina Djinovic Carugo, University of Vienna, Austria
Irene Farabella, Fondazione Istituto Italiano di Tecnologia (IIT), Italy
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Vera Moiseenkova-Bell, University of Pennsylvania, USA
Henrike Mueller-Werkmeister, University of Potsdam, Germany
Elena Papaleo - Danish Cancer Society Research Center & Technical University of Denmark
Camilo Perez, University of Basel, Switzerland
Malene Ringkjobing Jensen, Institut de Biologie Structurale, Grenoble
Vahid Sandoghdar, Max Planck Institute for the Science of Light, Germany
Tessa Sinnige, Utrecht University, Netherlands
Barbora Spackova, Chalmers University of Technology, Sweden
Ana Teixeira, Karolinska Institutet, Sweden
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Gijs Wuite, Vrije Universiteit Amsterdam, Netherlands
Julia Yeomans, University of Oxford, UK

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Žibrat, Nika – *Slovenia*
Zulueta Díaz, Yenisleidy De Las Mercedes – *Denmark*

Scientific Program

Due to advanced printing submission, the exact timings and locations should be considered preliminary and may change slightly.

The exact timings and locations are in the accompanying Program Schedule, delivered to participants at the site of the meeting.

Content:

Plenary, Keynote and Session abstracts are presented in the order of the meeting.

Poster abstracts are arranged in alphabetic order by the presenting author within each session.

MONDAY 31 JULY
Aula Magna
13.00 15.30
Registration
15.30 16.00
Opening, Welcome
16.00 17.00
Plenary Speaker:
Molly Stevens, Imperial College London, Karolinska Institutet
Designing and translating new biomaterials to heal the body and detect diseases earlier
17.00 17.15
Break
17.15 18.15
Honorary Plenary Speaker:
David Julius, University of California San Francisco (UCSF)
Structural insights into TRP channel gating: visualizing a polymodal signal integrator
18.15 19.30
Welcome Reception
Exhibition opening hours:
11:00-17:00

TUESDAY 1 AUGUST

	Aula Magna A + B	Lecture hall 1	Lecture hall 2	Lecture hall 3
08.30 10.30	**Protein dynamics** **Session chairs** - Mikael Akke & Pétur Orri Heidarsson **Lynn Kamerlin** - Loop Dynamics and the Evolution of New Enzyme Functions **Malene Ringkjøbing Jensen** - Visualizing the multi-step folding trajectory of an intrinsically disordered scaffold protein upon binding to the small GTPase Rac1 **Short talks** Alexandra Teslenko, David Scheerer, Rafael Tapia-Rojo & Zhaowei Liu	**Single-molecule biophysics** **Session chairs** - Victoria Birkedal & Mike Heilemann **Chirlmin Joo** - SPARXS: Single-molecule Parallel Analysis for Rapid eXploration of Sequence space **Julie Biteen** - An experimental framework to assess phase separation in bacteria **Short talks** Annie Sahota, Jan Christoph Thiele, Sabine Straathof & Veronika Frank	**Microfluidics and organ-on-a-chip for biophysics** **Session chairs** - Pawel Sikorski & Bjørn Torger Stokke **Tuomas Knowles** - Kinetics of protein phase transitions **Anna Herland** - Combining Stem Cell and Device Engineering for In vitro Models of Human Physiology **Short talks** Aitor Manteca, Christina Tringides, Enrico Turato & Oliver Vanderpoorten	**Biophysics of viruses** **Session chairs** - Sarah Butcher & Nicola Abrescia **Charlotte Uetrecht** - Flying viruses - mass spectrometry meets X-rays **Pedro de Pablo** - Physical Virology with atomic force and fluorescence microscopies: seeing and touching viruses and protein cages **Short talks** Carolina C. Buga, Daniel Ziemianowicz, Gyula Hoffka & Marta Bally
10.30 11.00	**Coffee and exhibition**			
11.00 12.00	**Aula Magna A + B** **Plenary Speaker: Lewis Kay,** University of Toronto Structural biology After AlphaFold- What is left? NMR is the answer			
12.00 13:30	**Lunch Break**			
13:30 14.15	**Aula Magna A** **Elizabeth Villa** Opening Windows into the Cell: Bringing structure to cell biology using cryo-electron tomography	**Aula Magna B** **Claus AM Seidel** Integrative dynamic structural biology with multi-modal fluorescence spectroscopy and nanoscopy: From single molecules to live cells		
14.15 14.45	**Coffee and exhibition**			
	Aula Magna A	**Aula Magna A**	**Lecture hall 2**	**Lecture hall 3**
14.45 16.45	**Activation and modulation of membrane proteins** **Session chairs** - Ville Kaila & John Seddon **Vera Moiseenkova-Bell** - Structural Pharmacology of TRPV channels **Joachim Heberle** - Watching ions moving across the biomembrane at utmost temporal and spatial resolution **Short talks** Adam Lange, Burkhard Bechinger, Maria Florencia Sánchez & Shreyas Kaptan	**Intrinsically disordered proteins and liquid-liquid phase separations** **Session chairs** - Jean Baum & Ylva Ivarsson **Richard Kriwacki** - The Role of Phase Separation in Biology and Disease **Kristina Djinovic Carugo** - Order from Disorder: Towards molecular architecture of the muscle Z-disk assembly by integrative structural biology **Short talks** Agustin Mangiarotti, Francesco Luca Falginella, Johanna Huftman & Sheung Chun Ng	**Neutrons in Life Sciences and Biophysics** **Session chairs** - Trevor Forsyth & Esko Oksannen **Henning Tidow** - Structural studies of integral membrane proteins using stealth carrier systems **Hanna Wacklin** - Probing amphotericin B mechanism and resistance in human pathogenic yeast using neutron reflection **Short talks** Andreas Stadler, Hanna Barriga, Marie Lycksell & Georg Pabst	**Protein design** **Session chairs** - Aitziber L. Cortajarena & Ingemar André **Sarel Fleishman** - Design of enzyme repertoires **Charlotte Deane** - Modelling and comparing the binding sites of immune receptor proteins to enable design **Short talks** Anastassia Vorobieva, Francesco Antonio Aprile, Laura Perez Chirinos & Sara Fortuna
16.45 19.00	**Poster Session 1**			

WEDNESDAY 2 AUGUST

	Aula Magna A	Aula Magna B	Lecture hall 2	Lecture hall 3
08.30 10.30	**Computational biophysics** **Session chairs** - Ilpo Vattulainen & Himanshu Khandelia **Helmut Grubmüller -** Three routes to molecular movies **Julia Yeomans** - Mesoscale models of mechanobiology **Short talks** Forbes Burkowski, Lukáš Sukeník, Maryam Majdolhosseini & Maxim Igaev	**Biophysics of RNA and ribosomes** **Session chairs** - Katja Petzold, Giovanni Bussi & Maria Selmer **Alan Chen** - A tale of two riboswitches: How ligand binding alters RNA folding pathways in atomistic detail **Short talks** Ankush Singhal, Neva Caliskan, Ruchi Anand, Shreya Pundir, Valerio Piomponi & Vinoth Sundar Rajan	**Bionanophotonics** **Session chairs** - Haichun Liu & Artur Bednarkiewicz **Xiaogang Liu** - Photon Conversion Nanocrystals: Transforming Imaging and Assistive Technology **Hamid Dehghani** - Applications of diffuse optics for detection and characterisation of disease **Short talks** Erik Olsén, Francesco Reina, Ivan N. Unksov & Lucia Gardini	**Molecular motors and machines** **Session chairs** - Sebastian Deindl & Dea Slade **Gijs Wuite** - Nonlinear mechanics of human mitotic chromosomes **Nynke Dekker** - Adventures in DNA and chromatin replication using single-molecule biophysics **Short talks** Borja Ibarra, Line Mørkholt Lund, Stavros Azinas & Thomas C.R. Miller
10.30 11.00	**Coffee and exhibition**			
	Aula Magna A		**Aula Magna B**	
11.00 11.45	**Simon Scheuring** High-speed atomic force microscopy captures conformational transitions and the kinetics of rare, transient conformational states		**Harald Schwalbe** NMR studies to support RNA targeting by small molecules	
11.45 14.00	**Lunch break and Poster session 2**			
	Aula Magna A + B			
14.00 15.00	**Plenary Speaker: Karen Fleming,** Johns Hopkins University Chaperoning Unfolded Outer Membrane Proteins			
15.00 15.30	**Coffee and exhibition**			
	Aula Magna A + B	**Lecture hall 1**	**Lecture hall 2**	**Lecture hall 3**
15.30 17.30	**Protein structure and function** **Session chairs** - Elisabeth Sauer-Eriksson & Lynn Kamerlin **Laura Itzhaki** - Engineering the folding and function of tandem-repeat proteins: Teaching old proteins new tricks **Gilad Haran -** The Secret Ultrafast Motions of Protein Nanomachines **Short talks** Darian Yang, Dominique Madern, Kristyna Pluhackova & Silvia Trigüis	**Mechanobiology in health and disease** **Session chairs** - Christelle Prinz & Christine Selhuber-Unkel **Jochen Guck** - High-throughput mechanical phenotyping for diagnostic applications **Ada Calvalcanti-Adam** - Receptor-mediated regulation of molecular and cellular mechanics **Short talks** Alejandro Jurado Jiménez, Christian Nehls, Daniel Aili & Poul Martin Bendix	**Biophysics of the green transition** **Session chairs** - Daniel Otzen & Doris Ribitsch **Neel Joshi** - Biologically fabricated materials from engineered microbes **Ren Wei** - Enzymatic Plastic Recycling and Upcycling: A Promising Strategy for Green Transition **Short talks** Caterina Medeot, Christofer Lendel, Mai Bay Stie & Miguel A. Soler	**Biophysics in Industry and Biotech** **Session chairs -** Elena Pohl & Pål Stenmark **In progress - to be announced**
19.00 21.00	**CITY HALL RECEPTION**			

Springer

THURSDAY 3 AUGUST

	Aula Magna A + B	Lecture hall 1	Lecture hall 2	Lecture hall 3
08.30 10.30	**Membranes and membrane proteins** **Session chairs** - Gunnar von Heijne & Anthony Watts **Camilo Perez** - Mechanism of cell wall transporters and role in the adaptation of bacterial pathogens **Irep Gözen** - Membrane self-organization in flat land: Rise of the vesicles **Short talks** Agnes Koerfer, Christine Doucet, Luke Chao & Roberto Covino	**Protein folding, assembly and disease** **Session chairs** - Jan Johansson & Cláudio Gomes **Tessa Sinnige** - The biophysics of polyglutamine aggregation in a multicellular animal model **Stefano Gianni** - Folding of multi-domain proteins - folding intermediates, hidden kinetic traps and cryptic functional features **Short talks** Arantza Muguruza-Montero, Axel Abelein, Ismahene Mesbah & Sara Ribeiro	**Biophysics of redox biology** **Session chairs** - Giuseppe Filomeni & Elias Arnér **Elena Papaleo** - S-nitrosylation under the lens of molecular dynamics **Graham George** - Probing Toxic Metals and Metalloids in Redox Biology using Synchrotron X-rays **Short talks** Francesco Stellato, Giuseppe Filomeni, Michael Di Gioacchino & Tania Sousa	**Bioenergetics and biological thermodynamics** **Session chairs** - Karim Fahmy & Daumantas Matulis **Daniel Harries** - Macromolecular Crowding Beyond Hard-Core Repulsions **Dorothee Kern** - Aiming Higher – In Energy and Functional Understanding of Proteins **Short talks** Anaïs Biquet-Bisquert, Daniel Dornbusch, Joana V. Ribeiro & Kohki Okabe
10.30 11.00	**Coffee and exhibition**			
	Aula Magna A + B			
11.00 12.00	**Plenary Speaker: Hao Wu,** Harvard Medical School Inner workings of the inflammasome engine elucidated by cryo-EM			
12.00 14.15	**Lunch Break and Poster session 3**			
	Aula Magna A		**Aula Magna B**	
14.15 15:00	**Ville Kaila** Deciphering Long-Range Charge Transport Mechanisms of Biological Energy Transduction		**Julie Forman-Kay** Regulating biochemical processes by modulating disordered protein phase separation	
15.00 15:30	**Coffee and exhibition**			
	Aula Magna A	**Aula Magna A**	**Lecture hall 1**	**Lecture hall 3**
15.30 17.30	**Biophysics of biological barriers** **Session chairs** - Jesus Perez Gil & Emma Sparr **Chiara Autilio** - Lung surfactant at the respiratory air-liquid interface: from biophysics to translational medicine **Jonathan Brewer** - Uncovering the Nanoscopic Molecular Pathway and Mechanism of Penetration Enhancement by Lipid-Based Nanoparticles in Human Skin **Short talks** Lucrezia Caselli, Lukasz Cwiklik, Maria J. Sarmento & Nicky Tam	**Self-organised and biomimetic systems** **Session chairs** - Björn Högberg & Hendrik Dietz **Ana Teixeira** - Mapping and targetting membrane protein nanoenvironments **Kerstin Göpfrich** - Engineering a synthetic model cell with DNA nanotechnology **Short talks** Ioanna Smyrlaki, Johanna Utterström, Lukasz Piatkowski & Raviv Dharan	**Novel methods for cell biophysics** **Session chairs** - Jonas Tegenfeldt & Sarah Köster **Vahid Sandoghdar** - iSCAT: from detection of very small proteins to characterisation of extracellular vesicles and 3D imaging of cellular nanostrcutures **Helena Knowles** - Simultaneous nanorheology and nanothermometry using intracellular diamond quantum sensors **Short talks** Daniel Midtvedt, Guillem Marín-Aguilera & Soohyen Jang	**Time-resolved structural biology** **Session chairs** - Arwen Pearson & Gisela Brändén **Ulrich Lorenz** - Microsecond Time-Resolved Cryo-EM **Henrike Mueller-Werkmeister** - Multiscale time-resolved spectroscopy and serial crystallography for studies of protein dynamics **Short talks** Guillaume Mas, Luiz Schubert, Nandan Haloi & Sebastian Jaksch
19:00 22:00	**CONGRESS DINNER**			

FRIDAY 4 AUGUST

	Aula Magna A	Aula Magna B	Lecture hall 1	Lecture hall 3
08.30 10.30	**50 Years of Pure and Applied Biophysics in Italy** **Session chairs** - Alberto Diaspro & Velia Minicozzi **Irene Farabella** - Investigating genome plasticity at the nanoscale **Short talks** Armando Carpaneto, Elena Ferraguzzi, Lorenzo Stella, Mauro Manno, Sajedeh Kerdegari & Tomaso Zambelli	**New and notable** **Session chairs:** - Robert Gilbert & Maria Sunnerhagen **Luke Chao** - Ancestral sequence reconstruction exploration of the mitochondrial cristae junction **Katherine Stott** How do different linker histones direct chromatin to liquid- or fibre-like states? **Pernilla Wittung Stafshede** - Amyloids of alpha-synuclein catalyze chemical reactions **Sebastian Deindl** - Massively multiplexed single-molecule fluorescence microscopy **Dorothee Kern** - Evolution at play – from primordial oscillators to AI-assisted substate prediction **Nicholas Pearce** - Intuitively extracting structural dynamics from macromolecular disorder	**Breakthrough methods in molecular biophysics** **Session chairs** - Isabel Alves & Tomasz Kobiela **Zdenek Lansky** - Tau envelopes regulate access to microtubules **Barbora Spackova** - Label-free Microscopy for Investigation of Biomolecular Interactions at Single Molecule Level **Enrica Bordignon** - Dynamics of the prepore-to-pore transition of a Tc toxin **Short talks** Giulio Bianchi, Helena Danielson & Taras Sych	**Supporting Biophysics – into the future!** **Sessions chairs** - Martha Carroni & Göran Karlsson **Janne Salo** - European Research council: Funding for frontier research in Europe **Annika Jensen** - SciLifeLab and EMBL: Collaboration projects **Jacques Jestin** – ILL Neutrons for society: an update of specific applications in structural biology and biophysics **Brinda Vallat** –Disseminating Integrative and Hybrid Structures through PDB-Dev **Harald Schwalbe** – Instruct-ERIC: Current and future approaches and challenges
10.30 11.00	**Coffee and exhibition**			
	Aula Magna A		**Aula Magna B**	
11.00 11.45	**Syma Khalid** Computational microbiology: a focus on bacterial cell envelopes		**Hendrik Dietz** Virus traps and other molecular machines of the future	
11.45 13.15	**Lunch Break**			
	Aula Magna A+B			
13.15 14.15	**Plenary Speaker:** **Thorsten Wohland,** National University Singapore Fluorescence Correlation Spectroscopy as a tool in developmental biology: Morphogen dynamics and interactions in live zebrafish embryos			
14.15 15.30	**Award lectures: EBSA Young Investigator and Avanti Lipid Prize recipients** Poster and Talk awards			
15.30 16.00	**Closing Ceremony** Introducing the EBSA Congress in Rome 2025			

Plenary Lectures

O-1
Designing and translating new biomaterials to heal the body and detect diseases earlier
Molly Stevens[1]
[1] Imperial College London, UK

In this talk I will discuss highlights of our nanomedicine portfolio including nanosensors for diagnosing and monitoring infectious and non-communicable diseases, and high molecular weight polymer carriers for enhanced delivery of saRNA therapeutics. I will present advances in Raman spectroscopy for high-throughput label-free characterization of single nanoparticles (SPARTA™) that allow us to integrally analyse a broad range bio-nanomaterials such as polymer particles, liposomes and extracellular vesicles without any modification. SPARTA™ has become an integral tool for the design of nanotherapeutics, with recent examples including DOPC-containing lipid nanoparticles for nucleic acid delivery and dendrimersome-based systems for controlled delivery of antibacterial drugs, and for profiling extracellular vesicles (EVs) for detection of breast cancer through a minimally invasive liquid biopsy. I will also discuss our cell interfacing nanoneedle platforms for multiplexed intracellular biosensing at sub-cellular resolution and modulation of biological processes. I will explore how these versatile technologies can be applied to transformative biomedical innovations.

O-2
Structural insights into TRP channel gating: visualizing a polymodal signal integrator
David Julius[1]
[1]University of California San Francisco (UCSF), USA

The capsaicin receptor, TRPV1, is a heat-activated ion channel that is modulated by numerous inflammatory agents, including some (e.g., extracellular protons and bioactive lipids) that interact with the channel directly and others (e.g., NGF, bradykinin, or ATP) that mediate their effects indirectly through activation of cytoplasmic second messenger signaling pathways. This ability of TRPV1 to serve as a complex polymodal signal integrator underlies its critical role in both acute and persistent pain. We have used electron cryo-microscopy to capture TRPV1 in distinct functional states, with the goal of elucidating structural and allosteric mechanisms by which inflammatory agents and drugs regulate channel function. Insights from these studies will be presented and discussed.

O-3
Structural biology After AlphaFold- What is left? NMR is the answer
Lewis Kay[1]
[1] University of Toronto, Canada

There is tremendous excitement in the structural biology community reflecting recent developments in both computational and experimental methods for establishing accurate static three-dimensional folds of biological molecules. But biological molecules are not static and both function and misfunction in many cases can only be explained on the basis of dynamics. In this talk I will describe how NMR spectroscopy can contribute to an increased understanding of molecular function, emphasizing that the structure-function paradigm must be changed to structure-dynamics-function. NMR studies of a number of molecular machines using methyl-TROSY based techniques will be described, and the unique insights provided by NMR will be highlighted.

O-4
Chaperoning Unfolded Outer Membrane Proteins
Karen Fleming
[1] Johns Hopkins University, USA

The periplasmic chaperone network ensures the biogenesis of bacterial outer membrane proteins (OMPs). Because the periplasm is devoid of external energy (e.g. ATP), this chaperone network relies only on the thermodynamics and kinetics of binding to unfolded OMPs to maintain these clients in unfolded but folding-competent conformations. We use a combination of solution biophysics, binding, hydrodynamics, scattering analysis, computational flux modeling and integrative structural modeling to investigate how the three main soluble chaperones accomplish their function. Our results show that distinct members of the uOMP conformational ensemble are recognized by different chaperones. Skp captures the majority compact forms of the intrinsic uOMP ensemble whereas SurA recognizes the more expanded but rarer expanded forms. These structural differences highligh the different functions of the chaperone network.

O-5
Inner workings of the inflammasome engine elucidated by cryo-EM
Hao Wu[1,2]
[1] Harvard Medical School, USA [2] Boston Children's Hospital, USA

Inflammasomes are supramolecular complexes that activate caspase-1 and other inflammatory caspases. Upon caspase activation, pro-cytokines in the interleukin-1 family and the pore-forming protein GSDMD are proteolytically processed, resulting in cytokine maturation, and formation of GSDMD pores to release cytokines and to induce the lytic cell death pyroptosis. In this talk, I will present a few examples on how inflammasome structures revealed interesting biology and cellular trafficking in inflammasome activation. Methodologically, our studies offer examples of integrative cryo-EM and structure prediction (AlphaFold or others) as a general approach for attaining more precise models of megadalton protein complexes from medium-resolution density maps.

O-6
Fluorescence Correlation Spectroscopy as a tool in developmental biology: Morphogen dynamics and interactions in live zebrafish embryos
Thorsten Wohland[1]
[1] National University Singapore, Singapore

The development of an organism from a single cell to its fully grown form is a carefully orchestrated process, guided by a set of signaling molecules or morphogens. They determine, through long-distance signaling, cell fate and migration in a concentration dependent manner. How morphogens are distributed and perform their function in the developing embryo is still not fully understood. Here we use Fluorescence Correlation and Cross-Correlation Spectroscopy (FCS and FCCS) and imaging FCS, to investigate the transport, dynamics, and interactions of two stereotypical morphogens, Wnt3 and Squint, in live zebrafish embryos. We selected these two morphogens as they exert their functions at very different developmental stages and tissues and act in very different environments. Squint was measured at 5 hours post fertilization (hpf), a stage with large intercellular spaces and rapid cell movement. Wnt3 is measured at ~36 hpf in the zebrafish brain, where the intercellular space cannot be resolved by optical microscopy, and where cells are much less mobile. FCS with single molecule sensitivity allows us to follow both morphogens from secretion to diffusion, interactions in intercellular spaces and receptor binding. Here, we quantify the receptor binding affinities for both morphogens and determine their local and global diffusion coefficients and the factors governing this transition. Surprisingly, despite the molecular and tissue differences, the dynamics of both morphogens seem to be governed by similar general mechanisms that allow the establishment of morphogen gradients in the early embryo in the case of Squint and govern Wnt3 distribution in brain tissue.

Keynote Lectures

O-7

Opening Windows into the Cell: Bringing structure to cell biology using cryo-electron tomography

Elizabeth Villa[1,2]
[1] University of California San Diego, United States
[2] HHMI

To perform their function, biological systems need to operate across multiple scales. Current techniques in structural and cellular biology lack either the resolution or the context to observe the structure of individual biomolecules in their natural environment, and are often hindered by artifacts. Our goal is to build tools that can reveal molecular structures in their native cellular environment using the power of cryo-electron tomography to image biomolecules at molecular resolution in situ. I will show how we used these techniques to discover how jumbo phage build a proteinaceous compartment that separates its replicating genome from the rest of the cell to avoid host defenses that acts akin to a eukaryotic nucleus.

O-8

Integrative dynamic structural biology with multi-modal fluorescence spectroscopy and nanoscopy: From single molecules to live cells

Claus Seidel[1]
[1] Heinrich-Heine-University Duesseldorf, Germany

Multimodal fluorescence spectroscopy and microscopy with multiparameter detection provide rich insights on biomolecular systems under ambient / live cell conditions, including spatial, structural and kinetic information. In a comparative single-molecule study, we assessed the accuracy of Förster Resonance Energy Transfer (FRET) measurements. We studied two protein systems with distinct conformational changes and dynamics and obtained an interdye distance precision of ≤2 Å and accuracy of ≤5 Å. Considering cellular studies, we introduced a framework for quantitative high throughput FRET image spectroscopy. We measured the time-evolution of pairwise homo- or hetero-interactions of the Guanylate binding proteins and the membrane receptor CD95 in live cells with 0.8% fraction precision. Finally, I will present a novel integrated framework for FRET nanoscopy that provides seamless resolution at a molecular scale, accessing distances down to 5 nm with a precision < 0.7 nm. We obtained these numbers in a quantitative benchmark study of various DNA origamis, double-stranded DNA rulers and oligomeric protein assemblies. Most importantly, to enable dissemination of these results, we introduce the flrCIF data representation, which extends established data standards from the Protein Data Bank and allows for archiving fluorescence-aided integrative structures for multiple states together with associated kinetic data on exchange in the PDB-Dev repository. This is crucial for making fluorescence data Findable, Accessible, Interoperable, and Reusable (FAIR). In view that protein structure prediction with AlphaFold has reached the single-structure frontier, we must tackle the next level of complexity by linking structural dynamics of biomolecules with their cellular function and localization.

O-9

HIGH-SPEED ATOMIC FORCE MICROSCOPY CAPTURES CONFORMATIONAL TRANSITIONS AND THE KINETICS OF RARE, TRANSIENT CONFORMATIONAL STATES

Simon Scheuring[1]
[1] Weill Cornell Medicine New York, USA

High-speed atomic force microscopy (HS-AFM) is a powerful technique that provides dynamic movies of biomolecules at work. We successfully used HS-AFM to determine structures and dynamics of membrane trafficking proteins, transporters, and channels. I will briefly review our recent developments to break current temporal limitations to characterize molecular dynamics by developing HS-AFM line scanning (HS-AFM-LS) and HS-AFM height spectroscopy (HS-AFM-HS) [1,2,3,4], and resolution limitations by developing Localization AFM (LAFM) [5]. Currently, we exploit these new possibilities for the analysis of conformational transitions and the discovery of rare, transient conformational states. As I will show, the dynamic single-molecule imaging capability of HS- AFM, avoiding temporal and ensemble averaging, opens new avenues in membrane biology [6]. References: [1] Heath et al. Nature Communications, 2018, 9(1):4983, High-Speed AFM Height Spectroscopy (HS-AFM-HS): Microsecond dynamics of unlabeled biomolecules. [2] Perrino et al., Nature Communications, 2021 12(7225), doi.org/10.1038/s41467-021-27580-2, Single molecule kinetics of bacteriorhodopsin by HS-AFM. [3] Jiao et al., Nature Communications, 2022, 13(5039). doi.org/10.1038/s41467-022-32757-4, Perforin-2 clockwise hand-over-hand pre-pore to pore transition mechanism [4] Jiang et al., Nature Communications, 2022, 13(7373), doi.org/10.1038/s41467-022-35202-8, Membrane-mediated protein interactions drive membrane protein organization [5] Heath et al. Nature, 2021, 594(7863):385–390, doi:10.1038/s41586-021-03551-x, Localization Atomic Force Microscopy. [6] Lansky et al., in preparation

O-10

NMR studies to support RNA targeting by small molecules

Harald Schwalbe[1]
[1] Goethe University Frankfurt, Germany

RNA structure exhibits large conformational dynamics that occur on time scales spanning several orders. RNA riboswitches represent key examples where allosteric conformational changes on the millisecond-to-second time scale involve coupled order-to-disorder and disorder-to-order transitions are functionally highly relevant. Further, non-canonical RNA structural elements involve recognition sides for protein-RNA but also small molecule-RNA interaction. In this contribution, we will discuss the NMR spectroscopy investigation of RNA functional dynamics and ways to target dynamic RNA elements identified in the genome of SARS-CoV-2 by small molecules.

O-11
Deciphering Long-Range Charge Transport Mechanisms of Biological Energy Transduction
Ville Kaila1
[1] Department of Biochemistry and Biophysics, Stockholm University, Sweden

Cellular respiration is catalyzed by large membrane-bound protein complexes that transduce redox energy into a trans-membrane proton gradient, powering the synthesis of ATP. Yet, despite major advances in recent years, the molecular principles of these charge transport reactions remain poorly understood, and a major challenge for biophysics. In this talk, I describe our integrative biophysical, computational, and structural approach to derive a molecular understanding of long-range charge-transport mechanisms in biological systems, with a focus on the highly intricate Complex I superfamily. Our findings reveal how catalysis triggers conformational and hydration changes in the proteins, how protonation signals propagate across the membrane domain triggering action-at-a-distance effects, and how various modular adaptations and supercomplex assemblies modulate the functional dynamics. Despite distinctly different molecular architectures, our findings suggest that the proteins operate by similar electric field effects, with striking physical similarities across different biological systems.

O-12
Regulating biochemical processes by modulating disordered protein phase separation
Julie Forman-Kay[1,2]
[1] University of Toronto, Canada [2] Hospital for Sick Children

The role of protein phase separation in sub-cellular organization is increasingly appreciated. Intrinsically disordered proteins (IDPs) and protein regions (IDRs), which make up over 60% of residues in the human proteome, are key modulators of this phase separation. The resulting cellular biomolecular condensates are critical regulators of biochemical processes, from transcription, nucleocytoplasmic transport, RNA processing, translation, and signaling. In neurons, the regulation of translation at the synapse in response to synaptic activity underpins learning, memory and neurodevelopment. Neuronal mRNA transport granules, biomolecular condensates that reversibly respond to synaptic activity to regulate translation, contain numerous RNA binding proteins with phase-separating IDRs. Our work demonstrated how in vitro phase separation of IDRs from two of these proteins, FMRP and CAPRIN1, controls translation and deadenylation rates. We also showed that phase separation is dependent on post- translational modifications linked to synaptic activity. With collaborators, we extended these biophysical insights to demonstrate cellular impacts on neuronal translation impacted by neurodevelopmental and neurodegenerative disease variants. In addition, we proposed how IDR variants, including common variants, can lead to pathology in complex diseases such as autism spectrum disorder and cancer through aberrant properties of biomolecular condensates controlling cellular processes.

O-13
Computational microbiology: a focus on bacterial cell envelopes
Syma KHALID[1]
[1] University of Oxford, UK

Bacterial cell envelopes are compositionally complex and crowded and while highly dynamic in some areas their molecular motion is very limited in others. Thus, studying them at high resolution across a range of temporal and spatial scales requires a number of different techniques. Details at atomistic to molecular scales for up to tens of microseconds are now within range for molecular simulations. Here we discuss how molecular dynamics simulations have contributed to our current understanding of the molecular arrangements within the cell envelopes of Gram-negative bacteria - in particular how they are providing insights into the molecular organisation of the outer membrane of E. coli. Simulations that incorporate as much of the native molecular complexity as is possible/pragmatic are also enabling us to generate new hypotheses on the behaviour of antimicrobials within the cell envelope. In conclusion, a perspective will be provided on advancing the complexity of molecular simulations such that they enable a 'computational microbiology' approach to studying bacterial cell envelopes.

O-14
Virus traps and other molecular machines of the future
Hendrik Dietz[1]
[1] Technische Universität München, Germany

Our research is focused on understanding and creating molecular devices and machines that can perform specific tasks as directed by users. We draw inspiration from natural macromolecular assemblies such as viruses and molecular motors and seek to implement similar principles in synthetic molecular machinery. DNA origami is a particularly promising technique that we use for programmable molecular self-assembly. We employ computational design and cryo-electron microscopy to enhance the accuracy and complexity of our synthetic molecular creations, which lead to several interesting accomplishments: (1) By studying viruses, we have successfully programmed DNA blocks to self-assemble into icosahedral shells, which have potential applications as programmable antiviral drugs. These virus traps can be designed to neutralize targeted viruses. (2) We have developed DNA origami structures capable of carrying genetic instructions that can be read by mammalian cells. This opens up possibilities for a wide range of applications, including gene therapy and targeted drug delivery. (3) We have constructed nanoscale assemblies with controllable movements, such as autonomous, power-generating rotary DNA motors and turbines driven by ion flux across membranes. These machines offer potential for executing energy-consuming tasks in synthetic cells.

Protein Dynamics

Session chairs: Mikael Akke, Lund University & Pétur Orri Heidarsson, University of Iceland

O-15 Invited speaker
Loop Dynamics and the Evolution of New Enzym Functions
Shina Caroline Lynn Kamerlin[1,2]
[1] Georgia Institute of Technology, Atlanta, USA [2] Uppsala University, Sweden

Two decades have passed since James & Tawfik posited their so called "New View" of proteins, emphasizing the importance of conformational diversity to the emergence and evolution of new enzyme functions. Despite the passage of substantial time since then, and while there has been a wealth of studies of links between conformational dynamics and enzyme catalysis, the importance of such dynamics to enzyme evolution has only recently started to become more broadly appreciated. In particular, there is ever increasing evidence that catalytic loops are not only highly conformationally plastic, but that this plasticity, and it's regulation, plays an important role in regulating the evolution of enzyme activity, selectivity, and even the pH dependency of catalysis. In addition to being important drug targets, protein tyrosine phosphatases (PTPs) provide an excellent model system with which to probe links between loop dynamics and catalysis, as they are allosterically regulated by the motion of multiple correlated loops that cover the active site, with direct links between loop motion and turnover rates. This talk will explore recent studies of a range of PTPs, showcasing the importance of loop dynamics to evolution in these biomedically critical enzymes.

O-16 Invited speaker
Visualizing the multi-step folding trajectory of an intrinsically disordered scaffold protein upon binding to the small GTPase Rac1
Malene R. Jensen[1], Lenette F. Kjær[1], Francesco S. Ielasi[2], Luiza M. Bessa[1], Laura Marino Pérez[1], Agneta Kiss[1], Elisabetta Boeri Erba[1], Guillaume Bouvignies[3], Andrés Palencia[2]
[1] Institut de Biologie Structurale, Grenoble, France [2] Institute for Advanced Biosciences, Grenoble, France [3] École Normale Supérieure, Paris, France

Activation of the pro-apoptotic c-Jun N-terminal kinase (JNK) cell signalling pathway is initiated by binding of the small GTPase Rac1 to the intrinsically disordered scaffold protein POSH (Plenty Of SH3s). Here we identify a novel recognition mode for Rac1 binding to a non-canonical CRIB motif in the intrinsically disordered region of POSH. Using NMR nuclear relaxation rates to delineate the precise binding site, we demonstrate that the interaction involves two molecular recognition elements (MRE1 and MRE2) covering an impressive 55 amino acids of POSH. Using high-throughput crystallization screening, CrystalDirect harvesting and automated crystal diffraction at the MASSIF-1 beamline at the ESRF, Grenoble, we obtained the crystal structure of the POSH-Rac1 complex at 1.2 Å resolution showing complete folding of both MREs of POSH upon binding to Rac1. Using an extensive set of NMR chemical exchange saturation transfer (CEST) experiments, we map the kinetic details of the folding trajectory of POSH upon binding to Rac1. We show that the interaction initially proceeds through binding and instantaneous folding of MRE1 followed by a reversible folding event of MRE2 on the second time scale on the surface of the GTPase. Our work provides insight into the complexity of binding mechanisms employed by intrinsically disordered proteins and offers novel structural insight into effector recruitment by Rac1.

O-17 Short talk
Single-molecule enzymology of chromatin ubiquitination
Alexandra Teslenko[1], Beat Fierz[1]
[1] Laboratory of Biophysical Chemistry of Macromolecules (LCBM), École Polytechnique Fédérale de Lausanne (EPFL), Switzerland

Epigenetic gene regulation via post-translational modifications (PTMs) on histone proteins defines the cell-type-specific gene expression of multicellular organisms. In particular, the primary ubiquitination of H2A catalyzed by the E3 ligase Polycomb repressive complex 1 (PRC1) recruits downstream Polycomb proteins which together establish gene repressive loci. It remains unknown how the enzymatic activity of PRC1 correlates with the dynamic propagation of the repressed chromatin state. Here, we present a method to study chromatin ubiquitination by PRC1 in real time on the single-molecule scale. First, we dissect the recruitment dynamics of the PRC1 complexes on immobilized synthetic chromatin in the absence of preinstalled PTMs. Then, we introduce preloaded E2~Ub which drives the PRC1 ligase to ubiquitinate H2AK119. The observed binding and enzymatic kinetics are used to build a mechanistic model of chromatin 'reading' and 'writing' in real time. This allows us to identify protein-protein interactions that are key for allosteric control of PRC1 and reveal chromatin states that are specifically targeted. In the following, we correlate the activity of PRC1 to its subunit composition and to its dynamics on chromatin with preinstalled PTMs, i.e. ubiquitin. The presented approach can be used to identify drug effects on the binding and enzymatic kinetics of E3 ligases.

O-18 Short talk
Substrate Inhibition of an Enzyme: Are Ultrafast Motions Affecting Catalytic Activity?
David Scheerer[1], Bharat Adkar[2], Sanchari Bhattacharyya[2], Dorit Levy[1], Marija Iljina[1], Inbal Riven[1], Orly Dym[1], Gilad Haran[1], Eugene Shakhnovich[2]
[1] Weizmann Institute of Science, Israel [2] Harvard University, USA

Proteins often harness large-scale motions of domains and subunits to promote their function. The enzyme adenylate kinase (AK) has served as a playground for testing the potential relation between conformational dynamics and catalytic activity. This three- domain enzyme plays a crucial role in maintaining cellular energy homeostasis by catalyzing phosphotransfer from ATP to AMP. Efficient catalysis is ensured by a large-scale domain motion that encloses the bound substrates. Single-molecule FRET spectroscopy combined with H2MM, a photon-by-photon hidden Markov model analysis, has revealed that this process occurs within tens of microseconds, two orders of magnitude faster than the enzyme's turnover. In the present work, we demonstrate that it is the equilibrium between open and closed state that dictates turnover, rather than the explicit interconversion rates. This is tested through the effect of AMP as a substrate inhibitor. We further show that inhibition arises due to strong allosteric communication between the domains, which manifests in a highly cooperative closure. The results allow us to develop a model that properly accounts for AK's kinetics by combining conformational dynamics and biochemical steps. In this model, the order of binding of ATP and AMP affects the kinetics; AMP binding generates an overpopulation of a dead-end closed state, which can reduce activity. In conclusion, the control of conformational equilibria via very fast domain motions explains substrate-dependent effects in AK. Since ultrafast dynamics have recently been observed in several other proteins, this might be a universal mechanism to regulate activity.

O-19 Short talk
Enhanced statistical sampling reveals microscopic complexity in the talin mechanosensor folding energy landscape
Rafael Tapia-Rojo[1,2], Marc Mora[1,2], Sergi Garcia-Manyes[1,2]
[1] The Francis Crick Institute, UK [2] King's College London, London, UK

Proteins must fold into their native state to be functional. How proteins search and attain their native structure as they emerge from the ribosome and when they repeatedly unfold and refold in their physiological setting remains a pivotal question in biology. Statistical mechanics allow the description of the conformational ensembles that define the free-energy landscape of a folding protein. However, the challenge lies in capturing the entire repertoire of low-probability states separated by high kinetic barriers that define complex landscapes. Computationally, enhanced sampling techniques bias the system to drive the exploration of rare molecular events. Experimentally, accessing the complete protein's conformational space requires technological developments to expand the measuring times. We developed ultra-stable single-molecule magnetic tweezers to capture over a million individual transitions as a single talin protein unfolds and refolds under force in equilibrium. Over classically probed timescales, talin folds in an uncomplicated two-state manner. As our measuring time extends from minutes to days, the underlying energy landscape exhibits gradually larger signatures of complexity, involving a finite number of well-defined rare conformational states. We developed a fluctuation analysis method to propose plausible structures for each low-probability state and portray its energy landscape as a kinetic network, reminiscent of the Markov-state models standard in Molecular Dynamics, here experimentally obtained. The physiological relevance of each distinct conformation was connected to the binding of the cytoskeletal protein vinculin, suggesting an extra layer of complexity in talin-mediated mechanotransduction. Our experiments directly test the fundamental notion that equilibrium dynamics depend on the observation timescale.

O-20 Short talk
Unravelling the dynamics of replisome progression and histone transfer in eukaryotic DNA replication
Zhaowei Liu[1], Pang Yen Wang[1], Julien Gros[1], Theo van Laar[1], Edo van Veen[1], Daniel Ramírez Montero[1], Humberto Sánchez[1], Nerea Murugarren[1], Katinka Ligthart[1], Milos Cvetkovic[2], Samson Glaser[2], Alessandro Costa[2], John Diffley[2], Nynke Dekker[1]
[1] Delft University of Technology, Netherlands [2] The Francis Crick Institute, UK

During eukaryotic DNA replication, the replisome is assisted by histone chaperones to disassemble parental nucleosomes into histone dimers/tetramers and transfer them onto the nascent DNA to reassemble nucleosomes. However, the molecular mechanisms of this process are still poorly understood due to the lack of dynamic information. Here, we use integrated single-molecule force-fluorescence spectroscopy to extract spatiotemporal information of replication-coupled histone transfer. We purified and fluorescently labeled the central component of Saccharomyces cerevisiae (Sc.) replisome, CMG helicase, and loaded it on 17 kb dsDNA molecules in bulk. Individual dsDNA molecules loaded with CMG were optically trapped, and confocal scanning microscopy was used to image the CMG and monitor its dynamics. Consistent with previous reports, the CMG translocated unidirectionally in the presence of ATP, and two crucial replication factors, Mcm10 and MTC, significantly increased the efficiency of CMG loading on DNA and its translocation velocity. Other replisome components, including DNA polymerases, are subsequently added to assemble a full replisome. To reconstitute chromatinized DNA, we used fluorescently labeled Sc. histones to in vitro assemble a nucleosome array on the aforementioned dsDNA substrate. We optically trapped the chromatinized DNA molecules, and imaged the fluorescent nucleosomes, which were subsequently unwrapped through the application of controlled increasing stretching forces. We demonstrated that complete nucleosomes with well-controlled positioning were assembled on these dsDNA molecules. In the future, chromatinized DNA substrates will be integrated with active replisomes under various histone chaperoning conditions to monitor the dynamics of the replisome as well as histone chaperones.

Biophysics of Viruses

Session chairs: Sarah Butcher, University of Helsinki & Nicola Abrescia,CIC bioGUNE

Arranged in collaboration with Protein Society

O-21 Invited speaker
Flying viruses - mass spectrometry meets X-rays
Charlotte Uetrecht[1]
[1] Heinrich Pette Institut, Leibnitz, Germany

Viruses affect basically all organisms on earth. Some are detrimental to human development as we experience during the ongoing pandemic, whereas those targeting pathogenic bacteria or crop pathogens can be beneficial for us. An integral part of icosahedral viruses is the capsid protein shell protecting the genome. Many copies of the capsid protein often self-assemble into shells of defined size. Low binding affinity of individual subunits allows efficient assembly and gives rise to highly stable particles. These capsids can be studied by native mass spectrometry (MS), a single molecule like approach, in terms of stoichiometry, dynamics, assembly pathways and stability revealing coexisting states. However, the structural resolution provided is limited. Therefore, we built a prototype native mass spectrometer in the MS SPIDOC project to deliver select species to X-ray sources for gas phase SAXS and single particle imaging. First experiments reveal good performance of the MS setup. As opposed to icosahedral viruses, the hemorrhagic fever causing Lassa virus forms a helical, viral ribonucleoprotein particle (vRNP) that associates with the matrix protein Z to allow packaging into the budding enveloped virions. Using native MS, hydrogen/deuterium exchange MS and computational modelling, the first stages of vRNP assembly from nucleoprotein and RNA are revealed. Those initial forms are in line with Z association. Z association is sensitive to pH, which allows fine tuning between packaging and uncoating.

O-22 Invited speaker
Physical Virology with atomic force and fluorescence microscopies: seeing and touching viruses and protein cages
Pedro J. de Pablo[1]
[1] Universidad Autonóma de Madrid, Spain

The basic architecture of a virus consists of the capsid, a shell made up of repeating protein subunits, which packs, shuttles and delivers their genome at the right place and moment. Viral particles are endorsed with specific physicochemical properties which confer to their structures certain meta-stability whose modulation permits fulfilling each task of the viral cycle. These natural designed capabilities have impelled using viral capsids as protein containers of artificial cargoes (drugs, polymers, enzymes, minerals) with applications in biomedical and materials sciences. Both natural and artificial protein cages must protect their cargo against a variety of physicochemical aggressive environments, including molecular impacts of highly crowded media, thermal and chemical stresses, and osmotic shocks. Viral cages stability depends not only on the structure of the external capsid, which relies on the interactions between protein subunits, but also on the nature of the cargo. During the last decade our lab has focused on the study of protein cages with Atomic Force Microscopy (AFM). We are interested in establishing links of their mechanical properties with their structure and function. Mechanics provide information about the cargo storage strategies of both natural and virus-derived protein cages. Mechanical fatigue has revealed as a nanosurgery tool to unveil the strength of the capsid subunit bonds. This allows to unveil ageing effects on virus structures, in a similar way to ageing in materials science.

O-23 Short talk
Unveiling the molecular mechanism of membrane fusion mediated by the parainfluenza fusion peptide
Carolina C. Buga[1,2], Mariana Valério[1], Miguel A.R.B. Castanho[2], Cláudio M. Soares[1], Ana Salomé Veiga[2], Diana Lousa[1]
[1] Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal [2] Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal

Parainfluenza viruses are responsible for a significant number of infections worldwide and a major burden of disease in children. These enveloped viruses enter the cell through the fusion of the viral envelope with the host cell membrane mediated by the fusion glycoprotein. The fusion peptide (FP), exposed after the proteolytic cleavage of the fusion glycoprotein, is believed to play a major role in the fusion process, but the exact mechanism by which it promotes membrane fusion is not fully understood. Previously, we found that the parainfluenza FP (PIFP) forms water-permeable porelike structures in the membrane, inducing not only lipid head intrusion and lipid tail protrusion but also promoting membrane leakage and fusion. Moreover, we have pinpointed key PIFP amino acid residues for the membrane fusion. Here, we dissect the impact of those residues in the membrane fusion process through the combination of biophysical and computational methods. Our results showed that the PIFP N-terminus is essential for membrane interaction and induction of leakage and fusion of lipid vesicles. Furthermore, we found that in the PIFP porelike structure, the Q120 residue located in the hydrophobic core of the membrane might be involved in the mediation of the water passage by establishing a key network of peptide-peptide interactions. Overall, our work contributes to the understanding of the PIFP-induced membrane fusion process and ultimately to the development of viral entry inhibitors.

O-24 Short talk
How Influenza A Virus Solves the Nuclear Escape Room
Daniel Ziemianowicz[1,2], Ying Wang[1,2], Per Haberkant[1], Frank Stein[4], Rene Rosc[2,5], Nikenza Viceconte[1], Kay Grünewald[1,2,6], Mikhail Savitski[1], Jan Kosinski[1,2,]
[1] European Molecular Biology Laboratory, Hamburg, Germany [2] Centre for Structural Systems Biology (CSSB), Hamburg, Germany [3] Germany [4] Max Planck Institute of Biophysics, Frankfurt, Germany [5] University of Hamburg, Hamburg, Germany [6] Leibniz Institute of Virology, Hamburg, Germany

Influenza A virus, notorious for its resistance to antivirals and vaccines, relies on host cell components for viral ribonucleoprotein (vRNP) replication. The host nuclear pore complex (NPC) is vital for vRNP nuclear import and export—the virus potentially exploits host molecular pathways to modify NPC structure and function. However, the molecular mechanisms underlying vRNP nuclear export remain unclear. Our approach integrates systems and structural biology, utilizing techniques like thermal proteome profiling with mass spectrometry, cellular cryo-electron tomography (cryoET), fluorescence microscopy imaging, and cellular assays with drugs targeting influenza replication to investigate these mechanisms and their temporal dynamics. We confirm that influenza vRNPs accumulate in the host cell nucleus during the first 7 hours of infection, with nuclear export commencing at 7-8 hours. CryoET data reveal a 15 nm (~15%) enlargement of NPC pore diameter at 8 and 15 hours post-infection, a compression of the nuclear and cytoplasmic rings toward the middle, and additional structural changes in the nuclear ring. Proteomics analysis indicate perturbed protein thermal stability across the NPC, and hundreds of other proteins, across the host proteome from 7 hours onward. Notably, components of the nuclear lamin—a network structurally associated with the NPC—and cytoskeleton were affected. Systems analysis, based on the proteomic data, suggests molecular pathways that are potentially causal in disrupting the NPC during infection. This research provides valuable insights into the molecular mechanisms of influenza A virus infection and replication, presenting new host candidates for disrupting IAV infection by combining systems and structural biology perspectives.

O-25 Short talk
Combined computational study of the binding of nirmatrelvir to SARS-CoV-2 main protease: insight into resistance mechanism
Gyula Hoffka[1,2], Mohamed Mahdi[1], József Tőzsér[1], János András Mótyán[1]
[1] Laboratory of Retroviral Biochemistry, Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary [2] Doctoral School of Molecular Cell and Immune Biology, University of Debrecen, Debrecen, Hungary

To treat the COVID-19 disease in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infected individuals, Pfizer has developed Paxlovid, an antiviral combination that includes nirmatrelvir, a covalent inhibitor of the SARS-CoV-2 main protease (Mpro). Recent experimental studies have shown how mutations may lead to the emergence of resistance of Mpro against nirmatrelvir. The aim of our study was to model the reaction of Mpro with a substrate and with the inhibitor, as well as to study the molecular background of resistance development. Multiple crystal structures were analyzed to map the enzyme-substrate and enzyme-inhibitor interactions, while the conformational space and non-covalent interactions were studied by molecular dynamics simulations. In order to compare the substrate cleavage and nirmatrelvir binding, we have applied the hybrid QM/MM ONIOM method. To examine the resistance, we have modelled previously described resistant variants as well as novel, computationally generated mutants. Our results help better understanding how individual residues of Mpro contribute to substrate cleavage and inhibitor binding at atomic level. We propose how individual mutations of the active site may lead to the development of resistance against nirmatrelvir. The work was supported in part by TKP2021-EGA-20 (Biotechnology) and POST-COVID2021-16 projects.

O-26 Short talk
Dynamic investigations of norovirus-glycosphingolipid interactions using cell-membrane mimics
Marta Bally[1,2], Konrad Thorsteinsson[1,2], Inga Rimkute[3], Hudson Pace[1,2], Victoria Tenge[4], Jonas Nilsson[3], Mary Estes[4], Göran Larson[3]
[1] Umeå University, Sweden [2] Wallenberg Centre for Molecular Medicine, Sweden [3] Gothenburg University, Sweden [4] Baylor College of Medicine, USA

The initial recruitment of a virion to the cell membrane is a highly dynamic process which needs to be tightly regulated to ensure successful navigation of the virion to its entry point and efficient uptake. While much research effort is devoted to receptor identification, less is known on how virus attachment, detachment and diffusion is modulated at the cell surface. Here, we investigate the mechanisms regulating the interaction of human norovirus (hNoV) at the cell surface and aim at establishing a relation between interaction dynamics and susceptibility. hNoV has been shown to interact with histo-blood group antigens (HBGAs) decorating the gastrointestinal tract. Here, we use TIRF microscopy to study the binding kinetics of individual virus particles on HBGA-glycolipid-containing bilayers. Using membranes produced from human intestinal enteroids, we reveal significant differences in the binding dynamics of the virus, depending on the individual's HBGA profile and susceptibility to infection. Furthermore, we systematically study how the membrane environment affects the hNoV-HBGA interaction and pinpoint the role of specific lipid components in modulating binding kinetics. These results highlight the importance of considering the dynamics of virus-membrane interactions when studying viral entry and provide new insights into tropism and the initial stages of hNoV infection.

Microfluidics and organ-on-a-chip for biophysics

Session chairs: Pawel Sikorski & Bjørn Torger Stokke, Norwegian University of Science and Technology, NTNU

O-27 Invited speaker
Kinetics of protein phase transitions
Tuomas Knowles[1]
[1] University of Cambridge, UK

Protein molecules can access different physical phases, including solid amyloid aggregate phases and liquid condensate phases. The transitions from the normal solution state into these phases has consequences for biological function and malfunction. While liquid condensate phases of proteins are often functional, the solid amyloid state of proteins is most commonly associated with malfunction, including notably in the context of neurodegenerative disorders such as Alzheimer's and Parkinson's diseases. This talk will outline our efforts to discover, characterise and understand the molecular mechanisms that underpin the transition of proteins from one phase to another. A particular focus will be the different nucleation mechanisms, including secondary nucleation, that trigger amyloid formation from the solution state of proteins.

O-28 Invited speaker
Combining Stem Cell and Device Engineering for In vitro Models of Human Physiology
Anna Herland [1,2]
[1] Department of Neuroscience, Karolinska Institutet, Sweden [2] SciLifeLab Division of Nanobiotechnology Department of Protein Science, KTH Royal Institute of Technology, Sweden

Mammalian cell in vitro studies have poor translation to human in vivo processes and treatment outcomes. The low resemblance between classical cell culture, a monolayer, static culture of cell lines and the dynamic, three-dimensional, and multicellular in vivo environment explains this gap. The demand to accurately model human physiology and pathophysiology has, however, risen with insights into inter-species differences and with the development of human-specific treatments. Engineered human microfluidic Organ-on-Chip models have emerged as a promising new pre-clinical technology to meet this demand. In Organ-on-Chips, cells are cultured in connected microcompartments with perfusion. We developed a 10-Organ Chip platform to recapitulate human Body-on-Chip with physiological vascular coupling. This Body-on-Chip system allowed studies of intestinal uptake, intravenous injection, first-pass metabolism and excretion, organ-specific responses, and accurate in vitro-to-in vivo extrapolation (IVIVE). We are developing these systems from three perspectives: first, to increase human physiological relevance through stem cell engineering and relevant three-dimensional microenvironment, second, to incorporate non-disruptive real-time monitoring of cells, and third, to make them user-friendly and cost-effective. Our specific focus is the neurovascular unit (NVU), the restrictive barrier that lines the capillaries in the brain and spinal cord. Our NVU-on-Chip models are populated with human pluripotent stem cell- derived vascular and neural cells. The design and material of the NVU-on-Chip have been tailored to study barrier penetration of small drugs and biopharmaceuticals, as well as cellular interactions and inflammatory responses in real time.

O-29 Short talk
Neoepitope libraries construction and screening by Natural Language Processing tokenization and droplet microfluidics
Aitor Manteca[1], Elena Lopez-Martinez[1], Noelia Ferruz[2], Rémi Sieskind[3], Thomas Beneyton[4], JC Baret[4,5], Aitziber L. Cortajarena[1,6]
[1] Centre for Cooperative Research in Biomaterials, Basque Research and Technology Alliance, Donostia-San Sebastián, Spain. [2] Institut de Biologia Molecular de Barcelona IBMB, Barcelona, Spain. [3] Pasteur Institute, Paris, France [4] University of Bordeaux, Pessac, France [5] Institut Universitaire de France, Paris 75005, France. [6] IKERBASQUE, Basque Foundation for Science, Bilbao, Spain.

Epitopes are small protein regions on virus and bacteria that can be recognized by antibodies. The discovery of novel epitopes is crucial to speed up the development of vaccines and immunotherapies. However, the discovery of these molecular recognition sites in real pathogens is not straightforward since epitopes are not always lineal and can adopt complex structural conformations. To overcome this issue, screening artificial epitope libraries against certain antibodies can be helpful. Here, applied conventional computational studies and Natural Language Processing (NLP) to identify specific sequence patterns in epitope databases that can serve as building blocks for universal epitope libraries. Our results reveal that amino acid frequency in epitopes varies from the rest of the genome. While aromatic residues increase in frequency, the presence of cysteines is practically null. Byte Pair Encoding tokenization shows high frequencies of tryptophan in tokens of 5, 6, and 7 amino acids. These results have been applied to reduce the diversity of epitope libraries in orders of magnitude. The libraries were fused to GFP and encapsulated in water-in-oil drops. Epitope-antibody recognition was detected by two fluorescent methods: (i) conjugating the antibody with a microbead that leads to a peak in the fluorescence of the drop when this recognition takes place and (ii) measuring the fluorescence anisotropy of the drop that increases when the epitope binds the antibody. Finally, the binding epitopes were selected using fluorescence activated droplet sorting (FADS).

O-30 Short talk
Developing tissue-inspired systems to enable physiologically-mimicked 3D neuronal cultures
Christina Tringides[1,2], Janos Vörös[1], David Mooney[2], Blandine Clément[1], Marjolaine Boulingre[2]
[1] ETH Zurich, Switzerland [2] Harvard University, USA

Biomaterial scaffolds have emerged as a tool to build 3D cultures of cells which better resemble biological systems, while advancements in bioelectronics have enabled the modulation of cell proliferation, differentiation, and migration. Here, we first describe a porous conductive hydrogel with the same mechanical modulus and viscoelasticity as neural tissue. Electrical conductivity is achieved by incorporating low amounts (<0.3% weight) of carbon nanomaterials in an alginate hydrogel matrix, and then freeze-drying to self-organize into highly porous networks. The mechanical and electrical properties of the material can be carefully tuned and used to modulate the growth and differentiation of neural progenitor cells (NPCs). With increasing hydrogel viscoelasticity and conductivity, we observe the formation of denser neurite networks and a higher degree of myelination. To investigate the functionality of neurite networks in 3D, we begin by placing a polydimethylsiloxane (PDMS) microstructure on an underlying multielectrode array (MEA). We then explore different materials and techniques to integrate hydrogels into the PDMS microstructures, such that the hydrogel can facilitate neurons to form 3D networks while still confined by the PDMS. This platform is compatible with various methods to assess neuronal functionality (e.g. MEA electrical recordings), and can be used to understand the effect(s) of hydrogel properties on the resulting neuronal networks. Both described biomaterial platforms can support the growth of neuronal cells for over 6 weeks and could be used to investigate neuronal development and disease progression.

O-31 Short talk
Enhanced DNA Mixing with Viscoelastic Waves
Enrico Turato[1], Oskar Ström[1], Jason Beech[1], Jonas Tegenfeldt[1 1]
Lund University, Sweden

At high DNA concentrations and high flow velocities, instabilities in the stream of DNA solutions have been shown to appear and to have a detrimental effect on the microfluidic sorting using deterministic lateral displacement. These fluctuations in concentrations and flow direction have been recently characterized. We believe that viscoelastic DNA waves can be used to enhance the mixing in microfluidic channels at low Reynolds numbers, where mixing is usually a challenge due to laminar flow. The idea behind our type of mixing is that we use elastic turbulence to enhance diffusion by lowering diffusion lengths and diffusion times relying on wave-induced DNA transport. To experimentally test the degree of mixing for pressure-driven flows of 48 kbp λ-DNA solutions with concentrations of the order of hundreds ng/μL in micropillar arrays structures, we stained two DNA solutions with YOYO-1 and YOYO-3 respectively and used a two-way image splitter (Optosplit) to visualize the flow of the two DNA samples independently as they were injected side by side at the entrance of the device and as they start to mix along the microfluidic channel. Microfluidic devices were made using PDMS bonded to glass using standard soft lithography. We show how two initially parallel streams of DNA, coming from two different inlets, move through our pillar structures and intertwine. We observe enhanced mixing of the DNA for increasingly higher flow rates as the DNA moves through the device.

0-32 Short talk
Nanofluidic diffusional sizing of single molecules and nanoparticles in solution
Oliver Vanderpoorten[1]
[1] The Arctic University of Norway, UiT, Norway

Nanofluidic devices find tremendous application potential in various research fields ranging from renewable energy to human health. They allow researchers to use a completely new toolset of physical effects on the nanoscale to design their experiments, such as nanofiltration of e.g. protein aggregates related to neurodegenerative diseases (htau, α-syn), diffusional sizing of single molecules using fluorescence and label-free methods (TIRFM, iSCAT), or nanoencapsulation of biomolecules for drug delivery. These nanofluidic chips are conventionally manufactured following fabrication approaches of a clean-room environment, which involves reactive ion etching and electron beam lithography. Hybrid 2-photon lithography with soft lithography allows to circumvent these limitations and facilitates scalable nanofluidic PDMS device fabrication. Their functionality for diffusional sizing at the single molecule level is demonstrated using total-internal-reflection-fluorescence microscopy and confocal microscopy on various samples related to protein misfolding diseases. By mean-square-displacement (MSD) analysis of single molecules (e.g. Rhodamine 6G, GFP, hTau, α-synuclein oligomers, exosomes, liposomes) in nanochannels/nanotraps it is possible to estimate their hydrodynamic radius using the Stokes-Einstein relation at single molecule sensitivity - without permanent surface immobilization of the protein for detection. These results showcase a versatile pathway for the improvement of single molecule- based protein studies in biological laboratories with limited access to nanofabrication facilities.

Single-molecule biophysics

Session chairs: Viktoria Birkedal, Aarhus University & Mike Heilemann, Goethe-University Frankfurt

O-33 Invited speaker
SPARXS: Single-molecule Parallel Analysis for Rapid eXploration of Sequence space
Chirlmin Joo[1]
[1] Delft University of Technology, Netherlands

Single-molecule fluorescence studies reveal valuable structural and functional information on biomolecules. However, conventional single-molecule methods have limited sequence diversity due to time, effort and cost constraints. To overcome this, we combine surface-based single-molecule fluorescence with next generation sequencing, enabling massively parallel single-molecule experiments for millions of sequences. Our technique provides sequence-dependent biophysical and biochemical properties at the single molecule level, advancing our understanding of molecular structures and functions and enabling more accurate models in biomolecular engineering.

O-34 Invited speaker
An experimental framework to assess phase separation in bacteria
Julie Biteen[1]
[1] University of Michigan, USA

Biocondensates, which form when molecules phase separate in solution to form a condensed phase with material properties distinct from the surrounding dilute phase, has emerged as an organizing principle in bacterial cell biology. However, the small cell size creates a key hurdle specific to the study of these membraneless organelles in bacteria. To address this challenge, we have developed an experimental framework to assess phase separation in bacteria based on how biocondensates form, dissolve, tune their shape and size, and transition between phase states. Our experimental and analytical framework leverages super-resolution microscopy and single-molecule tracking to identify the material state of a fluorescent focus in bacteria, and we demonstrate its use in assessing the phase separation activity of heterologously expressed proteins in Escherichia coli.

O-35 Short talk
Nanoscale tweezers for spatially resolved single-cell subcellular analysis
Annie Sahota[1], Binoy Paulose Nadappuram[2], Michael Devine[3], Aleksandar Ivanov[1], Joshua Edel[1]
[1] Imperial College London, UK [2] University of Strathclyde, Scotland [3] Francis Crick Institute, UK

Profiling the single-cell omics of live cells could aid our understanding of intracellular mechanisms and how these are affected in disease. Subcellular sampling methods have the potential to achieve this as they maintain cells in their standard environment and do not require cell lysis or fixation. Many of these methods, however, are limited in performing dynamic measurements of the same cell because they involve the aspiration of cytoplasmic fluid which may cause alterations in the cell. Here, we present the nanotweezer: a low-cost, minimally invasive sampling tool that can trap biomolecules directly from living cells via dielectrophoresis, avoiding the need to remove large volumes from cells to access their contents. In this work, we show that nucleic acids and individual organelles can be biopsied from subcellular compartments of live primary neurons, achieving repeat sampling from the same cell, high spatial resolution, and minimal impact on cell viability. Further, we demonstrate mRNA and single mitochondrion analysis from individual biopsies and highlight the significance of this technique for precise quantification and mapping of biomolecules in the same cell. We propose the nanotweezer as an innovative tool for dynamic subcellular sampling and tracking in living cells which could ultimately transform the fields of spatial transcriptomics and personalized medicine.

O-36 Short talk
Single-protein holography
Jan Christoph Thiele[1,2], Emanuel Pfitzner[1,2], Philipp Kukura[1,2]
[1] Physical and Theoretical Chemistry Laboratory, Department of Chemistry, University of Oxford, UK [2] Kavli Institute for Nanoscience Discovery, Oxford, UK

Light scattering by nanoscale objects is a fundamental physical property defined by their scattering cross-section. Over the past decade, a variety of approaches have demonstrated single molecule sensitivity, by interfering the coherent scattering from the object of interest with a reference field. This approach enables mass measurement of single biomolecules in solution owing to the linear scaling of the image contrast with the molecular polarisability. Nevertheless, all approaches to date cannot separate and independently tune the reference and scattered light field, meaning that the underlying polarisability cannot be quantified. Here, we present a dark-field scattering microscope which, similar to a Mach-Zehnder interferometer, separates the scattering and reference light into separate arms, enabling us to introduce distinct phase shifts in four parallel detection channels, in a fashion that is insentive to absolute pathlength changes, enabling highly sensitive phase measurements. Combination of these four interference images allows us to reconstruct the complex scattering field. We calibrate our instrument with gold nanoparticles and demonstrate detection and mass measurement of single proteins below 100 kDa. Importantly, holographic detection separates the amplitude and phase information, which yields direct information on sample identity and the first experimental determination of the scattering cross-section of single biomolecules.

O-37 Short talk
Protein sizing using 15-nm conical nanopores YaxAB
Sabine Straathof[1], Giovanni Di Muccio[2], Maaruthy Yelleswarapu[1], Melissa Alzate Banguero[2], Carsten Wloka[1], Nieck Van der Heide[1], Mauro Chinappi[2], Giovanni Maglia[1]
[1] University of Groningen, Netherlands [2] University of Rome Tor Vergata, Italy

Nanopores are promising single-molecule tools for the electrical identification and sequencing of biomolecules. However, the characterisation of proteins, especially in real-time and in complex biological samples, is complicated by the sheer variety of sizes and shapes in the proteome. Here, we introduce a large biological nanopore YaxAB for folded protein analysis. The 15 nm cis-opening and a 3.5 nm trans-constriction describe a conical shape that allows the characterisation of an unprecedented wide range of proteins. Molecular dynamics showed proteins are captured by the electroosmotic flow and the overall resistance is largely dominated by the narrow trans constriction region of the nanopore. Conveniently, proteins in the 33-125 kDa range remain trapped within the conical lumen of the nanopore for a time that can be tuned by the external bias. Contrary to cylindrical nanopores, in YaxAB the current blockage decreases with the size of the trapped protein, as smaller proteins penetrate deeper into the constriction region than larger proteins. These characteristics are especially useful for characterising large proteins, as shown for pentameric C-reactive protein (125 kDa), a widely used health indicator, which shows a unique signal that could be identified in real-time and with a background presence of proteins from depleted blood.

O-38 Short talk
Detection and quantification of ultrafast molecule-spanning dynamics in a multi domain protein by single-molecule fluorescence
Veronika Frank[1], Benedikt Sohmen[1], Steffen Wolf[2], Jean-Benoît Claude[3], Jérôme Wenger[3], Thorsten Hugel[1,4]
[1] Institute of Physical Chemistry, University of Freiburg, Freiburg, Germany [2] Institute of Physics, University of Freiburg, Freiburg, Germany [3] Fresnel Institute, CNRS, Aix-Marseille University, Marseille, France [4] BIOSS and CIBSS Signalling Research Centres, University of Freiburg, Freiburg, Germany

Protein functions are closely linked to their dynamics, which take place on a wide variety of time and length scales. Up to now, protein dynamics on the microsecond to minute time scale are associated with (large) conformational changes, while picosecond dynamics have been linked to side chain movements. The nanosecond time scale domain is still not well studied. Combining single-molecule fluorescence techniques and MD simulations, we reveal fast protein fluctuations on the hundreds of nanoseconds time scale, which surprisingly span the whole multi-domain heat shock protein 90 (Hsp90). We currently investigate how these molecule-spanning fluctuations are affected by helper proteins (cochaperones) and substrates (clients) of Hsp90. In parallel, we develop the use of zero-mode waveguide nanoapertures to improve the signal-to-noise ratio of these measurements and to cut down very long measurement times. Finally, these experiments will guide us from a pure descriptive picture of the molecule- spanning dynamics to an understanding of their functional relevance.

Activation and modulation of membrane proteins

Session chairs: Ville Kaila, Stockholm University & John Seddon, Imperial College London

O-39 Invited speaker
Structural Pharmacology of TRPV channels
Vera Moiseenkova-Bell[1]
[1] Perelman School of Medicine, University of Pennsylvania, United States

Transient Receptor Potential channels from the vanilloid subfamily (TRPV) are a group of cation channels that play a critical role in a variety of physiological and pathophysiological processes. They are modulated by a variety of endogenous stimuli as well as a range of natural and synthetic compounds. Their roles in human health make them of keen interest, particularly from a pharmacological perspective. However, despite this interest, the complexity of these channels has made it difficult to obtain high resolution structures until recently. With the cryo electron microscopy (cryo-EM) resolution revolution, our laboratory produced several TRPV channels structures and determine mechanisms of TRPV channels activation, inhibition and desensitization. This newly obtained information could guide us towards the design of novel TRPV channles specific therapeutic molecules.

O-40 Invited speaker
Watching ions moving across the biomembrane at utmost temporal and spatial resolution
Joachim Heberle[1]
[1] Freie Universität Berlin, Germany

Proteins are flexible nanomachines. Biological pumps that use retinal isomerization to move protons across a me mbrane have been studied extensively, but the mechanisms involved in moving sodium and chloride ions, which have both a different charge and different coordination requirements, are less well understood. We combined time-resolved X-ray crystallography by applying free electron laser sources, molecular spectroscopy, and QM/MM simulations to provide direct molecular insight into the dynamics of active ion transport across biological membranes. By these means, molecular movies were generated of ion transport through two microbial rhodopsins: The sodium pump rhodopsin 2 from Krokinobacter eikastus (KR2) and the chloride-pumping halorhodopsin from Nonlabens marinus (NmHR). Tracing these structural alterations over the entire chemical time range from femtoseconds to seconds provides an atomic-level understanding of how ions can be pumped against a concentration gradient. It is also demonstrated how spectroscopy and simulations can provide essential information not accessible to X-ray crystallography. Comparing the mechanism of these two ion pumps reveal similarities that have not been previously anticipated. These results provide insights to understand and improve tools for optogenetic silencing of neurons. An intimate coupling between lipids and proteins is observed for mechanosensitive channels, which open and close in response to changes in the lateral pressure of the lipid bilayer. The latter can be optically controlled with synthetic photoswitches, which are integrated into lipids (photolipids) and influence transmembrane proteins directly, via lipid/protein interactions, or indirectly, by altering the structure and dynamics of the membrane that hosts the protein.

O-41 Short talk
Cation Channels and Rhomboid Proteases Studied by Solid-State NMR
Adam Lange[1]
[1] Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP), Germany

Our lab develops novel solid-state NMR (ssNMR) based methods for efficient protein structure determination and the accurate measurement of protein dynamics. We apply these methods to a wide range of different biological systems, with a major focus on membrane proteins. Distinct from other methods in structural biology, ssNMR makes it possible to study membrane proteins in native-like lipid bilayers at room temperature and under physiological buffer conditions. This allows us to address basic biophysical principles of membrane proteins that are not easily accessible by other methods. Additionally, our group combines results from ssNMR and cryo-EM in order to elucidate the atomic structures of flexible supramolecular assemblies. In terms of membrane proteins, the focus lies on the one hand on cation channels and on the other hand on rhomboid proteases. For instance, we have recently shed light on the controversial question whether water molecules are involved in potassium conduction through the potassium channel selectivity filter. More recently, we could directly detect ions in the selectivity filter using ammonium NMR. Recent results on the rhomboid protease GlpG using tailored proton-detected ssNMR experiments developed in our group revealed a previously unobserved kink in the central part of the gating helix TM5. In addition, relaxation dispersion experiments suggested that TM5 is in conformational exchange between an open and a closed state. Additionally, we characterized the interaction of GlpG with a range of inhibitors. We have also recently started to screen for novel rhomboid protease modulators in cooperation with our in-house screening unit.

O-42 Short talk
Mechanism of action and lipid-mediated synergistic interactions of antimicrobial peptides
Burkhard Bechinger[1]
[1] University of Strasbourg, France

Biophysical and structural studies of peptide-lipid interactions, peptide topology and dynamics have changed our view how antimicrobial peptides insert and interact with membranes. Clearly, both the peptides and the lipids are highly dynamic, change and mutually adapt their conformation, membrane penetration and detailed morphology on a local and a global level. Consequently, the peptides and lipids can form a wide variety of supramolecular assemblies in which the more hydrophobic sequences preferentially, but not exclusively, adopt transmembrane alignments and have the potential to form oligomeric structures like those suggested by the transmembrane helical bundle model. In contrast, charged amphipathic sequences tend to stay intercalated at the membrane interface, where they have been found to adopt mesophase structures in a lipid dependent manner. Although the membranes are soft and can adapt, at increasing peptide density they cause pronounced disruptions of the phospholipid fatty acyl packing. At increasing local or global concentrations the peptides result in transient membrane openings, rupture and ultimately lysis. Interestingly mixtures of peptides such as magainin 2 and PGLa which are stored and secreted naturally as a cocktail exhibit considerably enhanced antimicrobial activities when investigated together in antimicrobial essays but also in pore forming experiments applied to biophysical model systems. A investigations reveal that these peptides do not form stable complexes but act by specific lipid-mediated interactions and through the nanoscale properties of phospholipid bilayers. Notably, a quantitative idea about the strength of the lipid packing interactions can be obtained when comparing the peptide topologies in DMPC and POPC bilayers.

O-43 Short talk
Ligand-free in situ confinement for GPCR activation and signal transduction
Maria Florencia Sánchez[1], Marina S. Dietz[2], Sylvia Els-Heindl[3], Annette G. Beck-Sickinger[3], Ulrike Müller[4], Julian Weghuber[4], Karl Gatterdam[1], Ralph Wieneke[1], Mike Heilemann[2], Peter Lanzerstorfer[4], Robert Tampé[1]
[1] Institute of Biochemistry, Goethe University Frankfurt, Germany [2] Institute of Physical and Theoretical Chemistry, Goethe University Frankfurt, Germany [3] Faculty of Life Sciences, Leipzig University, Germany [4] School of Engineering and Environmental Sciences, University of Applied Sciences Upper Austria, Wels, Austria

Cell-cell communication and signal transduction rely on the assembly of receptor-ligand complexes at the plasma membrane. The spatiotemporal receptor organization plays a pivotal role in evoking cellular responses. Nevertheless, the mechanism for cluster formation and how its localization within the plasma membrane influence cell responses is not fully understood. Thus, tools which allow to modulate receptor networks with high spatiotemporal control are required. Here, we controlled neuropeptide Y2 hormone receptor (Y2R) clustering in situ either via a chelator nanotool or via an ultrasmall photo-tool. Within seconds, receptor clustering can be modulated in size, location, and density. Y2R enrichment in clustered areas triggered ligand-independent downstream signaling determined by an increase in cytosolic calcium, cell spreading, and migration. These signaling events are analogous to the ones induced by the natural ligand revealing a ligand-independent receptor response upon clustering. This approach and the developed tools represent versatile instruments for tracking of biophysical processes at the plasma membrane and depict now the possibility to elucidate unexplored mechanisms in cell signaling and mechanotransduction.

O-44 Short talk
Competing wooers: A regulatory mechanism of the β2-adrenergic receptor based on competition between cholesterol and polyunsaturated lipids
Shreyas Kaptan[1] , Felix Eurasto[1], Waldemar Kulig[1], Ilpo Vattulainen[1]
[1] University of Helsinki, Finland

G-protein-coupled receptors (GPCRs) are integral membrane proteins, whose dysfunction is associated with the emergence of numerous diseases. The structures of GPCRs have allosteric binding sites for lipids, especially cholesterol, and this binding has been argued to be a biologically relevant mechanism for regulating the GPCR function. This regulation is also influenced by polyunsaturated fatty acids (PUFAs), which favor the rough surface of GPCRs and facilitate their partitioning on the lipid membrane. Abnormalities of PUFA levels in the synaptic membranes of the brain are a common feature in many neurological diseases (e.g., Alzheimer's disease, schizophrenia), which supports the idea that PUFA lipids also have a significant effect on the activity of GPCR proteins, even though the allosteric binding of PUFA lipids to GPCR proteins has not been reported. In this work, we apply machine learning (ML) methods to approximately millisecond long datasets produced by atomic- simulation models and show that the activity of β2-adrenergic receptor, (β2AR) a member in the GPCR family, is regulated by competition between cholesterol and PUFA lipids. We show that the β-adrenergic receptor family contains a hitherto unreported and highly conserved cholesterol binding site whose function, when occupied is ligand-free activation of signaling. This is compensated by PUFAs, for which the analysis reveals a specific binding site on the surface of β2AR, through which the bound PUFA lipid can suppress the ligand-free activation of the β2AR. Using ML methods, we demonstrate an atomistic resolution mechanism of how lipid binding is communicated to β2AR activation.

Intrinsically disordered proteins and liquid-liquid phase separations

Session chairs: Jean Baum, Rutgers University & Ylva Ivarsson, Uppsala University

Arranged in collaboration with Protein Society

O-45 Invited speaker
The Role of Phase Separation in Biology and Disease
RICHARD KRIWACKI[1,2], David Baggett[2], Bappaditya Chandra[2], Priyanka Priyanka Dogra[2], Mylene Ferrolino[2], Eric Gibbs[2], Katelyn Jackson[2], Suparna Khatun[2], Snigdha Maiti[2], Qi Miao[2], Aaron Phillips[2], Brittany Pioso[2], Hazheen Shirnekhi[2], Swarnendu Tripathi[2]
[1] Department of Microbiology, Immunology and Biochemistry, University of Tennessee Health Sciences Center, Memphis, USA [2] Department of Structural Biology, St. Jude Children's Research Hospital, Memphis, USA

Phase separation organizes biomolecules within membraneless condensates that mediate diverse biological processes. Importantly, phase separation is altered in these processes in human diseases, including cancer. The abundant nucleolar protein, Nucleophosmin (NPM1), phase separates with ribosomal components, mediating assembly of 40S and 60S subunits. We will discuss how the many biomolecules mediate multi-phase behavior that creates the granular component (GC) region of the nucleolus. We will also discuss the Arf tumor suppressor, a small disordered protein that binds NPM1 and alters the liquid nature of the GC, inhibiting both ribosome biogenesis and ribosome function. Another topic will be phase separation by fusion oncoproteins (FOs), which are oncogenic drivers in diverse human cancers. We discovered relationships between the physicochemical features of FOs and their propensity to form condensates, sub-cellular localization and biological functions. Using Machine Learning (ML), we extended our analyses to understand the phase separation behavior of ~3,000 other FOs. We are currently identifying intrinsically disordered regions (IDRs) within FOs that contribute to phase separation and are developing a ML model to identify phase separation-prone IDRs within cancer-associated human proteins. Collectively, our findings highlight the importance of biomolecular phase separation in both suppressing and promoting cancer in humans.

O-46 Invited speaker
Order from Disorder: Towards molecular architecture of the muscle Z-disk assembly by integrative structural biology
Kristina Djinovic Carugo[1,2]
[1] Department of Structural and Computational Biology, Max Perutz Labs, University of Vienna, Campus Vienna Biocenter 5, A -1030 Vienna, Austria[2] 1European Molecular Biology Laboratory (EMBL) Grenoble, France

The sarcomere is the smallest contractile unit in cardiac and skeletal muscle, where actin and myosin filaments slide past each other to generate tension. This molecular machinery is supported by a subset of highly organised cytoskeletal proteins that perform architectural, mechanical, and signalling functions. Sarcomere ultrastructure is highly organised and delimited by Z-disks, which play a critical role in mechanical stabilisation and force transmission. To investigate the molecular structural architecture of the Z- disk, the assembly hierarchy, and structure-function relationships, we are employing an integrative structural biology strategy that combines molecular biophysics, structural, and biochemical approaches. FATZ proteins interact with α-actinin and five other core Z-disk proteins, contributing to the assembly and maintenance of myofibrils as a hub for protein interactions. I will present our studies on the interaction of the major Z-disk protein α-actinin-2 with FATZ-1 and Z-portion of titin, forming dynamic fuzzy complexes, and discuss findings in view of asymmetric sorting of α-actinin and sarcomeric Z-disk architecture and assembly. Furthermore, our recent discover that FATZ-1 can phase-separate and form biomolecular condensates with α-actinin-2 and other three Z-disk proteins raises the intriguing question of whether FATZ proteins can create an interaction hub for Z-disk proteins during myofibrillogenesis via membrane-less compartmentalization.

O-47 Short talk
Biomolecular condensates in contact with membranes: interaction mechanism, wetting, and complex remodeling
Agustin Mangiarotti[1], Nannan Chen[1,2], Ziliang Zhao[1,3,4], Macarena Siri[1], Leonel Malacrida[5], Reinhard Lipowsky[1], Rumiana Dimova[1]
[1] Max Planck Institute of Colloids and Interfaces, Germany [2] Guangzhou Medical University, China [3] Leibniz Institute of Photonic Technology e.V., Germany [4] Institute of Applied Optics and Biophysics, Germany [5] Institut Pasteur de Montevideo, Uruguay

Membrane wetting by biomolecular condensates recently emerged as an important phenomenon in cell biology. It plays a vital role in a diverse range of processes across different organisms. However, the potential of membrane remodeling and the underlying mechanisms are not well understood. Here, we performed a systematic analysis of the interaction of protein and polymer condensates with giant unilamellar vesicles as model membranes. We demonstrate that these interactions can lead to striking morphological transformations, which are elucidated by theoretical analysis. A remarkable phenomenon, namely fingering or ruffling of the condensate-membrane interface is observed when sufficient membrane area is available. We resolve these intricately curved structures with STED. The observed morphologies are governed by the interplay of adhesion, membrane elasticity, and interfacial tension. Using nano-environmental sensors and state-of-the-art microscopy techniques combined with phasor analysis, we find that biomolecular condensates can modulate membrane lipid packing and hydration by wetting. This discovery has important implications for understanding how cells regulate their membrane properties and could pave the way for new approaches to the manipulation of cellular processes.

O-48 Short talk
Phase separation of the C-terminal domain of RNA Pol II: a code in the code
Francesco Luca Falginella[1,2], Katerina Linhartova[1,2], Richard Stefl[1,2], Robert Vácha[1,2]
[1] Central European Institute of Technology, Czech Republic [2] Masaryk University, Czech Republic

Liquid-liquid phase separation (LLPS) is now considered to be the major driving force behind the formation of membraneless organelles. These biomolecular condensates, which exhibit liquid-like properties, are known to be involved in the spatial and temporal regulation of many cellular processes. A significant contribution to LLPS originates from multivalent interactions between intrinsically disordered proteins/regions. A prominent member of this protein class is the C-terminal domain (CTD) of RNA Pol II, a low complexity and unstructured region known to associate with several protein factors during the transcription cycle. Recently, CTD phase separation has been proposed as an additional regulatory mechanism of DNA transcription. However, the sequence determinants and multivalent interactions underlying this process remain largely unclear. To fill the gap, we combined all-atom and coarse-grained molecular dynamics simulations, microscopy experiments, and biochemical assays of various CTD mutants. We determined the role of individual amino acids and phosphorylation modifications in LLPS of CTD. These results not only advance our understanding of the regulation of DNA transcription, but also provide a guide for predicting the biophysical properties of proteins with similar amino acid sequence.

O-49 Short talk
The dynamic interaction of the N-Myc oncoprotein and the protein kinase Aurora A
Johanna Hultman[1], Vivian Morad[1], Alexandra Ahlner[1], Zuzanna Pietras[1], Björn Wallner[1], Maria Sunnerhagen[1]
[1] Division of Chemistry, Department of Physics, Chemistry and Biology, Linköping University, Sweden

The intrinsically disordered N-Myc protein is a master regulator involved in numerous pathways important for cellular growth and function. However, when deregulated it becomes a key cancer driver, especially in aggressive brain tumors in children. Interaction with the protein kinase Aurora A increases cellular N-Myc levels, but the nature of this interaction remains elusive. By crystallography, only the interaction between Aurora A and a short, non-conserved N-Myc region (AIR) has been characterized. In our work, we apply a range of biophysical techniques to describe the structure and dynamics of the N-Myc interaction with Aurora A. By solution-state NMR, we have for the first time characterized the N-Myc transactivation domain and shown that the kinase domain of Aurora A binds directly to N-Myc's conserved MBo and MBI in a highly dynamic manner, displaying characteristics of a "fuzzy complex" while still forming a stable 1:1 interaction as judged by SEC-MALS, nano-DSF and SAXS. Our ITC and NMR data jointly show that the interactions of N-Myc and Aurora A is independent of the AIR previously identified by crystallography. Further, HDX-MS and SAXS data, together with molecular modelling, jointly show that N-Myc binds to the N-lobe of Aurora A, anchoring above the active site.

O-50 Short talk
Barrier-properties of Nup98 FG phases ruled by FG motif identity and inter-FG spacer length
Sheung Chun Ng[1], Abin Biswas[2,3], Trevor Huyton[1], Jürgen Schünemann[1], Simone Reber[2], Dirk Görlich[1]
[1] Max Planck Institute for Multidisciplinary Sciences, Germany [2] Humboldt-Universität zu Berlin [3] Max Planck Institute for the Science of Light

The permeability barrier of nuclear pore complexes (NPCs) controls nucleocytoplasmic transport. It restricts inert macromolecules >30 kDa but allows passage of nuclear transport receptor proteins (NTRs) that shuttle cargoes in or out of nuclei. The barrier is described as a condensed protein-rich phase assembled from cohesive FG (Phe-Gly) repeat domains, which are intrinsically disordered regions at the central channel of NPCs. FG domains include foremost the Nup98 FG domains and several distinct subtypes. Nup98 FG domains comprise hydrophobic FG motifs, typically GLFG motifs, connected by more hydrophilic, uncharged spacers. These FG motifs bind NTRs passing NPCs. We previously showed that the cohesive Nup98 FG domains phase-separate from dilute solutions to form gel-like phases ("FG phases") with NPC-like selectivity: They favour the partition of NTRs/NTR-cargo complexes but exclude inert large proteins. However, determinants of this barrier property were unclear. In this study we report that shortening inter-FG spacers enhances cohesion, increases FG phase density, and tightens such barrier - all consistent with a sieve-like phase. Phase separation tolerates mutating the GLFG motifs, provided domain-hydrophobicity remains preserved. NTR-entry, however, is sensitive to deviations from canonical FG motifs, suggesting co-evolutionary adaptation. Changing GLFG to FSFG motifs, which are common in FG domain subtypes other than Nup98, makes the FG phase hypercohesive, precluding NTR- entry. Extending inter-FG spacers relaxes hypercohesion. Thus, antagonism between cohesion and NTR-FG interactions is key to transport selectivity.

Neutrons in Life Sciences and Biophysics

Session chairs: Trevor Forsyth, Lund University & Esko Oksannen, European Spallation Source and Lund University

O-51 Invited speaker
Structural studies of integral membrane proteins using stealth carrier systems
Henning Tidow[1]
[1] University of Hamburg, Germany

Structural studies of integral membrane proteins (IMPs) are challenging, as many of them are inactive or insoluble in the absence of a lipid environment. We recently pioneered an approach making use of fractionally deuterium labelled 'stealth carrier' systems that are effectively invisible to low-resolution neutron diffraction and enable structural studies of IMPs in a lipidic native-like solution environment. I will illustrate the potential of the method in a joint small-angle neutron scattering (SANS) and X-ray scattering (SAXS) study of the ATP-binding cassette (ABC) transporter protein MsbA solubilized in stealth carriers. The data allow for a direct observation of the signal from the solubilized protein without contribution from the surrounding lipid nanodisc. Not only the overall shape but also differences between conformational states of MsbA can be reliably detected from the scattering data, demonstrating the sensitivity of the approach and its general applicability to structural studies of IMPs.

O-52 Invited speaker
PROBING AMPHOTERICIN B MECHANISM AND RESISTANCE IN HUMAN PATHOGENIC YEAST USING NEUTRON REFLECTION
Hanna Wacklin-Knecht[1,2], Robin Delhom[2,3,4], Olena Ishchuk[3], Juan Manuel Orozco Rodriguez[3], Katarina Koruna[3], Anna Shifferdecker[3], Anna Andersson Rasmussen[3], Valerie Laux[4], Michael Haertlein[4], Giovanna Fragneto[2,4]
[1] Lund University, Sweden [2] European Spallation Source ERIC, Lund, Sweden [3] Lund University, Sweden [4] Institut Laue Langevin, Grenoble. France

Millions of people suffer life-threatening fungal infections, but there are only a few antifungal agents, whose application is often restricted by toxicity, resistance and low bioavailability. Most existing drugs target ergosterol, either by preferentially binding to it in fungal membranes, or by preventing its biosynthesis, but their precise mechanisms of action, toxicity and resistance are still unclear. A fuller understanding of the function of the current drugs is critical for designing better, safer therapeutics. Candida glabrata is a non-pathogenic yeast in healthy humans, but the number of infections it causes has increased, making it urgent to understand its virulence and resistance. We have combined RNA interference to produce C. glabrata strains (1) with well-defined genetic modifications with lipidomic analysis and structural characterization of the fungal membrane interaction with Amphotericin B. Amphotericin (AmB) is a WHO essential medicine that has the broadest antifungal spectrum and has been used as the last line of defense against systemic fungal infections for more than 50 years, but its mechanism of action and resistance are still not well understood. We have elucidated the AmB mechanism in model yeast membranes (2,3) and in yeast membranes from C. glabrata strains with increased or decreased AmB resistance. Our integrative approach demonstrates how neutron techniques can provide insight into the molecular basis of antimicrobial activity and resistance with the long-term aim to improve therapies. (1) Ishchuk et al. Front Microbiol (2019) doi:10.3389/fmicb.2019.01679. (2) de Ghellinck et al., BBA - Biomembranes, 2015 doi:10.1016/j.bbamem.2015.06.006 (3) Delhom et al. Nanomaterials 2020 doi:10.3390/nano10122439

O-53 Short talk
Synthetic myelin for biomimetic neuroscience
Andreas Stadler[1], Igor Graf von Westarp[1], Benjamin Krugmann[1], Aurel Radulescu[1], Marie-Sousai Appavou[1], Alexandros Koutsioubas[1], Laura Stingaciu[2], Martin Dulle[1], Stephan Förster[1]
[1] Forschungszentrum Julich, Germany [2] Oak Ridge National Laboratory

Myelin basic protein (MBP) and its interaction with lipids of the myelin sheath plays a crucial role for the formation of the myelin sheath in the human brain. Previous studies observed that changes in the myelin lipid composition during multiple-sclerosis (MS) conditions lead to instabilities and enhanced local curvature of MBP-lipid multilayer structures. We investigated the molecular origin of the instability using a combination of neutron and X-ray scattering techniques as well as cryo-transmission electron microscopy. We found that the MS lipid membrane has a 25% lower bending rigidity than the native one, thus destabilizing smooth> 1 µm curvature radius structures such as in giant unilamellar vesicles. MBP-mediated assembling of lipid bilayers proceeds in two steps, with a slow second step occurring over many days where native lipid membranes assemble into well- defined multilayer structures, whereas MS diseased lipid membranes form folded assemblies with high local curvature. For both native and diseased lipid mixtures we could show that MBP forms concentrated liquid-like phases on top of the lipid membranes mediating attractive membrane interactions. In further work, we could demonstrate as well that MBP performs a liquid-liquid phase separation (LLPS) under crowding conditions in vitro. Time-resolved small-angle neutron and X-ray scattering as well as dynamic light scattering experiments allowed us to follow the size evolution kinetics of LLPS condensates consisting of MBP for more than six orders of magnitude in time. Spinodal decomposition and subsequent Ostwald ripening appear to be main driving forces for LLPS droplet formation of MBP in vitro.

O-54 Short talk
Towards mapping the relationship between lipid nanoparticle structure and performance
Hanna Barriga[1], Isaac Pence[2], Margaret Holme[1], Catherine Saunders[2], Miina Ojansivu[1], James Doutch[3], Jelle Penders[2], Valeria Nele[2], Michael Thomas[2], Marta Carroni[4], Molly Stevens[2]
[1] Karolinska Institutet, Sweden [2] Imperial College London, UK [3] STFC Rutherford Appleton Laboratory, UK [4] Stockholm University, Sweden

Lipid nanocarriers are versatile structures with tunable physicochemical properties that are ideally suited for therapeutic applications. A key barrier to rational design is the challenge of relating composition and structure to cellular uptake, endosomal escape and performance. Several factors impact the structure of lipid nanocarriers including formulation methods, lipid composition, payload, surface labelling and intracellular processing. By combining small angle scattering (SAXS / SANS) with bulk fluorescence measurements and super resolution microscopy we are aiming to map the structural evolution of lipid nanocarriers in biological environments to understand how structure impacts performance. One key aspect has been understanding the structural evolution of LNPs during interactions with lipid modifying enzymes - phospholipases. Phospholipases can specifically alter the phospholipids contained within lipid nanocarriers. The phospholipase D (PLD) family are found in the endosomal pathway and are dysregulated in breast cancer. PLD cleaves phosphocholine headgroups releasing choline and generating anionic lipids within the lipid bilayer. By combining bulk and single particle analysis using Single Particle Automated Raman Trapping Analysis (SPARTA®) and small angle scattering (SAXS / SANS) we were able to couple nanoparticle structure to dynamic measurements of lipid composition to study their interaction with PLD. Our analysis demonstrates that PLD, a key intracellular trafficking mediator, can access the entire lipid substrate contained within structured lipid nanoparticles and generate anionic LNPs. PLD activity on vesicles with matched amounts of substrate was an order of magnitude lower. This highlights the fundamental importance of understanding the relationship between lipid nanocarrier structure and performance.

O-55 Short talk
Small-angle neutron scattering of a pentameric ligand-gated ion channel reveals a dynamic regulatory domain
Marie Lycksell[1], Urška Rovšnik[2], Anton Hanke[3], Anne Martel[4], Rebecca Howard[2], Erik Lindahl[2]
[1] Umeå University, Sweden [2] Stockholm University [3] Heidelberg University [4] Institut Laue-Langevin

Pentameric ligand-gated ion channels (pLGICs) perform electrochemical signal transduction in organisms ranging from bacteria to humans. Among the prokaryotic pLGICs there is an architectural diversity involving N-terminal domains (NTDs) not found for the eukaryotic relatives, exemplified by the calcium-sensitive channel DeCLIC. Here, we characterized DeCLIC structure using cryogenic electron microscopy (cryo-EM), small-angle neutron scattering (SANS), and molecular dynamics (MD) simulations. In both the presence and absence of calcium, cryo-EM reconstructions were similar to a previously reported calcium-bound x-ray structure. The NTDs exhibited lower local resolution than the canonical unit, consistent with these domains being relatively mobile. The small-angle scattering profile revealed a feature not explained by the available structures, indicating that further conformational diversity is available to DeCLIC. MD simulations indicated that this profile is largely attributable to rigid-body motions of the NTDs relative to the protein core, including conformations similar to those in experimental structures, as well as more expanded and asymmetric conformations. Using these expanded conformations, it was possible to fit the previously unexplained SANS feature, indicating the presence of such conformations under solution conditions. This work reveals the range of motion available to the DeCLIC NTDs, expanding the conformational landscape of the pLGIC family; and demonstrates the power of combining low-resolution, high-resolution, and simulations data in the study of protein structure.

O-56 Short talk
Distributing Aminophospholipids Asymmetrically Across Leaflets Causes Anomalous Membrane Stiffening
Georg Pabst[1], Moritz Frewein[1,2], Paulina Piller[1], Enrico Semeraro[1], Orsolya Czakkel[2], Yuri Gerelli[3,4], Lionel Porcar[2]
[1] Biophysics, Institute of Molecular Biosciences, University of Graz, Graz, Austria [2] Institut Laue-Langevin, Grenoble, France [3] CNR Institute for Complex Systems, Uos Sapienza, Roma, Italy [4] Department of Physics, Sapienza University of Rome, Roma, Italy

We studied the mechanical leaflet coupling of prototypic mammalian plasma membranes using neutron spin-echo spectroscopy. In particular, we examined a series of asymmetric phospholipid vesicles with phosphatidylcholine and sphingomyelin enriched in the outer leaflet and inner leaflets composed of phosphatidylethanolamine/phosphatidylserine mixtures. The bending rigidities of most asymmetric membranes were anomalously high, exceeding even those of symmetric membranes formed from their cognate leaflets. Only asymmetric vesicles with outer leaflets enriched in sphingolipid displayed bending rigidities in conformity with these symmetric controls. We performed complementary small-angle neutron and X-ray experiments on the same vesicles to examine possible links to structural coupling mechanisms, which would show up in corresponding changes in membrane thickness. We also estimated differential stress between leaflets originating from a mismatch of their lateral areas or spontaneous curvatures. However, no correlation with asymmetry-induced membrane stiffening was observed. To reconcile our findings, we speculate that an asymmetric distribution charged or H-bond forming lipids may induce an intraleaflet coupling which increases the weight of hard undulatory modes of membrane fluctuations and hence the overall membrane stiffness.

Protein design

Session chairs: Aitziber L. Cortajarena, CIC biomaGUNE & Ingemar André, Lund University

Arranged in collaboration with Protein Society

O-57 Invited speaker
Design of enzyme repertoires
Sarel Fleishman[1]
[1] Weizmann Institute of Science, Israel

We recently developed methods that combine phylogenetic analysis and Rosetta atomistic design calculations to design highly optimized variants of natural proteins. Our methods have been used by thousands of users worldwide to generate stable therapeutic enzymes, vaccine immunogens, and highly active enzymes for a range of needs in basic and applied research. We now present a machine-learning strategy to design and economically synthesize millions of active-site variants that are likely to be stable, foldable and active. We applied this approach to the chromophore-binding pocket of GFP to generate more than 16,000 active designs that comprise as many as eight mutations in the active site. The designs exhibit extensive and potentially useful changes in every experimentally measured parameter, including brightness, stability and pH sensitivity. We also applied this strategy to design millions of glycoside hydrolases that exhibit significant backbone changes in the active site. Here too, we isolated more than 10,000 catalytically active and very diverse designs. Contrasting active and inactive designs illuminates areas for improving enzyme design methodology. This new approach to high-throughput design allows the systematic exploration of sequence and structure spaces of enzymes, binders and other functional proteins.

O-58 Invited speaker
Modelling and comparing the binding sites of immune receptor proteins to enable design
Charlotte Deane[1]
[1] University of Oxford, UK

Immune receptor proteins play a key role in the immune system, our response to vaccines and have shown great promise as biotherapeutics. The development of new vaccines or biotherapeutics typically takes many years and requires over $1bn in investment. Computational methods and in particular machine learning have shown great promise for increasing the speed and reducing the cost of biotherapeutic development. In this talk I will describe some of the novel computational tools we are pioneering in the area of biotherapeutics from accurate rapid structure prediction to understanding the diverse binding preferences between different types of immune receptor proteins to allow us to better design them.

O-59 Short talk
De novo design of transmembrane beta-barrel nanopores
Anastassia Vorobieva[1,2], Carolin Berner[1,2], Samuel Berhanu[3], Sagardip Majumder[3], Alvaro Martin[1,2], James Whitehouse[4], Oleksandr Volkov[1,2], Sebastian Hiller[5], Sheena Radford[4], David Baker[3,6]
[1] Vrije Universiteit Brussel, Belgium [2] Flanders Institute of Biotechnology [3] University of Washington [4] University of Leeds [5] University of Basel [6] Howard Hughes Medical Institute

Biological nanopores are emerging as powerful tools for the single-molecule analysis of complex samples and for the sequencing of biopolymers. However, protein nanopores have evolved to fold in biological membranes and for very different functions than analyte sensing/sequencing. De novo design could enable the generation of nanopores with structures and properties specifically tailored for the detection of an analyte of interest. We developed computational methods for the de novo design of transmembrane beta-barrels (TMBs), a class of membrane proteins forming one beta-sheet closing on itself. TMBs form rigid and stable transmembrane channels with excellent nanopore properties. We show that negative design to delay folding of the individual beta- strands in water is critical to achieve folding in membranes. We describe an experimental pipeline for the characterization of the designs based on cytosolic expression in inclusion bodies, refolding in detergent micelles and a new 1D NMR screen to identify properly folded designs. In silico validation of designed sequences with AlphaFold2 results in an increase of the success rate from 10 to 50%. The de novo designed TMBs readily fold in detergent micelles from chaotropic denaturant and insert spontaneously from micelles to planar lipid membranes to form nanopores with stable ion conductance. Systematic variation of the nanopore diameter showed that open pore current scales well with the expected pore size. Our computational design method enables unprecedented level of control over the nanopore size, shape and chemical properties. It paves the way to programmable nanopore sensors, at the interface of biotechnology and nanoelectronics.

O-60 Short talk
Integrative Antibody Discovery to Target Challenging Protein Assemblies
Francesco Antonio Aprile[1,2], Ying Ge[1], Devkee Mahesh Vadukul[1], Rebecca Jade Thrush[1,2]
[1] Department of Chemistry, Molecular Sciences Research Hub, Imperial College London, London, UK [2] Institute of Chemical Biology, Molecular Sciences Research Hub, Imperial College London, London, UK

Antibodies have emerged as a powerful tool for biomedical research and clinical applications. Their ability to selectively bind to biomolecules makes them valuable detection probes and a promising therapeutic approach. However, developing antibodies against biologically relevant post-translationally modified proteins and molecular self-assemblies remains challenging, given the chemical and structural heterogeneity of these protein species. To address this challenge, we have developed an antibody discovery platform that integrates protein design, directed evolution, and multi-parametric screening to save cost and production time and expand the repertoire of target biomolecules. Our approach employs sequence-based prediction and directed evolution to generate antibody candidates, which are simultaneously screened based on epitope binding and functional assays. Using this method, we have successfully generated single-domain antibodies, i.e., nanobodies, targeting solid and liquid self-assemblies of the intrinsically disordered proteins amyloid-beta and alpha-synuclein, which are involved in Alzheimer's and Parkinson's, respectively. Our antibodies have enabled us to better understand the kinetic of formation, and structural and functional characteristics of these self-assemblies, both in vitro and in animal models of disease. Additionally, our approach has identified promising therapeutic targets within these proteins, providing potential avenues for future drug discovery efforts.

O-61 Short talk
Tuning the dimensionality of supramolecular materials through the design of peptide-protein co-assemblies
Laura Perez-Chirinos[1], Ivan R. Sasselli[1,2], Aitziber L. Cortajarena[1,3]
[1] Center for Cooperative Research in Biomaterials (CIC biomaGUNE), Basque Research and Technology Alliance (BRTA), Donostia San Sebastián, Spain. [2] Centro de Fisica de Materiales (CFM), CSIC-UPV/EHU, San Sebastián, Spain. [3] Ikerbasque, Basque Foundation for Science MaDíaz de Haro 3, Bilbao, Spain.

Peptide-based supramolecular assemblies have been widely studied for their inherent ability to self-organization, making them useful for applications in nanotechnology. Combining these structures with other molecules opens a new perspective for the design of complex functional materials. Here, we aim to build complex architectures combining a versatile engineered protein, which has potential applications in bioelectronics and nanotechnology, with self-assembled fibers. For this purpose, the protein is modified to incorporate one or two peptides in one or both termini, respectively. This modification will drive the co-assembly with the fibers, resulting in one-dimensional co-assemblies when the protein has one peptide, and an extended network on a second dimension when the protein has two peptides, acting as a fiber cross-linker. The material design was carried out by screening peptide sequences using molecular dynamics (MD) simulations to optimize their co-assembly with the engineered proteins. The resulting architectures were experimentally characterized to prove the change in dimensionality with the number of peptides linked to the protein. The combination of the experimental and computational techniques provided a better understanding of each component in the system, facilitating further rational functional modifications. Therefore, a versatile hybrid material has been created using biocompatible building blocks without introducing any artificial chemical groups or bonds. This material can be customized to suit specific needs in the fields of bioelectronics and biomedicine.

O-62 Short talk
A computational protocol for the in silico maturation of antibody fragments
Sara Fortuna[1], Patricio Barletta[1], Walter Rocchia[1], Miguel A. Soler[2]
[1] ISTITUTO ITALIANO DI TECNOLOGIA (IIT), Italy [2] University of Udine, Italy

We are working towards overcoming the limits of traditional approaches based on in vivo immunisation and in vitro selection of antibodies to timely discern among closely related targets. Indeed, an ideal binder should be capable of capturing with high affinity, sensitivity, and specificity a target molecule, such as an organic molecule or a biomolecule. Examples include antibodies typically optimised in vivo or vitro, and antibody fragments and peptides that can nowadays be optimised in silico. This latter opportunity offers invaluable advantages as computational design allows designing binders capable of capturing their target through a chosen binding site. Molecular modelling indeed allows addressing an important drawback of antibodies maturation: the control over their target binding site. This goal is now at reach as shown by our results in the ex-novo design of epitope-specific peptides and antibody fragments for protein recognition, already validated in wet lab. In this spirit and building up on both our and other group successes, we have recently developed an improved parallelised evolutionary algorithm for the computational optimization of generic aminoacid based binders. The code, through a combination of molecular dynamics simulation and free energy estimations, allows to iteratively improve epoch by epoch the predicted binding affinity of a pool of binders. Current benchmark on a number of HPC systems are showing its time-effectiveness, making it highly competitive to established methods.

Computational biophysics

Session chairs: Ilpo Vattulainen, University of Helsinki & Himanshu Khandelia, University of Southern Denmark

O-63 Invited speaker
Three routes to molecular movies
Helmut Grubmüller1, Maxim Igaev1, Lars V. Bock1, Steffen Schultze1
1 Max Planck Institute for Multidisciplinary Sciences, Germany

Combining atomistic simulation, cryo-electron microscopy, and serial crystallography, we will describe three routes towards molecular movies. (1) We used non-equilibrium atomistic simulations of whole microtubules to address the question how, despite their very similar structure of the tip, they switch between growing and shrinking phase. Our results show that and why the primary steps of microtubule tip flaring differ kinetically between GTP and GDP loaded states. (2) For improved ensemble refinement from cryo-electron microscopy data, we used continuum and atomistic simulations of shock freezing of whole fully solvated ribosomes to quantify how much of the physiological temperature structural heterogeneity and dynamics is preserved in electron microscopy samples at cryogenic temperatures. (3) We address structure refinement from single molecule femtosecond XFEL diffraction images, for which the signal to noise is in the extreme Poisson regime. Using synthetic data we have demonstrated via a rotationally invariant correlation approach that near-atomistic resolution is possible even for small proteins. With a rigorous Bayesian approach, even ensemble refinement can be achieved with much fewer images than expected from an information theoretical perspective.

O-64 Invited speaker
Mesoscale models of mechanobiology
Julia Yeomans[1]
[1] University of Oxford, UK

Living systems avoid equilibrium by taking chemical energy from their surroundings and using it to do work. Active systems, also exist out of thermodynamic equilibrium and the concepts of active matter are proving increasing useful in describing biological processes. In particular, dense active nematics show complex collective behaviour, mesoscale turbulence, flocking, coherent rotation and self-propelled topological defects. I shall describe phase field and continuum models of dense active systems and their applications to collective cell motility and morphogenesis.

O-65
Dynamic allostery in the PDZ3 domain: Deriving directed signals by assessing correlations of side chain dihedral angles perturbed by Brownian kicks
Forbes Burkowski[1]
[1] University of Waterloo, Canada

Over the last several years, a detailed description of intra-protein allosteric signals has remained elusive. Here I describe a residue network model for the PDZ3 domain that delineates internal pathways defined by correlated fluctuations of neighboring side chains. For reasons to be elaborated, I have avoided strategies such as normal mode analysis and principal component analysis of atom trajectories. Instead, the model is best described as a Brownian motion fluctuation-dissipation analysis at the atomic level. Signals are predominantly carried by the correlated motions of side chain atoms with one degree of freedom (atom positions dependent on the Chi 1 dihedral angle only). Each DoF1 atom is subject to driving forces that originate from other neighboring DoF1 atoms and atoms with higher degrees of freedom. If all the driving atoms have higher degrees of freedom, then motion of the driven atom still occurs but with lower probability because of the necessary coordination of these driving atoms. The driven atom will then encounter friction forces manifested as interactions with DoFn atoms ($n > 1$), but in many cases it will nonetheless impart a motion to a neighboring DoF1 atom. As a case study, I investigated the propagation of signals that originated from surface solvent exposed residues such as the charged residues, ending at the side chains defining the ligand binding pocket. Simulation studies demonstrated that after ligand binding, alterations in the disposition of charged residues changed the diffusion pattern of these signals in a manner that promoted the clamping functionality of this scaffold protein.

O-66
Elucidating the Mechanisms of Genome Release in Picornaviruses using Cryo-EM and Coarse-Grained Simulations
Lukáš Sukeník[1,2], Liya Mukhamedova[1], Michaela Procházková[1], Karel Škubník[1], Pavel Plevka[1], Robert Vácha[1,2,3]
[1] CEITEC – Central European Institute of Technology, Masaryk University, Brno, Czech Republic [2] Department of Condensed Matter Physics, Faculty of Science, Masaryk University, Brno, Czech Republic [3] National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Brno, Czech Republic

Genome release is a crucial step in the life cycle of picornaviruses. During virus intracellular transport in endosomes, exposure to low pH triggers a conformational change in the capsid necessary for genome release. As a result, some viruses form pores on symmetry axes, which have been proposed to facilitate slow release of the viral genome. In contrast, recent cryo-EM images have shown that viral capsids can crack open and release the genome rapidly. Thus, the mechanism of genome release remains elusive. We combined in vitro cryo-EM observations of the genome release from four viruses with coarse-grained simulations of generic virus-like nanoparticles to investigate the release pathways and virion stability. Here we show how the nature of interactions between capsid building blocks determines virion stability and genome release pathway. We found that preformed pores at the symmetry axes were not necessary for slow genome release. Rather, slow release occurred through transient pores when interactions between capsid subunits were long-range, and the interactions within the genome were weak. In contrast, rapid release was preferred when capsid interactions were short-range and/or the genome interactions were strong. These findings elucidate the genome release behavior of viruses and suggest a design strategy for virus-like nanoparticles for drug delivery.

O-67 Short talk
Which part of axonal membrane is the most vulnerable: A molecular dynamics/Finite Element study
Maryam Majdolhosseini[1], Zhou Zhou[1], Svein Kleiven [1], Alessandra Villa [2]
[1] KTH Royal institute of technology, Huddinge, Sweden [2] KTH Royal institute of technology, Stockholm, Sweden

Traumatic brain injury (TBI) is a major cause of death worldwide. Diffuse axon injury (DAI) is a type of TBI that affects the axons of the brain white matter and cannot be diagnosed by conventional imaging techniques. Among different theories about the mechanism of DAI, one hypothesis states that membrane poration of the axons can lead to DAI. To investigate this hypothesis, a finite element model of an axon is used to study the effects of deformation on local strain distribution of the axonal membrane. The observed membrane deformations are then investigated at the molecular level using molecular dynamics simulation and a coarse- grain (CG) description. In particular, we have built a CG membrane model to describe the axon's membrane based on the available experimental data. We simulate the bilayer under deformation, to identify the strain in which the poration occurs. We then compared the strength and mechanical properties of the axolemma model with other membrane models describing other subcellular parts of the axon, like the node of ranvier and the myelin. We show that the pore formation in the node occurs in lower strains than that of the axolemma, while the myelin ruptures at higher strains in comparison with the axolemma. This study can enhance our understanding of DAI at cellular and molecular levels by revealing the most vulnerable parts of the axonal membrane.

O-68 Short talk
Microtubule assembly as a molecular Brownian ratchet: from atomistic to super coarse-grained modeling
Maxim Igaev[1], Maksim Kalutskii[1], Helmut Grubmüller[1]
[1] Max Planck Institute for Multidisciplinary Sciences, Germany

Microtubules (MTs), mesoscopic cellular filaments, grow by the addition of GTP-bound tubulin dimers at their dynamic flaring ends. They operate as chemomechanical energy transducers with stochastic transitions between phases of growth and shortening upon hydrolyzing GTP to GDP. During this astounding process, known as "dynamic instability", MTs can push and pull on other cellular compartments. However, we do not yet fully understand exactly how MTs self-assemble and transmit mechanical force. Moreover, physical constraints present within the cell cause MTs to bend and fracture, resulting in intricate collective phenomena such as softening and self-repair, which are still elusive. Despite the importance of MTs, their highly dynamic nature makes it hard to study their structure and dynamics experimentally. On the other hand, there are no multiscale computational approaches to predict the impact of subtle tubulin changes driven by GTP hydrolysis on the large-scale behavior of MTs. To address this gap, we use atomistic, explicit-solvent simulations to scrutinize the microsecond dynamics of complete GTP- and GDP-MT end models. Our findings show that the post-hydrolysis MT end is exposed to higher activation energy barriers for straight lattice formation, which strongly reduces its probability to elongate. To extrapolate this knowledge to real-life timescales, we are now developing a novel coarse-grained model of MT assembly based on the discrete elastic rod representation and parametrized fully ab initio. Overall, our study provides an information-driven Brownian ratchet mechanism for MT self-assembly and offers new insights into the mechanoenzymatics of and force generation by MTs.

Bionanophotonics

Session chairs: Haichun Liu, KTH Royal Institute of Technology & Artur Bednarkiewicz, Wroclaw Research Centre EIT

O-69 Invited speaker
Photon Conversion Nanocrystals: Transforming Imaging and Assistive Technology
Xiaogang Liu[1]
[1] National University of Singapore, Singapore

Lanthanide doping in optical nanomaterials is a rapidly growing field of research, as it has the potential to significantly improve the efficiency of energy conversion processes and enable new applications in a wide range of technologies. One of the key advantages of using lanthanides as dopants is their ability to absorb and emit light at specific wavelengths, which can be precisely controlled by the choice of lanthanide ion and the doping concentration. This makes them useful for frequency conversion, where light of one wavelength is converted into light of another wavelength. One of the most promising areas of research in this field is in the area of photon upconversion, where low-energy photons are converted into higher-energy photons. In this talk, I will highlight recent advances in the development of these frequency conversion nanocrystals for multimodal imaging, bio-detection, therapy, and X-ray scintillation. and assistive technology. I will also present recent efforts to develop electronic assistive technology prototypes that demonstrate the power of technology to improve the lives of individuals with disabilities.

O-70 Invited speaker
Applications of diffuse optics for detection and characterisation of disease
Hamid Dehghani[1]
[1] University of Birmingham, UK

Diffuse optical imaging (DOI) of tissue is used to characterize molecular features for the detection, diagnosis, and/or management of diseases. This relies on the propagation of Near Infrared (NIR) light through tissue whose attenuation depends on the underlying tissue pathophysiology. Parameter recovery and/or image reconstruction from the measured data relies on a computation model used for optimization: Models of the tissue being imaged are constructed that best predict the measured data. Two examples will be presented where spectral NIR data are used to detect and diagnose different types of diseases: (1) Thyroid cancer and (2) Rheumatoid Arthritis. In the first example, the developed system, utilizing multi-spectral Time Resolved Spectroscopy (TRS) and Diffuse Correlation spectroscopy (DCS) will be outlined, together with model-based data analysis and parameter recovery. The utilization of multi-modal data analysis through novel development and utilization of computational algorithms will be discussed. It will be demonstrated that NIR and DCS data can be used to provide characteristic information regarding the tissue being imaged which is informative of state and function. In the second example, we will highlight the design of a system which includes a non- contact DOI tomographic system together with model-based 3D imaging capabilities. The novel aspect of a system whereby longitudinal studies can be used to monitor the effect of treatment will be highlighted. The importance inter/intra-subject variability of tissue function and pathology will be highlighted, demonstrating the importance of information-rich, non-invasive NIR sensing that is capable of providing continuous subject-specific treatment planning and monitoring.

O-71 Short talk
Microscopy-based label-free size and refractive index quantification of nanoparticles in unknown media using DAISY
Erik Olsén[1], Berenice García Rodríguez[2], Fredrik Skärberg[2], Petteri Parkkila[2], Giovanni Volpe[2], Fredrik Höök[1], Daniel Midtvedt[2]
[1] Chalmers University of Technology, Sweden [2] University of Gothenburg, Sweden

Traditional optical microscopy approaches for multiparametric quantification of nanoparticle properties such as size and refractive index assess the size via diffusivity and refractive index by combining the optical signal with the diffusivity-based size estimate. Consequently, such methods are inherently limited to suspended particles in a known medium. This has the consequence that nanoparticle characterisation using scattering microscopy in complex environments is currently limited to particle contrast measurements. To overcome this limitation, we have developed dual-angle interferometric scattering microscopy (DAISY), which offers full optical quantification of both size and refractive index for individual nanoparticles without relying on either their motion or media properties. We achieve this by combining twilight off-axis holography with interferometric scattering microscopy (iSCAT) and deep learning assisted signal quantification and demonstrate that the optical signal ratio relates to particle size with an insignificant dependence on particle and medium refractive index. We also show how this methodology can be used to investigate particles inside cells, for which preliminary data and analysis will be shown. Furthermore, in the case when the diffusivity-size relation is known, DAISY can also be used to elucidate the internal mass distribution of individual particles without the need for any prior information. This property of DAISY was used to characterise fetal bovine serum, in which case the detection events include both aggregates and homogeneous spherical particles. Combined, these results show that DAISY enables general characterisation of nanoparticles beyond the capabilities of traditional optical label-free scattering microscopy methods.

O-72 Short talk
Tracking membrane dynamics on stem cell-derived neurons using 3D MINFLUX
Francesco Reina[1], Jakob Rentsch[2], Bela Tristan Leander Vogler[3], Helge Ewers[2], Christian Eggeling[1,3,4]
[1] Leibniz Institute for Photonic Technologies, member of the Leibniz Centre for Photonics in Infection Research (LPI), Jena, Germany [2] Institute for Chemistry and Biochemistry, Freie Universität Berlin, Berlin, Germany [3] Institute of Applied Optics and Biophysics, Friedrich-Schiller-Universität Jena, Jena, Germany [4] MRC Human Immunology Unit, Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, UK

Actin rings are periodic cytoskeletal structures found in dendrites and neuronal cells that regulate the shape and stability of dendritic spines. They are also known to act as a semi-permeable barrier to membrane diffusion. However, detecting this effect in the diffusion of membrane-bound proteins has been challenging due to their subdiffraction-sized period and the need for simultaneous high localization precision and fast framerates. MINFLUX microscopy, a superresolution technique with unprecedented localization precision, has the potential to overcome these challenges. In this study, we used 3D MINFLUX to track fluorescent quantum dot and nanobody-labelled GPI-anchored proteins on the membrane of stem cell-derived neurons to detect anomalies in their diffusion and highlight the influence of the actin rings on the plasma membrane. Our results demonstrate the high-throughput single particle tracking capabilities of 3D MINFLUX on axon-like structures, leading to the quantification of their diffusion dynamics in 3D. The high localization precision and fast frame rates of MINFLUX microscopy enable researchers to track single fluorescent reporters in living cells and provide new insights into the dynamics of membrane-bound proteins and cytoskeletal structures within neurons. This study highlights the potential of 3D MINFLUX as a powerful single molecule tracking technique with potential applications in basic biology and infection research.

O-73 Short talk
Semiconductor nanowires for biosening
Ivan N. Unksov[1], Nicklas Anttu[2], Rubina Davtyan[1], Fredrik Höök[3], Christelle N. Prinz[1], Heiner Linke[1]
[1] NanoLund and Solid State Physics, Lund University, Box 118, 22100 Lund, Sweden [2] Physics, Faculty of Science and Engineering, Åbo Akademi University, FI-20500 Turku, Finland [3] Department of Physics, Chalmers University of Technology, 41296 Gothenburg, Sweden

Semiconductor nanowires are known to enhance the signal of fluorophores in their proximity. In our recent work, we optimized GaP nanowires to maximize the enhancement of fluorescence excitation. For that, we used optical microscopy to measure the fluorescence photobleaching rate on the nanowires, proportional to local excitation. From the measurements and modelling, we show for red fluorophores (excitation wavelength 640 nm) that nanowires with diameters of 90–130 nm enable enhancement of over a factor of 5 compared to bulk solution. We also demonstrate that a 10 nm oxide layer on the nanowires enables functionalisation using biotin-streptavidin chemistry without hindering the enhancement. Such nanowires were used in our laboratory to detect fluorescently-labelled proteins on a single molecule level. Now we employ these results for detection of down to nanomolar concentrations of DNA in solution, and in our poster, we discuss these biosensors.

O-74 Short talk
Single objective light-sheet PALM allows volumetric super-resolution imaging in bacterial biofilms
Lucia Gardini[1,2], Tiziano Vignolini[3], Marco Capitanio[2,4], Francesco Saverio Pavone[1,2,4]
[1] National Institute of Optics, National Research Council, Sesto Fiorentino, Italy [2] LENS, European Laboratory for Non-Linear Spectroscopy, Sesto Fiorentino, Italy [3] Institut Pasteur, Paris, France [4] University of Florence, Sesto Fiorentino, Italy

Single molecule (SM) imaging techniques, such as PALM and STORM, have opened the possibility to explore sub-cellular phenomena at the molecular scale thanks to the capability of localizing a single emitter with nanometer precision. However, up to date an effective application of SM imaging to three-dimensional thick samples has been challenged by the very low signal-to-background ratio which deteriorates the achievable localization accuracy. In this work, we propose a novel implementation of super-resolution PALM with single objective light-sheet excitation to study the spatial distribution of proteins in bacterial biofilms, which can grow up to tens of microns in height during their maturation. Thanks to the combination of our optical technique with precision genome editing, we directly visualized and quantified the endogenous expression level of AcrAB-TolC efflux pump within the biofilm volume at the sub-cellular level, with single-molecule sensitivity and during biofilm maturation. AcrAB-TolC is responsible for the extrusion of multi drugs from bacteria and, thus, its study is of great interest to uncover the molecular mechanism behind the increased antimicrobial resistance in biofilms. Through our method we obtained the first high resolution map of protein expression and distribution within a biofilm and, importantly, we set an imaging method that can be easily and fruitfully applied to other thick biological samples.

Biophysics of RNA and ribosomes

Session chairs: Katja Petzold, Uppsala University, Giovanni Bussi, SISSA, Trieste, & Maria Selmer, Uppsala University

O-75 Invited speaker
A tale of two riboswitches: How ligand binding alters RNA folding pathways in atomistic detail
Alan Chen[1]
[1] University at Albany, State University of New York, USA

One of the fascinating properties of RNA are its ability to act both as carrier of genetic information as well as form intricate, three-dimentional structures capable of highly discriminating feats of molecular recognition. Riboswitches are naturally occuring regulatory switches found in prokaryotes where the RNA serves both functions at once, altering transcription/ translation in response to the binding of a specific target molecule to a highly structured untranslated region of the mRNA. We present all-atom molecular dynamics simulation studies of two prototypical riboswitches to compare and contrast how, in each case, the binding of the ligand results in transmitted information which is decoded as altered cotranscriptional folding pathways of the mRNA itself. In both cases, we find that specific tertiary interactions are stabilized which modulate the favorbility of a strand-invasion based conformational rearrangement mechanism.

O-76 Short talk
Predicting RNAs 3d structure and targeting RNA structure with small-drug molecules
Ankush Singhal[1], Susmit Chaudhary[1], Tyler Mrozowich[2], Trushar Patel[2], Karrisa Sanbonmatsu[1]
[1] Los Alamos national laboratory, USA [2] University of Lethridge, Canada

O-77 Short talk
Decoding SARS-CoV-2 frameshifting: Unraveling the orchestrated interplay of RNA Structures
Neva Caliskan[1,2], Lukas Pekarek[2], Matthias Zimmer[2], Anne-Sophie Gribling-Burrer[2], Stefan Buck[2], Redmond Smyth[2]
[1] Julius-Maximilians University Würzburg, Germany [2] Helmholtz Institute for RNA-based Infection Research (HIRI-HZI), Würzburg, Germany

The RNA genome of the SARS-CoV-2 virus comprises a frameshift stimulatory element (FSE) that grants access to an alternate reading frame via −1 programmed ribosomal frameshifting (PRF). The −1PRF efficiency relies on the presence of conserved RNA elements within the FSE, such as slippery sequence, spacer, and a secondary structure – often a hairpin or a pseudoknot. We have integrated ensemble chemical probing and single-molecule structural analysis of diverse SARS-CoV-2 frameshift RNA variants. We have employed dimethyl sulfoxide chemical probing and optical tweezers force spectroscopy, and have complemented these structural data with functional information obtained from dual-fluorescence-based experiments. Our findings affirm the critical role of the pseudoknot in efficient frameshifting and reveal that limiting base pairing with neighboring nucleotides can impact the formation of alternative folds as well as the pseudoknot. Notably, we reveal a conformational interplay between the 5′ and 3′ regions and the FSE and demonstrate that the extended FSE can exist in multiple conformations that can rapidly transform into a pseudoknot during translation. Our results highlight the cooperation of co-existing RNA structures in fine- tuning SARS-CoV-2 gene expression, thus paving the way for the design of specific inhibitors of viral frameshifting.

O-78 Short talk
Deciphering the mechanism of ribosomal methyltransferases mediated antibiotic resistance
Ruchi Anand[1], Ruchika Bujbalrao, Juhi Singh
[1] Indian Institute of Technology Bombay, India

Antibiotic resistance has become a silent epidemic that will result in more than 300 million deaths by 2050, if no appropriate action is taken. Repurposing of existing antibiotics and devising strategies to curb resistance is an uphill task and has become increasingly difficult. Towards addressing this grave problem here, we combat the problem of origin of resistance itself and focus on understanding the mechanisms by which pathogens become resistant to existing drugs. One of the prevalent mechanisms by which resistance is conferred is by post translationally modifying the protein synthesis machinery, the ribosome. Several antibiotics such as erythromycin bind to the ribosome and kill the pathogens by selectively stalling their protein synthesis. Ribosomal modifying enzymes such as methyltransferases (Mtases) do not allow certain antibiotics to bind the ribosome by methylating select ribosomal bases thereby, cause a steric clash at the antibiotic binding site, thus result in evading their action leading to antibiotic resistance. Here, we decipher the mechanism of action and selective targeting of these resistant conferring Mtases. We have used two enzymes KsgA and Erm both enzymes methylating adenine bases at the N6 position of select bases on 50S and 30S respectively. A combination of Cryo-EM, biochemical, fluorescence and MD approaches on both the Mtases as well as chimeric version of the enzymes revealed that apart from base flipping at the target site, that is crucial for methylation, base flipping at a distal allosteric site, within the Mtase is key in selective recognition of the target RNA. These studies serve as stepping-stone towards development of exclusive inhibitors that can aid in resisting resistance.

O-79 Short talk
How E167K RF2 compensates for the loss of RF1 – molecular insight with structure and function
Shreya Pundir[1], Daniel Larsson[1], Maria Selmer[1], Suparna Sanyal[1]
[1] Department of Cell and Molecular Biology, Uppsala University, Sweden

Bacterial class-I release factors read the stop codons in a semi-specific manner - RF1 reads UAA/UAG and RF2 reads UAA/UGA codons. However, the E167K mutant RF2, naturally evolved to compensate for the loss of RF1, is omnipotent in reading all the three stop codons and also UGG (Trp) codon, causing 'collateral toxicity'. To investigate how E167K RF2 terminates translation on UAG and UGG we have used a fast-kinetics based fluorescent peptide release assay in a fully reconstituted E. coli translation system. We find that E167K RF2 terminates translation with an overall higher efficiency than WT RF2, around 6-fold on cognate (UAA and UGA) and an impressive 220 to 530-fold on near-cognate (UAG and UGG) codons. The higher efficiency of E167K RF2 is associated with significant decrease in KM (Michaelis-Menten constant). It is also five times slower than WT RF2 in turnover on the ribosome. These results indicate higher affinity of E167K RF2 than WT to the ribosome. This is validated by our 2.8 Å resolution cryo-EM structure of E167K RF2 on the ribosome, which depicts stabilizing salt-bridge interactions of the lys-167 side chain with the rRNA backbone phosphates. In addition, thermal melting assay and SEC-SAXS analysis indicate that E167K, a charge-flip mutation, destabilizes the native compact conformation of RF2. We conclude that E167K RF2 compensates for the loss of RF1 by virtue of semi-open conformation which can bypass a fidelity checkpoint and more importantly, by tight-binding feature on the ribosome.

O-80 Short talk
Combining Molecular Dynamics and Solution Experiments to Investigate the Impact of RNA Modifications on Structural Dynamics.
Valerio Piomponi[1], Giovanni Bussi
[1] Scuola Internazionale superiore di Studi Avanzati (SISSA), Italy

RNA modifications have attracted increasing attention due to their crucial roles in various biological processes. These modifications involve biochemical alterations of nucleotides that can impact RNA structure and dynamics. Despite extensive study, the use of molecular dynamics (MD) simulations to investigate modified RNA remains limited. MD simulations are a powerful tool for accessing the structural dynamics of RNA at the atomistic level. The accuracy of these simulations largely depends on the quality of the force-field parameters utilized. Therefore, it is beneficial to combine simulations with experiments, such as by fitting parameters against experimental data or enforcing experimental averages with ensemble refinement methods. This talk will discuss how two modified RNA systems were characterized using these strategies. Firstly, the talk will address efforts to improve the quality of the force field for N6-methyladenosine (m6A) by fitting partial charges and a torsional potential to denaturation experiments performed on m6A-containing duplexes. The fitting allowed for the estimation of m6A syn/anti isomer populations, as predicted by NMR measurements. Alchemical free-energy calculations were used to estimate the destabilization induced by the methylation on duplexes. Secondly, the talk will present the use of ensemble refinement methods to investigate the structure of a 20-bp RNA helix containing Inosines. The maximum entropy principle, along with advanced enhanced sampling techniques, was used to generate an ensemble of structures compatible with NMR data. The presence of Inosines increased flexibility in the helix and allowed for the observation of sugar puckering in the C2'-endo conformation, which is not expected in ds-RNA.

O-81 Short talk
Studying the stability and conformational dynamics of the SARS-CoV-2 SL4 RNA hairpin combining base analogues and optical tweezers
Vinoth SUNDAR RAJAN[1], Anna Wypijewska del Nogal[1], Sune Levin[1], L. Marcus Wilhelmsson[1], Fredrik Westerlund[1]
[1] Chalmers University of Technology, Sweden

The SARS-CoV-2 virus demands selective packaging of its RNA genome (gRNA) from the abundance of other nucleic acids present in infected cells. Despite increasing evidence that the 5' UTR stem-loop 4 (SL4) mediates the initiation of this process by binding the nucleocapsid (N) protein, little is known about its stability and conformational dynamics. Optical tweezers is a single-molecule biophysical technique to investigate nucleic acid structure and dynamics at high spatial-temporal resolution. Here, for the first time, we combine RNA base analogues (BAs) with optical tweezers to explore structure stability, dynamics, and intramolecular interactions of the SL4 hairpin with base-pair resolution. We used a cytosine BA, tCO, that provides a way to locally modify the base stacking without perturbing hydrogen bonds or the native nucleic acid structure. We find that SL4 (un)folds mainly in a single step or through an intermediate (native pathways), encompassing nucleotides of the upper stem, from the central U bulge to the hairpin loop, along with non-native pathways. In the tCO-modified constructs, we report the occurrence of native and non-native pathways in different proportions, and additional non-native pathways depending on the site of tCO incorporation. Our conformational study of SL4 will help in a better understanding of SARS-CoV-2 packaging and in design of drugs targeting SL4. Furthermore, our strategy of using BAs to locally modify the properties of RNA (and DNA) is general and can be expanded to other nucleic acids as well as various BAs.

Molecular motors and machines

Session chairs: Sebastian Deindl, Uppsala University & Dea Slade, Max Perutz Labs, Vienna

O-82 Invited speaker
NONLINEAR MECHANICS OF HUMAN MITOTIC CHROMOSOMES
Gijs Wuite[1]
[1] Vrije Universiteit Amsterdam, Netherlands

The DNA in eukaryotes is organised into linear chromosomes. These structures are highly dynamic throughout the cell cycle and undergo dramatic compaction during mitosis to adopt the characteristic "X-shape". This metamorphosis is driven mainly by the combined action of condensins and topoisomerase IIα (TOP2A). Still there is little known about the structural organization of a mitotic chromosome. Here we introduce a workflow to interrogate the organization of human chromosomes based on optical trapping and manipulation. This allows high-resolution force measurements and fluorescence visualization of native metaphase chromosomes to be conducted under tightly controlled experimental conditions. We have used this method to extensively characterize chromosome mechanics and structure. Notably, we find that under increasing mechanical load, chromosomes exhibit nonlinear stiffening behaviour, distinct from that predicted by classical polymer models. To explain this anomalous stiffening, we introduce a hierarchical worm-like chain model that describes the chromosome as a heterogeneous assembly of nonlinear worm- like chains. Moreover, through inducible degradation of TOP2A5 specifically in mitosis, we provide evidence that TOP2A has a role in the preservation of chromosome compaction. The methods described here open the door to a wide array of investigations into the structure and dynamics of both normal and disease-associated chromosomes.

O-83 Invited speaker
Adventures in DNA and chromatin replication using single-molecule biophysics
Nynke Dekker[1], Daniel Ramirez Montero[1], Humberto Sanchez[1], Zhaowei Liu[1], Edo van Veen[1], Theo van Laar[1], Belen Solano[1], John Diffley[2]
[1] Delft University of Technology, Netherlands [2] Francis Crick Institute, UK

Many transactions on DNA are carried out by molecular machines that operate at the nanometer-scale. How they do so effectively is a question of long-standing interest. We are particularly interested in studying the dynamics of these molecular machines using single-molecule techniques. I will briefly highlight how the field of single-molecule biophysics has advanced such techniques over the past decades, allowing the dynamics of diverse motor proteins to be accurately followed. I will next describe how single- molecule techniques are being used to tackle new challenges, including the probing of complex molecular machines built up from many different components. The replisome that copies DNA is such a complex machine. While the overall outline of replisome assembly in eukaryotes such as ourselves is understood, little is known about the dynamics of the individual proteins on the DNA and how these contribute to the formation of proper replisomes. I will show that using integrated optical trapping and confocal microscopy, one can dissect how protein binding, diffusion, sequence recognition, and protein-protein interactions play important roles in replisome assembly and motion. I will then describe our recent efforts on replisome assembly in the context of chromatin, and close by presenting an outlook for the future.

O-84 Short talk
Single-molecule optical tweezers studies of human mitochondrial DNA replication: Unraveling the coordinated activities of PolG and mtSSB
Borja Ibarra[1], Ismael Plaza-GA[1], Fernando Cerron[1], Grzegorz Ciesielsky[2], Francisco J Cao-Garcia[3]
[1] IMDEA Nanociencia, Spain [2] Auburn University at Montgomery. USA [3] Universidad Complutense, USA

The replicative DNA polymerase PolG is responsible for the accurate replication of human mitochondrial DNA. The activities of PolG are modulated by partner proteins, such as the mitochondrial single-stranded DNA binding protein (mtSSB). Here, we present single-molecule optical tweezers assays to elucidate the mechanisms by which mtSSB modulates the primer-extension and strand displacement activities of PolG. On one hand, our results show that during primer extension conditions, elimination of template secondary structure by mtSSB binding promotes the maximum replication rate of the polymerase. Under these conditions, strong polymerase-SSB interactions are crucial to dislodge the tightly bound mtSSB from the template without compromising the instantaneous replication rate. On the other hand, we show that mtSSBs stimulate PolG's strand displacement activity through several mechanisms. mtSSB binding energy to ssDNA increases the destabilization energy at the DNA junction by ~25%. Furthermore, mtSSB interactions with displaced ssDNA reduce DNA fork reannealing pressure on PolG, in turn promoting control of the polymerase over the template and increasing productive polymerization by ~3-fold. Overall, these stimulatory effects are enhanced by species-specific functional interactions and have significant implications in the replication of human mitochondrial DNA.

O-85 Short talk
Unfolding dynamics of G-rich DNA knots control RecQ helicase processivity
Line Mørkholt Lund[1], Emil Laust Kristoffersen[1], Tomasz Kulikowicz[2], Vilhelm A. Bohr[2,3], Victoria Birkedal[1]
[1] Aarhus University, Denmark [2] DNA Repair Section, National Institute on Aging, NIH. Baltimore, MD 21224 [3] University of Copenhagen, Copenhagen, Denmark

RecQ helicases facilitate genome stability and are conserved from bacteria to humans. Mutations in three of the five human RecQ helicases, Bloom, Werner, and RECQ4, result in premature aging diseases, type 2 diabetes, atherosclerosis, and cancer. RecQ helicases unwind a variety of substrates and have affinity for G-quadruplex structures, knots that can form in guanine-rich DNA sequences such as found in telomeres and promotor regions. These knots must be resolved to avoid unsuccessful DNA replication or processing throughout the cell cycle, and it is believed that un-resolved G-quadruplexes in RecQ-mutated cells are involved in the increased genomic instability. We have investigated the ability of the Werner and Bloom helicases to unwind various G-rich sequences, that fold G-quadruplexes of different topology and stability, using both ensemble spectroscopic techniques and single-molecule FRET. Our kinetics studies of helicase unwinding, as well as characterization of the dynamics, topology, and stability of the G-rich DNA structures, show that the intrinsic unfolding dynamics of the G-quadruplexes themselves is important for RecQ helicases to unwind these DNA knots. These results point to a semi-passive regulation mechanism used to control the activity of the RecQ helicases at G-quadruplex structures.

O-86 Short talk
Cryo-EM conformational insights of bacterial dissagregase ClpG
Stavros Azinas[1,2], Lisa Engelhardt[1,2], Panagiotis Katikaridis[3], Timo Jenne[3], Axel Mogk[3], Marta Carroni[1,2]
[1] SciLifeLab, Sweden [2] Stockholm University, Sweden [3] Heidelberg University (ZMBH), Germany

Pseudomonas aeruginosa ClpG has emerged as a viable alternative to the canonical 70 kDa heat shock protein (Hsp70)-ClpB bichaperone in facilitating bacterial survival during lethal heat stress. Recent investigations have elucidated the molecular underpinnings of ClpG-mediated protein disaggregation, with the ClpG-specific N1 domain implicated in binding protein aggregates and peptide substrate binding shown to activate its ATPase activity. Furthermore, electron and fluorescence microscopy experiments have revealed that ClpG oligomerizes into large complexes comprised of multiple hexamers, while the Y541A mutation promotes hexamer dissociation into isolated entities. In this report, we present cryo-EM structures of different conformational states of the hexameric ClpG mutant Y541A, providing mechanistic insights into the role of ClpG in maintaining proteostasis in P. aeruginosa.

O-127 Short talk
Mechanism of MCM helicase loading and regulation by CDK revealed by cryo-EM
Thomas C.R. Miller[1,2], Chew Theng Lim[1], Kang Wei Tan, Phil East[1], Alessandro Costa[1], John F.X. Diffley
[1] The Francis Crick Institute, London, NW1 1AT [2] University of Copenhagen, Copenhagen, Denmark

Faithful genome replication is required for genome stability, controlled cell proliferation, and healthy aging. The hexameric MCM helicase forms the core of the eukaryotic replication machinery. Prior to initiating replication in S-phase, pairs of MCM helicases are loaded onto duplex DNA to form symmetrical MCM double hexamers, 'licencing' origins for bidirectional replication. Re- replication during S-phase is inhibited by the cyclin-dependent kinase, CDK, which phosphorylates helicase loading factors including the Origin Recognition Complex (ORC) to prevent MCMs from being loaded and activated on duplicated DNA. How CDK phosphorylation of ORC inhibits re-replication is not known. Using time-resolved cryo-EM and a novel approach to visualize complete origins of replication (Reconstitution in Silico; ReconSil), we previously identified new steps in the MCM loading pathway and defined a sequential, coordinated mechanism for MCM double hexamer formation. In our latest work, we have used our EM-based visual biochemistry approach to determine how CDK phosphorylation of ORC inhibits MCM double hexamer formation. Our work reveals novel insights into the fundamental mechanisms of MCM helicase loading, reveals how CDK phosphorylation of ORC prevents re-replication during S-phase, and explains why specific origins are prone to re-replication, even in the presence of ORC phosphorylation.

Protein structure and function

Session chairs: Elisabeth Sauer-Eriksson, Umeå University & Lynn Kamerlin, Georgia Institute of Technology and Uppsala University

Arranged in collaboration with Protein Society

O-88 Invited speaker
Engineering the folding and function of tandem-repeat proteins: Teaching old proteins new tricks
Laura Itzhaki[1]
[1] University of Cambridge, UK

Tandem-repeat proteins, such as tetratricopeptide repeats, comprise tandem arrays of small structural motifs that pack in a linear fashion to produce regular, elongated, quasi-one-dimensional architectures. The repetitive, modular organisation of this architecture makes it straightforward to both to dissect and redesign their biophysical properties. I will describe our work exploring the folding energy landscapes of tandem-repeat proteins and more recent studies aiming to exploit tetratricopeptide repeats to create a platform of synthetic binding molecules capable of displaying both single as well as multiple functions by grafting short linear motifs onto them in precise and pre-programmed geometries.

O-89 Invited speaker
The Secret Ultrafast Motions of Protein Nanomachines
Gilad Haran[1]
[1] Weizmann Institute of Science Rehovot, Israel

Multiple proteins function as nanomachines, alternating chemical steps with conformational transitions. Single-molecule FRET (smFRET) spectroscopy is a powerful tool to study the internal motions of protein machines during function. We developed a novel photon-by-photon maximum likelihood method to facilitate smFRET experiments with a microsecond time resolution, and studied several protein machines, finding in all cases surprisingly fast function-related dynamics. We first measured domain closing and opening times of the enzyme adenylate kinase, and found them to be two orders of magnitude shorter than the catalytic turnover time. We proposed that the enzyme uses these fast motions to optimize substrate orientation. We then studied internal dynamics of the AAA+ machine ClpB, which rescues proteins from aggregation. The pattern of the microsecond dynamics of the pore loops, which protrude into the central pore and pull in substrate proteins, suggested a Brownian ratchet mechanism for protein threading. Finally, we studied the chaperone GroEL and found that the population ratio of four fast-exchanging microstates determines the allosteric state of the chaperone. Thus, it seems that the proteins we have probed can be described with a mechanism based on two time scales. While the activity cycle of each of these proteins defines a relatively 'slow' time scale, their structural fluctuations constitute a second, much faster time scale, which is employed by the protein to affect the slow activity cycle.

O-90 Short talk
Characterization of an Alternate Conformation of the HIV-1 Capsid Protein CTD Dimer using 19F NMR and Weighted Ensemble MD
Darian Yang[1,2,3], Lillian Chong[3], Angela Gronenborn[2,3]
[1] University of Pittsburgh and Carnegie Mellon University, USA [2] University of Pittsburgh School of Medicine, USA [3] University of Pittsburgh, USA

The HIV-1 capsid protein assembles into a conical shell during viral maturation, encasing and protecting the viral RNA genome. The C-terminal domain (CTD) of the two-domain capsid protein dimerizes, and this dimer connects individual chains in the mature capsid lattice. Previous NMR studies have shown that different dimer arrangements can be formed; however, the structure and function of any alternate dimers are unknown. To explore the conformational landscape of the CTD dimer, we carried out atomistic molecular dynamics simulations using the weighted ensemble rare-events sampling strategy, generating an ensemble of conformations for the alternate dimer orientation. To assess whether the conformations detected experimentally match those in our simulations, we measured interconversion rates between the two alternate dimers using high sensitivity 19F NMR. Overall, the measured experimental rates agree with the rate constants calculated directly from our simulations, and the alternate CTD dimer interface may mimic the orientation of the CTD dimer that connects pentameric and hexameric subunits in the mature, fully assembled capsid. Our results demonstrate the advantages of pairing atomistic rare-event sampling with 19F NMR and may help elucidate the HIV-1 capsid assembly process resulting in the intrinsic structural polymorphism of capsid cores.

O-91 Short talk
Protein Conformational Space at the Edge of Allostery: Turning a Non-allosteric Malate Dehydrogenase into an "Allosterized" Enzyme Using
Evolution-Guided Punctual Mutations
Dominique Madern[1], Antonio Iorio[2], Céline Brochier-Armanet[3], Caroline Mas[1], Fabio Sterpone[2]
[1] Institut de BIologie Structurale, France [2] Laboratoire de Biochimie Théorique, France [3] Laboratoire de Biométrie Biologie Evolutive, France

Allostery is the process in which the protein catalytic efficiency is regulated by the binding of an effector at a specific distal site from the catalytic site. According to initial models, the process relies on a ligand-dependent conformational change between a tense inactive state (T-state) and a relaxed active state (R-state). Later, extensive analyses have shown that allosteric regulation is grounded in protein dynamics, leading to the general “ensemble model” of allostery. In it, the allosteric capacity of an enzyme depends on the reorganization of its conformational landscape, induced by various events such as interactions with ligands, and physico-chemistry variations of the environment etc. Protein super families encompassing allosteric and non-allosteric enzymes offer strong opportunities to disclose the molecular mechanisms of allosteric regulation. This is the case with the malate (MalDH) and lactate dehydrogenase (LDH) super family that is studied using a complementary structure-function-dynamics approach. By studying a large set of non-allosteric MalDHs and LDHs, which display homotropic and heterotropic activation it was possible to identify the various evolutionary steps allowing the allosteric regulation to emerge in this super family. The presentation will show i) how a first set of permissive mutations allowed to create various subgroups of MalDHs displaying different hidden pre-allosteric behavior ii) how the successive addition of dynamics-enhancing mutations have altered their conformational landscape, making transiently T-like (inactive) conformers, typical of allosteric LDHs, accessible. The work shows that mutations causing the enrichment of low-populated conformational sub states is the fundamental process by which new phenotypes are selected.

O-92 Short talk
Killing to survive - the many molecular mechanisms of programmed cell death
Kristyna Pluhackova[1]
[1] SC SimTech, University of Stuttgart. Germany

Programmed cell death is an essential process of eukaryotic life, enabling e.g., embryonic development, regeneration, or fighting pathogens. Depending on the needs of an organism, diverse molecular mechanisms of cell death exist, determining among others the speed of cell death, its extent and the impact on surrounding cells. It is therefore not surprising that dysregulation of cell death culminates in diverse diseases, the most prominent of all being cancer. Here, we unveil molecular details of three different cell death processes by complementing experiments with multiscaling molecular dynamics simulations. First, we reveal a novel functional link in apoptotic signaling by showing that the anti-apoptotic BCL-2 protein inhibits cell death mediated by pro-apoptotic BOK protein by interactions of their transmembrane domains. Strikingly, in contrast to other more thoroughly studied members of the Bcl-2 family, BAX and BAK, which cause mitochondrial outer membrane permeabilization, BOK and BCL-2 are localized in the endoplasmic reticulum. Next, we unveil how during pyroptosis gasdermins create medium-sized pores in the plasma membrane showing astonishing adaptability of the pore shape. At last, we resolve the mechanism through which ninjurin-1 disrupts membranes during plasma membrane rupture, the terminal event of many cell-death processes. Our data show that upon lytic cell death initiation, the extracellular α-helices of ninjurin-1 insert into the membrane and form a bridge to other ninjurin-1 molecules leading to formation of tightly packed fence-like filaments, which stably cap membrane edges.

O-93 Short talk
SAMase from bacteriophage T3 counteracts bacterial defence systems through SAM cleavage and inhibition of SAM
synthesis
Silvia Trigüis[1], Alexandr Andriianov[2], Konstantin Severinov[3], Artem Isaev[2], Maria Selmer[1]
[1] Uppsala University, Sweden [2] Skolkovo institute of Science and Technology [3] Waksman Institute

Bacteriophage T3 encodes a SAMase that through cleavage of S-adenosyl-methionine (SAM) circumvents the SAM-dependent Type I Restriction-Modification defence of the host bacterium Escherichia coli. Here, we show that the SAMase also allows phage T3 to evade BREX defence. The anti-BREX activity of the T3 SAMase is mediated by two independent mechanisms: enzymatic degradation of SAM and inhibition of SAM synthesis through direct interaction with the host SAM synthase MetK. We purified the native octameric complex of T3 SAMase with E. coli MetK and determined a 2.8 Å cryo-EM structure. The complex stoichiometry was confirmed with mass photometry and small-angle X-ray scattering. Structure guided mutagenesis of the SAMase-MetK interface revealed that the interaction with MetK stabilizes the T3 SAMase in vivo, thus further contributing to its counter-defence activity. This work provides insights in the versatility and intricacy of bacteriophage counter-defence mechanisms and highlights the role of SAM as an important cofactor of diverse phage-defence systems.

Mechanobiology in health and disease

Session chairs: Christelle Prinz, Lund University & Christine Selhuber-Unkel, Heidelberg University

O-94 Invited speaker
High-throughput mechanical phenotyping for diagnostic applications
Jochen Guck[1,2,3]
[1] Max Planck Institute for the Science of Light, Germany [2] Max Planck Zentrum für Physik und Medizin [3] Friedrich-Alexander Universität Erlangen-Nürnberg (FAU, Department of Physics

The mechanical properties of cells have long been heralded as a useful inherent and sensitive marker of disease. Despite many proof-of-concept demonstrations to this effect, a major roadblock to general application has been a paucity of appropriate tools for the convenient quantification of such properties. This has changed with the recent advent of high-throughput microfluidic techniques. As one such technique, we have introduced real-time deformability cytometry (RT-DC) which permits the continuous physical single-cell characterization of large populations (> 1.000.000 cells) with analysis rates of 1,000 cells/s — approaching that of conventional fluorescence-based flow cytometers. Using RT-DC we can sensitively detect physiological and pathological changes in cell function by image-based parameters such as size, shape, deformability, and any other information contained in an image. For example, we have recently demonstrated its utility for detecting blood mechanical changes in the context of Covid19, which could be causally involved in the progression of the disease. Combined with quick mechanical dissociation, also tissue biopsies are now accessible for RT-DC to diagnose solid tumors. In general, mechanical phenotyping adds a new functional, marker-free and unbiased dimension to flow cytometry with diverse applications in biology, biotechnology and medicine.

O-95 Invited speaker
Receptor-mediated regulation of molecular and cellular mechanics
Ada Cavalcanti-Adam[1]
[1] Max Planck Institute for Medical Research & Heidelberg University, Germany

Transmembrane receptors, such as integrins and cadherins, convey chemical and mechanical signals to the intracellular compartment. In the first part of my talk I will introduce several approaches, based on surface micro- and nanopatterning, which allow the control of receptor clustering and the assembly of cell-matrix adhesions during cell migration. Moreover, at the nanoscale, the regulation of molecular and cellular forces is closely related to rigidity sensing of the substrate. In the second part of my talk, I will discuss our recent development on the controlled assembly and mechanics of E-cadherins at cell-cell junctions, and how the crosstalk between cell-matrix and cell-cell adhesion might be coordinated at the nanoscale.

O-96 Short talk
Shaping the embryo: blastoderm stress maps reveal early mechanical symmetry breaking
Alejandro Jurado Jiménez[1], Leon Lettermann[1], Bernhard Wallmeyer[2], Timo Betz[1]
[1] Third Institute of Physics - Biophysics, University of Göttingen, Germany [2] Institute of Cell Biology, ZMBE, University of Münster, Germany

In this work we present a hydrodynamical analysis of early Zebrafish development which aims to understand the mechanical state of the tissue leading to its first symmetry breaking during epiboly: the shield formation. A full mechanical characterization of the blastoderm is achieved using a combination of Light-Sheet microscopy and state-of-the-art cell tracking of the cells nuclei, viscosity measurements and polyacrylamide beads as force sensors. The extraction of stress maps in the tissue is possible thanks to a custom-made software for the analysis of the bead deformation, which is presented here a versatile tool for similar stress analyses in other biological samples.
Our experimental analysis of the mechanical state of the embryo is supported and expanded by a model-driven extraction of the stress fields using NeuronalODEs. The NODEs system only necessitates the velocity field on the blastoderm to solve the hydrodynamic problem, optimizing up to 10^5 parameters and retrieving a full dynamical description of the embryo. Both the experimental and numerical analyses expose a stress asymmetry prior and during the shield formation, from which we can learn more about the mechanical origin of the first embryonal symmetry breaking.

O-97 Short talk
Visualization and force spectroscopy of mineral desert dust and associated microbes: Unraveling a bacterial long- distance propagation strategy
Christian Nehls[1], Carla Neitzke[1], Sofía Gómez Maqueo[2], Martinique Frentrup[3], Gerrit Bredeck[4], Eduardo Souza[2], Dietrich Althausen[2], Kerstin Schepanski[2,5], Roel Schins[4], Ulrich Nübel[3], Khanneh Wadinga Fomba[2], Thomas Gutsmann[1]
[1] Research Center Borstel – Leibniz Lung Center, Borstel, Germany [2] Leibniz Institute for Tropospheric Research, Leipzig, Germany [3] German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany [4] Leibniz Research Institute for Environmental Medicine GmbH, Düsseldorf, Germany [5] Freie Universität Berlin, Berlin, Germany

Dust storms can transport mineral dust (MD) from desert soil through the atmosphere to regions far from the dust origin. Respiratory, cardiovascular, and allergic diseases can be observed in affected individuals. Moreover, non-pathogenic and pathogenic microbes are also transported with the dust. These not only spread to new regions but may also lead to direct infections. The “Dust-Risk” consortium takes a highly interdisciplinary approach and aims to link the composition and properties of MD and associated microbes with its health risk. MD and health data have been collected in the Cape Verde Islands, which serve as a model region. In our project part, we use atomic force microscopy and scanning electron microscopy to visualize and characterize the collected MD. With force spectroscopy, we determine the strength and type of molecular bonds between MD and different bacterial species that have been identified on the MD. Different sampling sites are compared to distinguish between local and desert dust, and between microbes of local, marine, and desert origin. We have shown that microbial components play a significant role in the inflammatory potency of MD. Our biophysical experiments help to understand how bacteria interact with MD to travel long distances through the atmosphere. This knowledge will be complemented by physicochemical, microbiological, toxicological, modeling, clinical and epidemiological data from further project parts to generate a composition-based dust-health- risk-index. The Cape Verde weather service will use this risk index to provide precise and specific advice and warnings to the population.

O-98 Short talk
Modular and Dynamic Hydrogels for Mimicking the Tumor Microenvironment
Daniel Aili[1], Fatemeh Rasti Boroojeni[1], Sajjad Naeimipour[1], Charlotta Dabrosin[1], Annelie Abrahamsson[1]
[1] Linköping University, Linköping, Sweden

The extracellular matrix (ECM) is a complex, hydrated, and dynamic network of macromolecules that supports and organizes cells
in tissues and organs. The ECM plays a crucial role in influencing cell behavior, survival, and differentiation through various mechanisms, including physical, biochemical, and signaling pathways. Mimicking the cellular microenvironment using engineered hydrogels is challenging but can enable systemic studies of cell-matrix interactions and facilitate the development of biologically relevant tissue and disease models. We develop modular hydrogel systems that allow for control over biofunctionality, cross-linking density, and network topology, offering means to tailor hydrogel stiffness, stress relaxation, and cell response. Several strategies for hydrogel cross-linking have been developed and explored, using bioorthogonal covalent strategies, supramolecular interactions, and combinations thereof. The effects of multimodal hydrogel cross-linking were explored using a novel enzyme- activated thiol-based chemistry, allowing for on-demand generation of thiols that can both form intermolecular disulfide bridges and react with thiol-specific cross-linkers. The possibilities to combine multiple cross-linking chemistries allow for tuning of hydrogel properties and cell behavior. The hydrogels were used as bioinks for development 3D bioprinted mimics of the breast cancer tumor microenvironment that combine stromal and immune cells, in addition to breast cancer cells. These models enable detailed studies of the effects of cell-mediated matrix remodeling on tumor cell proliferation and can provide new valuable tools for cell biophysics.

O-99 Short talk
Active generation of twist in filopodia
Poul Martin Bendix[1], Natascha Leijnse[1], Younes Barooji[1], Mohammad Arastoo[1], Stine Sønder[1], Bram Verhagen[1], Lena Wullkopf[1], Janine Erler[1], Szabolcs Semsey[1], Lene Oddershede[1], Amin Doostmohammadi[1], Jesper Nylandsted[2]
[1] University of Copenhagen, Denmark [2] Danish Cancer Research Institute, Denmark

Filopodia are actin-rich dynamic tentacles displayed on the surface of eukaryotic cells which allow cells to explore their environment, generate mechanical forces or perform chemical signaling. We show that filopodia explore their 3D extracellular space by combining growth and shrinking with axial twisting and buckling. Importantly, the actin core inside filopodia performs a twisting or spinning motion which is observed for a range of cell types spanning from earliest development to highly differentiated tissue cells. Non-equilibrium physical modeling of actin and myosin confirm that twist is an emergent phenomenon of active filaments confined in a narrow channel which is supported by measured traction forces and helical buckles that can be ascribed to accumulation of sufficient twist. These results lead us to conclude that activity induced twisting of the actin shaft is a general mechanism underlying fundamental functions of filopodia.

Biophysics of the green transition

Session chairs: Daniel Otzen, Aarhus University & Doris Ribitsch, Graz University of Technology

O-100 Invited speaker
Biologically fabricated materials from engineered microbes
Neel Joshi[1]
[1] Northeastern University, USA

The intersection between synthetic biology and materials science is an underexplored area with great potential to positively affect our daily lives, with applications ranging from manufacturing to medicine. My group is interested in harnessing the biosynthetic potential of microbes, not only as factories for the production of raw materials, but as fabrication plants that can orchestrate the assembly of complex functional materials. We call this approach “biologically fabricated materials”, a process whose goal is to genetically program microbes to assemble materials from biomolecular building blocks without the need for time consuming and expensive purification protocols or specialized equipment. Accordingly, we have developed Biofilm Integrated Nanofiber Display (BIND), which relies on the biologically directed assembly of biofilm matrix proteins of the curli system in E. coli. We demonstrate that bacterial cells can be programmed to synthesize a range of functional materials with straightforward genetic engineering techniques. The resulting materials are highly customizable and easy to fabricate, and we are investigating their use for practical uses ranging from bioremediation and biodegradable bioplastics to engineered therapeutic probiotics.

O-101 Invited speaker
Enzymatic Plastic Recycling and Upcycling: A Promising Strategy for Green Transition
Ren Wei[1]
[1] University of Greifswald, Germany

The increasing amount of plastic waste and its adverse impact on the environment has led to a growing interest in developing sustainable solutions for plastic recycling. Enzymatic plastic degradation has emerged as a promising strategy for the circular plastic economy, offering a green and efficient alternative to traditional mechanical and chemical recycling methods. Various hydrolases have been discovered and engineered to allow for efficient enzymatic polyethylene terephthalate (PET) recycling at an industrial scale. In recent years, significant progress has been made in upcycling options using PET hydrolysates and its monomers to create value-added products through biological and chemo-biological approaches. This lecture will highlight the latest advances in this area of research, as well as the challenges and opportunities in developing enzymatic plastic degradation as a scalable and economically viable technology. By exploring the potential for enzymatic plastic degradation to drive the green transition, we can better understand how to tackle plastic waste challenges and mitigate environmental harm.

O-102 Short talk
Gold Nanoparticles green synthesis: the effect of natural compounds
Caterina Medeot[1,2,3], Ahmed Alsadig[2,4], Francesco D'Amico[5], Albano Cossaro[5,6,7], Hendrick Vondracek[2,5,8], Giovanni Birarda[5], Paola Posocco[9], Pietro Parisse[2,6], Loredana Casalis[2], Serena Bonin[1]
[1] University of Trieste, Italy [2] Elettra Sincrotrone Trieste S.C.p.A., Italy [3] University of Trieste, Italy [4] National Research Council (CNR-NANOTECH), Campus Ekotecne, Italy [5] Elettra-Sincrotrone Trieste S.C.p.A., Area Science Park, [6]National Research Council (CNR-IOM), Area Science Park, Italy [7] University of Trieste, Italy [8] Harwell Science and Innovation Campus, Didcot, Oxfordshire, UK [9] University of Trieste, Italy

Among various classes of nanomaterials, gold nanoparticles (AuNPs) have attracted enormous scientific attention, owing to their unique optical and electrical properties. Despite the great feasibility of standard wet chemistry, limitations are found in the difficulty to separate the produced AuNPs from the reaction mixture, including toxic chemicals. Nowadays the demand for developing environmental-friendly routes to synthetize AuNPs preventing wastes, and using instead safer solvents (e.g., water), is growing. Unfortunately, current studies lack standardized synthesis procedures, and the nature of the reducing reaction is still unclear. The work aims to compare AuNPs synthesis from different natural extracts focusing on finding the most efficient and environmental- friendly ones. A careful spectroscopic and morphological integrated analysis has been applied to infer the nature of the redox reaction driving the AuNPs formation and to identify the best protocol that should be adopted to generate small, clean, spherical and monodisperse AuNPs. Our best choice felt on spherical AuNPs with diameter of 10-11 nm successfully synthetized from cocoa extract. Spectroscopic analyses showed a large presence of fatty acids, proteins, and catechins on cocoa extract. The effective reducing agent are catechins which also surround cocoa AuNPs forming a spot layer which maintains AuNPs stability. Functionalization tests are under development on cocoa AuNPs via surface modification with alkanethiols, carboxyl and amine groups for coupling amino acid side chains on antibodies for a facile combination with biomolecules.

O-103 Short talk
Design of hierarchical protein materials for a sustainable society
Christofer Lendel[1], Mikael Hedenqvist[2], Maud Langton[3], Fredrik Lundell[4]
[1] Department of Chemistry, KTH Royal Institute of Technology, Stockholm, Sweden [2] Department of Fibre and Polymer Technology, KTH Royal Institute of Technology, Stockholm, Sweden [3] Department of Molecular Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden. [4] Department of Engineering Mechanics, KTH Royal Institute of Technology, Stockholm, Sweden

Protein-based materials could play important roles in the development towards a more sustainable society, as proteins are renewable resources and biodegradable. Moreover, protein materials can provide unique functionality in e.g. water remediation or bioelectronics devices and constitute a fundamental building block in food. Natural occurring protein materials, such as silk, have served as inspiration for manmade structures for long time but the challenge to produce synthetic materials with comparable properties from biobased resources remains. The key to achieve this is to gain control over the assembly of hierarchical structures from the protein building blocks. Amyloid-like protein nanofibrils (PNFs) have emerged as a promising foundation for the synthesis of novel bio-based materials for a variety of applications. Such nanofibrils are formed through self-assembly and have mechanical properties comparable to silk. We have demonstrated the formation of PNFs from a range of agricultural resources and how these fibrils can be assembled into ordered, hierarchical structures. In recent studies we have deciphered the formation mechanisms for nanofibrils with distinct mechanical properties. This allows us to explore the relationships between nanoscale- and macroscale structures in PNF-based fibres, films and foams. We have also developed an approach to create cross-linked protein materials with very good thermal- and chemical stability without any non-protein additives.

O-104 Short talk
Sustainable Waterborne Electrospinning of Protein-based Materials
Mai Bay Stie[1,2], Kleopatra Kalouta[1,2,3], Valeria Vetri[3], Vito Foderà[1,2]
[1] Department of Pharmacy, University of Copenhagen, Copenhagen, Denmark [2] Center for Biopharmaceuticals and Biobarriers in Drug Delivery, University of Copenhagen, Copenhagen, Denmark [3] Dipartimento di Fisica e Chimica, Università Degli Studi di Palermo, Palermo, Italy

Nanofibers have gained great interest as materials for textile production, food packaging, tissue engineering and drug delivery. During electrospinning, an electrical field drives the formation of fibers by intermolecular entanglement and solvent evaporation. To facilitate evaporation, organic solvents and surfactants are widely used; this compromising the safety of the electrospun materials and causing negative environmental impact. Proteins can be purified from renewable sources, are biodegradable and can partially or completely substitute synthetic polymers. We focused on electrospinning proteins of both animal and plant origin in water as a green and sustainable approach to fabricate innovative biomaterials. We have deciphered key parameters including protein solubility, intermolecular entanglement and humidity during electrospinning, to be critical for formation of fibers from proteins electrospun in water. The nanofibers of high protein-content were characterized by biophysical techniques including circular dichroism spectroscopy, Fourier-transform infrared spectroscopy, and fluorescence spectroscopy. Use of the nanofibers as drug delivery systems was demonstrated using in vitro cell models and ex vivo porcine tissue for the delivery of active pharmaceutical ingredients. We propose that our results may serve as a general workflow for the establishment of waterborne electrospinning of proteins as methodology to support the green transition for materials within pharmaceutical sciences**.**

O-105 Short talk
Enzymes in deep eutectic solvents: simulations of lipases for the biocatalysis of carbohydrate polyol esters.
Miguel A. Soler[1], Ozge Ozkilinc[1], Paolo Giannozzi[1], Federico Fogolari[1]
[1] University of Udine, Udine, Italy

Biocatalysis of renewable bio-based polymers has emerged as one of the most powerful strategies to fight against plastic pollution. Enzymes overcome the limitations of conventional chemical strategies by catalyzing, under highly selective and mild conditions, the synthesis or degradation of polymers. The selection of a suitable reaction media is then fundamental, as the enzymatic synthesis shows limitations in terms of enzyme inactivation and low reactivity with certain organic solvents, besides the toxicity problems that their use involves. The employment of natural deep eutectic solvents (NADES) is a promising solution. NADES are nontoxic, biodegradable and their preparation costs are usually low. In this work, molecular modelling followed by a combination of docking and molecular dynamics (MD) simulations have been performed to study the stability of the lipase B from Candida antarctica in three different carbohydrate polyol-based NADES for the biocatalysis of carbohydrate polyol esters. The innovative utilization of a simulated annealing protocol in the MD simulations allowed to predict the effect of NADES on the structural stability of the enzyme, and to support the experimental results concerning the selective formation of diesters in this medium. Moreover, through docking poses the plausibility of a diesterification reaction pathway could be demonstrated. These results open the way to further explore in silico the influence of solvent mixtures, as NADES, on the structural stability and activity of enzymes.

Membranes and membrane proteins

Session chairs: Gunnar von Heijne, Stockholm University & Anthony Watts, Oxford University

O-106 Invited speaker
Mechanism of cell wall transporters and role in the adaptation of bacterial pathogens
Camilo Perez[1]
[1] University of Basel, Switzerland

Bacterial infections represent a major public health problem augmented by the increasing occurrence of strains resistant to antibacterial agents. Gram-positive bacteria such as Staphylococcus aureus and Streptococcus pneumoniae are leading pathogens causing nosocomial and community-acquired infections. The bacterial cell wall is a complex structure that exerts fundamental protective functions against host defenses and antibiotics, contributing to bacterial survival and adaptation under adverse conditions. Teichoic acids are distinctive biopolymers found in the cell wall of Gram-positive bacteria. They play essential roles in immune evasion, adhesion, biofilm formation, and protection against antimicrobials. Multiple membrane transporters are key players in the biosynthesis pathways of teichoic acids, participating in the translocation of lipid and water-soluble precursors and translocation of fully assembled cell wall components. We are interested in understanding the mechanism of these proteins and in revealing their contribution to bacteria adaptation strategies. We use a combination of X-ray crystallography, single-particle cryo-EM, Solid-Supported-Membranes electrophysiology, nanobodies selection, and several biochemical and biophysical in vitro assays.

O-107 Invited speaker
Membrane self-organization in flat land: Rise of the vesicles
Irep Gözen[1]
[1] University of Oslo, Norway

My team recently discovered astonishing biosurfactant assemblies developing autonomously on flat solid interfaces. The assemblies are non-trivial and adopt morphologies ranging from compartments interconnected with nanotubes to microbial colony-like structures. In my talk, I will explain how we exploit the tiny energy gain arising from contact with solid interfaces to drive the architecture, communication and transport properties of membrane compartment populations in a 'flat world'. The lecture will highlight the implications of the new findings for synthetic cell design, and argue that materials properties-driven autonomous processes on solid interfaces might have had a greater role in the development of life than currently considered.

O-108 Short talk
Measuring lipid dynamics and packing with MINFLUX microscopy
Agnes Koerfer[1,2], Christian Eggeling[1,2], Pablo Carravilla[2,3]
[1] Friedrich Schiller University Jena, Jena, Germany [2] Leibniz Institute of Photonic Technology eV, Jena, Germany [3] Karolinska Institutet, Solna, Sweden

The eukaryotic cell plasma membrane is highly complex, and its organization is critical to multiple biological processes, such as virus infection. Still, the role of membrane heterogeneity remains poorly understood due to the lack of techniques showing both high spatial and temporal resolution. Here, we assess the capacity of MINFLUX (minimal photon fluxes) microscopy to investigate lipid membrane organization quantitatively. We show that MINFLUX imaging combined with exchangeable solvatochromic dyes allows quantifying lipid packing in live cell plasma membranes with nanometre resolution. Further, we use MINFLUX in combination with fluorescent lipid analogues to follow their path over space and time. We explore the time resolution of such single lipid tracking experiments as well as apply it to investigate Gag-induced lipid sorting on HIV virus assembly sites at the plasma membrane. To separate lipids diffusing in the plasma membrane from those located in a virus budding site, we develop an open- source MINFLUX track segmentation and analysis pipeline based on a confocal fluorescent image reference. In conclusion, we highlight the applicability of MINFLUX imaging and tracking to investigate lipid organization and dynamics in live cell membranes.

O-109 Short talk
Structure and assembly of Nuclear Pore Complexes by correlative AFM-dSTORM
Christine Doucet[1], Anthony Vial, Emilie Costes, Luca Costa
[1] Centre de Biologie Structurale, France

Nuclear Pore Complexes (NPCs) are the only gateways between the cytoplasm and the nucleus. They are located at fusion sites between the two concentric bilayers that make up the nuclear envelope (NE). The NPC core channel, which is highly symmetric across the NE, is made of three stacked rings that line the highly curved pore membrane. It is capped by two asymmetric domains, the nuclear basket and the cytoplasmic filaments, whose structures are poorly defined. In dividing cells, new pores assemble during interphase in an intact NE. This process involves extensive membrane remodeling and the recruitment of hundreds of proteins. Assembly initiates on the inner nuclear membrane, where a large protein structure assembles, while the two nuclear membranes bend until they fuse and the protein assembly adopts its final conformation. For a highly symmetric complex to assemble in an asymmetric fashion must involve large rearrangements of the proteins and the nascent structure, but this is not understood yet. We prepared NEs from cultured human cells and imaged the nuclear side of NPCs by correlative fluorescence / Atomic Force Microscopy. We first showed that the nuclear basket is highly plastic and mechanically soft. It explores a continuous conformational landscape, plunging into the central channel or protruding towards the nucleoplasm. We also observed distinct stages of pore assembly. We identified several typical conformations, illustrating limiting steps encountered along the assembly pathway. Correlative dSTORM data help us proposing a sequence of events, from seeding of initial components to the sequential assembly of rings.

O-110 Short talk
In situ architecture of Opa1-dependent mitochondrial cristae remodeling
Luke H. Chao[1,2], Paula P. Navarro[1,2], Qin Xingping [3], Zintes Inde[3], Virly Y. Ananda[1,2], Camila Makhlouta Lugo[1,2], Pusparanee Hakim[1,2], Bridget E. Luce[1,2], Yifan Ge[1,2], Julie L. McDonald[1,2], Ilzat Ali[1], Leillani L. Ha[1,2], Benjamin P. Kleinstiver[1,2], David C. Chan[4], Kristopher A. Sarosiek[3], Michelle Y. Fry[1,2]
[1] Harvard Medical School, USA [2] Massachusetts General Hospital, USA [3] Harvard School of Public Health, USA [4] Caltech, USA

Cristae membrane state plays a central role in regulating mitochondrial function and cellular metabolism. The protein Optic atrophy 1 (Opa1) is an important crista remodeler that exists as two forms in the mitochondrion, a membrane-anchored long form (l-Opa1) and a processed short form (s-Opa1). The mechanisms for how Opa1 influences cristae shape have remained unclear due to the lack of native 3D views of cristae morphology. We perform in situ cryo-electron tomography of cryo-focused ion beam milled mouse embryonic fibroblasts with well-defined Opa1 states to understand how each form of Opa1 influences cristae architecture. In our tomograms, we observe elongated mitochondria with a notable stacking phenotype, as well as an absence of tubular cristae, when only l-Opa1 is present. In contrast, when mitochondria contain mainly s-Opa1, we observe irregular cristae packing, an increase in globular cristae, and decreased matrix condensation. Notably, we find the absence of l-Opa1 results in mitochondria with wider cristae junctions. BH3 profiling reveals that absence of l-Opa1 reduces cytochrome c release in response to pro- apoptotic stimuli and protects cells from apoptosis induced by anti-cancer agents. We discuss the implications Opa1-dependent cristae morphologies in cell death initiation.

O-111 Short talk
A unique Amphipathic alpha-helix Drives Membrane Insertion and Activity of ATG3
Roberto Covino[1], Taki Nishimura[2,3], Gianmarco Lazzeri[1], Sharon Tooze[4]
[1] Frankfurt Institute for Advanced Studies, Germany [2] The University of Tokyo, Japan [3] PRESTO, Japan Science and Technology Agency, Japan.
[4] The Francis Crick Institute, UK.

Autophagy is a fundamental cellular mechanism that maintains homeostasis by degrading cytoplasmic materials and damaged organelles. Autophagy involves dynamic membrane remodeling processes driven by autophagy-related (ATG) proteins. These proteins interact with membranes in a strictly controlled manner and sense local membrane environments during membrane reorganization. Amphipathic alpha-helices (AHs) are common lipid-binding modules that sense membrane charge, curvature, unsaturation, and lipid composition. ATG3, indispensable for LC3 lipidation, has an AH required for the protein's function in autophagy. However, the dynamics of ATG3 on autophagic membranes and how it recognizes membrane lipids via its AH are poorly understood. Therefore, we investigated the essential lipid-binding of ATG3 using machine learning, molecular dynamics (MD) simulations, and in vivo experiments. The analysis of more than 1800 AHs revealed that ATG3-type AHs contain less hydrophobic and less bulky amino acids than unrelated AHs. This biophysical feature of the ATG3 AH is highly conserved from yeast to mammals, and it is required for ATG3 to achieve efficient LC3 lipidation in cells. Molecular dynamics (MD) simulations of the ATG3-LC3 complex showed that the biophysical features of AHATG3 are fine-tuned to regulate its dynamics and substrate accessibility. Live cell imaging analysis revealed a transient membrane association of ATG3 with autophagic membranes. ATG3's AH fine-tuned biophysical features are fundamental to its central role. The conceptual and technical framework we present here can serve as a general platform to better understand the role of AHs in regulating key phenomena in the cell.

Biophysics of redox biology

Session chairs: Giuseppe Filomeni, Danish Cancer Society Research Center Copenhagen & Elias Arnér, Karolinska Institutet

O-112 Invited speaker
S-nitrosylation under the lens of molecular dynamics
Elena Papaleo[1,2], Matteo Tiberti[2], Matteo Arnaudi[1], Chiara Pecorari[2], Fiorella Faienza[3], Lisa Cantwell[2], Kristine Degn[1], Francesca Pacello[3], Andrea Battistoni[3], Matteo Lambrughi[2], Giuseppe Filomeni[2,3]
[1] Technical University of Denmark, Denmark [2] Danish Cancer Society Research Center, Denmark [3] University of Tor Vergata, Italy

Nitric oxide (NO) has an essential role in redox signaling in normal and pathological cellular conditions. NO modifies cysteine residues of proteins in a process known as S-nitrosylation, which is a selective and reversible post-translational modification with many diverse effects on protein stability and function. Despite its importance, only a few experimental studies are available for S-nitrosylated proteins. In this context, computational studies can help to investigate the multifaceted role of this redox post-translational modification. Our group has been developing high-throughput bioinformatic analyses to find targets of interest interested by S-nitrosylation and characterize them with different simulation approaches [2,3]. In particular, my group recently started to address the gap of structural knowledge on S-nitrosylation using biomolecular simulations to: i) benchmark parameters to study this modification using molecular dynamics simulations for the most commonly used force fields (i.e., physical models used in the simulations); ii) modeling of the mechanisms induced by S-nitrosylation on key proteins for human health, such as chaperones [2,3] and kinases.

O-113 Invited speaker
Probing Toxic Metals and Metalloids in Redox Biology using Synchrotron X-rays
Graham George[1], Ingrid Pickering, Emérita Mendoza Rengifo, Natalia Dolgova[2], Qing Cheng[3], Elias Arnér[3]
[1] University of Saskatchewan, Canada [2] Calibr, Scripps Research, California, USA [3] Karolinska Institutet, Sweden

Some of the compounds of the environmentally problematic elements arsenic and mercury are thought to act through interference with systems that are critical to redox biology. X-ray absorption spectroscopy (XAS) can provide details of the local physical structure and electronic structure of heavier elements in a wide variety of samples. One unique advantage of XAS is that it can be used to probe chemistry in situ in a range of systems with little or no pre-treatment. As such, XAS has been used to develop an understanding of diverse systems including metalloenzyme active sites, solution intermediates, intact biological tissues or organisms, and environmental samples. Recent developments using newly-available advanced X-ray spectroscopy methods have allowed greater spectroscopic resolution and access to the ultra-dilute concentration regime (ca. 100 nM). We have used XAS of selenium, arsenic and mercury to probe the mechanisms of interaction of arsenic and mercury compounds with the selenoenzyme thioredoxin reductase. This presentation will review these results, discuss the advantages and limitations of XAS, and highlight how these biophysical methods can be used to probe the complex interplay of mercury and arsenic compounds with the essential element selenium.

O-114 Short talk
The "In-Between" State of the Copper-Amyloidβ Complex studied by X-ray Absorption through Partial Thermal Relaxation after Photoreduction
Francesco Stellato[1,2], Enrico Falcone[3,4], Germano Nobili[1,2], Michael Okafor[4], Olivier Proux[5], Giancarlo Rossi[1,2,6], Silvia Morante[1,2], Peter Faller[4,7]
[1] University of Rome Tor Vergata, Italy [2] INFN [3] University of Nottingham [4] University of Strasbourg - Institut de Chimie [5] University Grenoble Alpes - Observatoire des Sciences de l'Univers de Grenoble [6] Centro Fermi – Museo Storico della Fisica e Centro Studi e Ricerche Enrico Fermi [7] Institut Universitaire de France, Paris

The redox activity of Cu ions bound to the amyloid-β (Aβ) peptide is implicated as a source of oxidative stress in the context of Alzheimer's disease. In order to explain the efficient redox cycling between Cu(II)-Aβ (distorted square-pyramidal) and Cu(I)-Aβ (digonal) resting states, the existence of a low-populated "in-between" state, prone to bind Cu in both oxidation states, has been postulated. We exploited the partial X-ray induced photoreduction at 10 K, followed by a thermal relaxation at 200 K, to trap and characterize by X-ray Absorption Spectroscopy (XAS) a partially reduced Cu-Aβ1–16 species. The combination of X-ray induced photoreduction and thermal relaxation unravels the structure of an intermediate of the Cu(II)/Cu(I) Aβ1–16 redox cycling and yield a structure different from the resting states and featuring an imidazole ring and a bi-dentate Asp residue in the Cu coordination site. The XAS spectrum measured at the end of the irradiation-heating-cooling protocol is well-fitted by a previously proposed model of the "in-between" state, hence providing the first direct spectroscopic characterization of this intermediate state. The present approach could be used to explore and identify the catalytic intermediates of other relevant metal complexes.

O-115 Short talk
It takes two to tango: Change in the quaternary structure and oncogenicity of TRAP1 via cysteine oxidation.
Giuseppe Filomeni[1,2], Fiorella Faienza[1], Claudio Laquatra[3], Andrea Rasola[3], Giovanni Chiappetta[4], Matteo Castelli[5], Giorgio Colombo[5], Chiara Pecorari[2]
[1] University of Rome Tor Vergata, Rome, Italy [2] Danish Cancer Society Research Center, Copenhagen, Denmark [3] University of Padua, Padua, Italy
[4] Université PSL, Paris, France [5] Department of Chemistry, University of Pavia, Pavia, Italy

The mitochondrial chaperone TRAP1 exerts protective functions in cells exposed to oxidative stress, ensuring cell survival and metabolic adaptation in both physiological and pathological contexts. Despite the fact that TRAP1 functions as a homodimer, new evidence indicates that it can also form tetrameric structures, the effects of which remain unclear. Here, we show that purified TRAP1 forms redox-sensitive oligomers with the same apparent molecular weight as a tetramer. We collected compelling evidence indicating that these oligomers are held together by disulfide bonds between C261 and C573. In tumor cells, the double mutant C261S/C573R does not undergo oligomerization, and does not inhibit succinate dehydrogenase (SDH), a well-known pro-neoplastic activity of TRAP1, suggesting that tetramers might have a prominent role in modulating the metabolic effects of TRAP1 and its oncogenic function. In line with these results, malignant peripheral nerve sheath tumor (sMPNST) cells expressing the C261S/C573R mutant of TRAP1 produce smaller spheroids with a reduced invasion capacity, further indicating that TRAP1 tetramers confer malignant properties to cancer cells. Accordingly, the expression of C261S/C573R TRAP1 markedly reduces the invasion of the aggressive sarcoma cell model sMPNST.

O-116 Short talk
Raman spectroscopy probes oxidative stress and nodules evolution in cytological thyroid cancer samples
Michael Di Gioacchino[1], Martina Verri[1,2], Anda Naciu[2], Alessio Paolucci[1], Alessandra di Masi[1], Chiara Taffon[2], Armida Sodo[1], Andrea Palermo[3], Anna Crescenzi[2], Maria Antonietta Ricci[1]
[1] Università degli Studi Roma Tre, Italy [2] University Hospital Campus Bio-Medico, Italy [3] Department of Endocrinology and Diabetes, Campus Bio-Medico University, Italy

Thyroid gland nodules are one of the predominant endocrine system pathologies and worldwide it is constantly increasing. Clinical guidelines impose that patients with doubtful ultrasound features or with large nodules undergo ultrasound-guided biopsy, followed by cytological examination. This increment results faster than setting of new reliable techniques for early diagnosis, knowledge, and control of thyroid tumorigenesis. Raman spectroscopy (RS) is a powerful candidate for in vivo thyroid cancer diagnosis, being a non-invasive label-free technique. Recently, it has been observed that RS allows to well discriminate healthy and cancerous thyroid samples, both on histological and on cytological ones. Moreover, different fingerprints have been collected for each kind of investigated neoplasia, giving the possibility to distinguish them. By applying RS to cytological samples, it is also possible to follow the disease evolution during the checking follow up. The recorded spectra show different nodules progression in patients with surgically proved papillary thyroid carcinoma (PTC). Cancer cells are characterized by high levels of oxidative stress, exerted by reactive oxygen species (ROS), which accumulate in cells as a consequence of an imbalance between their generation and elimination. RS evidences the presence of carotenoids bands whose uptake may be activated in order to mitigate the increase of ROS, as suggested by the intensity ratio of the peaks assigned to oxidized/reduced cytochromes. Our results support the hypothesis of a correlation among oxidative stress, carotenoids uptake, and cancer progression. Moreover, the presence of fatty acids droplets in PTC samples is evidenced as an additional pathway against oxidative stress.

O-117 Short talk
How abundant are superoxide and hydrogen peroxide in the vasculature lumen, how far can they reach?
Tânia Sousa[1], Marcos Gouveia[1], Rui Travasso[1], Armindo Salvador[1]
[1] Universidade de Coimbra, Coimbra, Portugal

Paracrine superoxide ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2) signaling critically depends on these substances' concentrations, half-lives and transport ranges in extracellular media. Here we estimated these parameters for the lumen of human capillaries, arterioles and arteries using reaction-diffusion-advection models. These models considered $O_2^{\bullet-}$ and H_2O_2 production by endothelial cells and uptake by erythrocytes and endothelial cells, $O_2^{\bullet-}$ dismutation, $O_2^{\bullet-}$ and H_2O_2 diffusion and advection by the blood flow. Results show that in this environment $O_2^{\bullet-}$and H_2O_2 have half-lives <60 ms and <40 ms, respectively, the former determined by the plasma SOD3 activity, the latter by clearance by endothelial cells and erythrocytes. H_2O_2 concentrations do not exceed the 10 nM scale. Maximal $O_2^{\bullet-}$ concentrations near vessel walls exceed H_2O_2's several-fold when the latter results solely from $O_2^{\bullet-}$ dismutation. Cytosolic dismutation of inflowing $O_2^{\bullet-}$ may thus significantly contribute to H_2O_2 delivery to cells. $O_2^{\bullet-}$ concentrations near vessel walls decay to 50% of maximum 12 μm downstream from $O_2^{\bullet-}$ production sites. H_2O_2 concentrations in capillaries decay to 50% of maximum 22 μm (6.0 μm) downstream from $O_2^{\bullet-}$ (H_2O_2) production sites. Near arterioles' (arteries') walls, they decay by 50% within 6.0 μm (4. μm) of H_2O_2 production sites. However, they reach maximal values 50 μm (24 μm) downstream from $O_2^{\bullet-}$ production sites and decrease by 50% over 650 μm (500 μm). Arterial/olar endothelial cells might thus signal over a mm downstream through $O_2^{\bullet-}$ -derived H_2O_2, though this requires nM-sensitive H_2O_2 transduction mechanisms.

Bioenergetics and biological thermodynamics

Session chairs: Karim Fahmy, Helmholtz-Zentrum Dresden-Rossendorf & Daumantas Matulis, Vilnius university

O-118 Invited speaker
Macromolecular Crowding Beyond Hard-Core Repulsions
Daniel Harries[1]
[1] The Hebrew University of Jerusalem, Israel

In contrast to the highly crowded solution inside cells, proteins are almost always studied in dilute aqueous buffer. I will review some of the experimental evidence that crowding affects the equilibrium thermodynamics of protein stability and protein association and discuss the theories employed to explain these observations. Theories based on hard-core interactions predict only crowding-induced entropic stabilization. However, experiment-based efforts conducted under physiologically relevant conditions show that crowding can destabilize proteins and their complexes. Furthermore, quantification of the temperature dependence of crowding effects produced by both large and small cosolutes, including osmolytes, sugars, synthetic polymers, and proteins, reveals enthalpic effects that stabilize or destabilize proteins. The emergent role of chemical interactions between and among the protein, cosolutes, and water augments the known effect of hard-core interactions and explains the thermodynamic footprint of crowding on protein stability and association. Applications to dilute and concentrated solutions will be discussed.

O-119 Invited speaker
Aiming Higher – In Energy and Functional Understanding of Proteins
Dorothee Kern[1]
[1] Brandeis University, USA

Modern structural methods describe macromolecules with atomic resolution, but they primarily capture the lowest energy structures. However, macromolecules transiently populate high-energy substates unseen in these static snapshots. Excursions into these minorly populated states appear pivotal for macromolecular function. First, I will describe a novel NMR method for high-resolution structure determination of minorly populated states by coupling pseudocontact shifts with Carr-Purcell-Meiboom-Gill relaxation dispersion. Applying our methodology to adenylate kinase during catalysis, we determine the previously elusive high-energy structure, which solves a longstanding controversy about conformational interconversions that are rate-limiting for catalysis. In contrast to cryo-EM X-ray crystallography, this NMR method allows characterizing the entire free-energy landscape as it additionally defines the kinetics and thermodynamics of high-energy excursions. Second, we will climb even higher in energy by visualizing the structures of transition-state ensembles (TSEs) that has been stymied due to their fleeting nature. TSE are arguably the most important states as they dictate the speed of biological processes. We determined the transition-state ensemble in Adk by a synergistic approach between high-pressure NMR relaxation during catalysis and MD simulations. Third, we discovered how directed evolution reshapes energy landscapes in enzymes to boost catalysis by nine orders of magnitude relative to the best computationally designed biocatalysts. The underlying molecular mechanisms for directed evolution, despite its success, had been illusive, and the general principles discovered here open the door for large improvements in rational enzyme design. Finally, I describe a new method that expands AlphaFold2's capability to predict these higher energy conformational substates.

O-120 Short talk
Unveiling the Dynamic Nature of the Proton Motive Force in Single Escherichia coli cells: Temporal and Spatial Characterization
Anais Biquet-Bisquert[1,2,3,4], Baptiste Carrio[1,2,3,4], Nathan Meyer[1,2,3,4], Thales Fernandes[1,2,3,4], Manouk Abkarian[1,2,3,4], Axel Magalon[3,5,6,7,8], Ashley L. Nord[1,2,3,4], Francesco Pedaci[1,2,3,4]
[1] Centre de Biologie Structurale, France [2] Université de Montpellier [3] CNRS [4] INSERM [5] Aix Marseille Université [6] Laboratoire de Chimie Bactérienne [7] IMM [8] IM2B

The proton motive force (PMF) is an essential electro-chemical potential established across the membranes of bacteria, mitochondria, and chloroplasts. It serves as a bioenergetic currency of living systems that powers an impressive range of physiological processes. While traditionally considered homeostatic, recent experiments have shown that PMF exhibits rich temporal dynamics at the single-cell level. Rapid membrane depolarizations observed on single cells, related to mechanosensing are indications of a temporally dynamical PMF. Furthermore, polar clustering of respiratory complexes suggests the possibility of spatial heterogeneity of the PMF at the single-cell level. Such spatial heterogeneity has been recently shown along the inner membrane of individual mitochondria. However, the spatial and temporal dynamical behavior of the PMF in bacteria remains poorly characterized and understood. Here, we characterized the dynamics of PMF on E. coli both temporally and spatially. We employed their flagellar motors as local sensors on the membrane and spatio-temporally structured laser excitation on single bacteria expressing the light-driven proton pump proteorhodopsin to produce an excess of PMF. We resolved temporal dynamics on the millisecond time scale and observed an asymmetrical capacitive response of the cell. Using localized perturbations, we found that PMF is rapidly homogenized along the entire cell, faster than proton diffusion can allow. Instead, the electrical response can be explained in terms of electrotonic potential spread, similar to passive neurons and described by cable theory. This implies a global coupling between PMF sources and consumers in the bacterial membrane, excluding a sustained spatial heterogeneity while enabling fast temporal dynamics.

O-121 Short talk
Unravelling the Interplay of DNA Origami and Chaotropic Agents: Anion-Specific Stability and Water-Driven Effects
Daniel Dornbusch[1,2], Christoph Hadlich[1], Marcel Hanke[3], Andre Rossberg[1], Niklas Hansen[3], Guido Grundmeier[3], Satoru Tsushima[1,4], Adrian Keller[3], Karim Fahmy[1]
[1] Helmholtz-Zentrum Dresden-Rossendorf, Germany [2] Dresden International Graduate School for Biomedicine and Bioengineering [3] Paderborn University, Germany [4] Technische Universität Dresden

DNA origami is a bottom-up approach for fabricating complex nanostructures of arbitrary shape with high precision by self-assembly. These nanostructures can be further functionalized with proteins, nanoparticles, or specific DNA structures. One application is the immobilization of individual proteins for single-molecule studies. Chemical denaturants like urea and guanidinium chloride are commonly used to study protein stability. Here, we investigated the stability of DNA origami by comparing atomic force microscopy images of denaturant-exposed DNA origami triangles with circular dichroism spectra. Unexpectedly, our findings show that DNA origami nanostructures undergo anion-specific changes in secondary structure, leading to breaks at the vertices due to anion-dependent properties of the hydration water, which affects the interaction between DNA and guanidinium. The altered water properties are further supported by MD simulations and correlate with anion-dependent heat capacity changes of DNA origami melting derived from temperature-dependent spectral analyses. Our results help to optimize DNA origami as a substrate for denaturant-induced folding dynamics of individual proteins and improve the fundamental understanding of the effects of water structure on DNA. Furthermore, we will present our recent findings on the effect of chemical denaturants on different 2D and 3D origami systems and the role of varying magnesium concentrations. The data provides important insights into the interaction between DNA origami and chaotropic agents and demonstrate their potential for studying protein folding and improving the design of nanostructures for various applications.

O-122 Short talk
Potential regulatory role of succinylation on electron transfer flavoprotein, a key protein in mitochondrial metabolism
Joana V. Ribeiro[1,2], Pedro R. Magalhães[1,2], Hélio Faustino[3], Pedro M. P. Góis[3], Bruno L. Victor[1,2], Cláudio M. Gomes[1,2], Bárbara J. Henriques[1,2]
[1] University of Lisbon, Lisbon, Portugal [2] BioISI – Biosystems and Integrative Sciences Institute, Lisbon, Portugal [3] Universidade de Lisboa, Lisboa, Portugal

Non-enzymatic post-translational acylations have recently been uncovered as a new regulatory node of mitochondria metabolism, and despite of the growing numbers of reports of acylated mitochondrial proteins, the structural and functional impact of these modifications remains to be clarified. Electron transfer flavoprotein (ETF) is a crucial mitochondrial protein that participates in fatty acid, amino acid and choline metabolism, and recently it has been identified as a target of succinylation. To evaluate the effect of succinyl-CoA, an intermediary metabolite of the tricarboxylic acid cycle (TCA), on ETF structure and function we resorted to a multidisciplinary approach, that combines both in vitro and in silico methods. We have successfully succinylated ETF in vitro, and by LC-MS/MS identified 8 modified lysines, two of which are located in the flavin binding pocket. ETF succinylation abolishes protein catalytic activity, without causing major structural changes on the native structure. Through mutagenesis analysis, we confirmed that the presence of negative charges in the cofactor binding site alters the flavin properties and impairs ETF function. And, in silico studies demonstrated that electron transfer in the succinylated protein is less efficient, a likely explanation for the compromised protein function. Furthermore, the importance of this regulation in vivo was established by showing that succinylated ETF is a substrate of sirtuin5, a NAD+-dependent protein deacylase which is a known regulator of acylation levels in mitochondria. Altogether, our results unveil how acylations can mediate the interplay between different metabolic pathways through new regulatory networks.

O-123 Short talk
Elucidation of the mechanism of intracellular temperature variation by high-speed temperature mapping
Kohki Okabe[1], Masaharu Takarada[1], Takashi Funatsu[1]
[1] The University of Tokyo, Japan

Temperature, a key regulator of biochemical reactions, has an impact on many physiological functions of organisms. We have previously developed a method for the visualization of the intracellular temperature distribution using a fluorescent polymer thermometer (FPT). The images of intracellular temperature distribution of mammalian cells revealed the temporal and spatial variation associated with cellular organelles and their functions, shedding light on an intriguing hypothesis: temperature changes inside a cell are fundamentally involved in cell functions. However, tracking intracellular temperature distribution is challenging due to low spatio-temporal resolution, hindering the elucidation of its mechanisms. Here, we developed a method for tracking intracellular temperature mapping using FPT and high-speed fluorescence lifetime imaging microscopy. By heating with infrared laser irradiation, we tracked changes in temperature distribution in cells in a location-specific manner. The temperature relaxation of single cells was significantly slower than in liposomes of comparable size and was influenced by intracellular structures and molecules. In addition, we demonstrated the existence of intracellular temperature relaxation that does not depend solely on heat conduction, which may explain the mechanism of temperature variation in cells that are unresolved by our current understanding. Besides elucidating heat dissipation mechanisms, this method should also contribute to the thermal biology of diverse rapid physiological phenomena.

Protein folding, assembly and disease

Session chairs: Jan Johansson, Karolinska Institutet & Cláudio Gomes

O-124 Invited speaker
The biophysics of polyglutamine aggregation in a multicellular animal model
Tessa Sinnige[1], Vera van Schijndel[1], Myrthe Franken[1], Delano Remkes[1]
[1] Bijvoet Centre for Biomolecular Research, Utrecht University, Netherlands

Protein aggregation is a hallmark of a wide variety of human diseases, including Alzheimer's, Parkinson's and Huntington's diseases. The molecular mechanisms underlying the conversion of soluble proteins into fibrillar aggregates have been mostly investigated in vitro, revealing that this process consists of primary nucleation, fibril elongation, and amplification of the fibril mass by secondary mechanisms. However, it is not yet clear to what extent the same biophysical principles govern protein aggregation in complex biological environments, where protein quality control is tightly regulated. Here, we demonstrate the use of C. elegans as a model system to quantitatively analyse protein aggregation kinetics in a multicellular animal. C. elegans models expressing fluorescently tagged polyglutamine, which is related to Huntington's and other repeat expansion disorders, display robust protein aggregation above the pathogenic length threshold of ca. 40 glutamine residues. We show that aggregation occurs in a concentration-dependent manner through a mechanism of stochastic nucleation in individual cells, followed by rapid aggregate growth. This mechanism applies to both the muscle tissue as well as the nervous system of C. elegans. Altogether, our results highlight that polyglutamine aggregation can be described by the same biophysical principles known from in vitro studies, even in the complex environment of a multicellular organism.

O-125 Invited speaker
Folding of multi-domain proteins - folding intermediates, hidden kinetic traps and cryptic functional features
Stefano Gianni[1]
[1] Sapienza University of Rome, Italy

Although more than 75% of the proteome is composed of multi-domain proteins, current knowledge of protein folding is based primarily on studies of isolated domains. In my presentation I will describe the folding of multi domain complexes with particular emphasis on the hidden functional features of misfiled intermediates

O-126 Short talk
Calmodulin is critical for folding of the Kv7.2 calcium responsive domain as the nascent peptide exits the ribosome
Arantza Muguruza-Montero[1], Sara M-Alicante[1], Ane Metola[2], Eider Nuñez[1], Janire Urrutia[3], Igone Campos-Zarraga[1], Cova Malo[1], Gunnar von Heijne[2], Alvaro Villarroel[1]
[1] Instituto Biofisika (CSIC-UPV/EHU), Spain [2] Stockholm Universitet, Sweden [3] University of the Basque Country, Spain

Protein folding has been historically analysed following in vitro unfolding and refolding approaches, that fall short of replicating co- translational folding events occurring in cells. Thus, little is known about co-translational folding of ion channels and other proteins. We have demonstrated that calmodulin (CaM) is essential for vectorial folding of the Calcium Responsive Domain (CRD) of the Kv7.2 channel. The force exerted on the nascent chain during the early folding events can be assessed using state-of-the-art Force Profile Analysis (FPA). We describe here the force profile of the CRD during translation. We find that CaM is required to generate early folding events of the CRD at critical places: the IQ site, helix TW and helix B. Surprisingly, there is not a hierarchical requirement for folding, which is not significantly affected in permuted variants. This investigation provides new insights into how a critical Kv7.2 channel domain acquires its final functional conformation during co-translational synthesis.

O-127 Short talk
Structural insights into aggregation hotspots on Alzheimer's associated amyloid-β fibrils blocked by the BRICHOS chaperone
Axel Abelein[1], Rakesh Kumar[1], Tanguy Le Marchand[1], Laurène Adam[1], Gefei Chen[1], Jekabs Fridmanis[2], Nina Kronqvist[1], Henrik Biverstål[1], Kristaps Jaudzems[2], Jan Johansson[1], Guido Pintacuda[3]
[1] Karolinska Institutet, Sweden [2] Latvian Institute of Organic Synthesis [3] Universite de Lyon

In Alzheimer's disease (AD), the most prevalent form of dementia, the pathogenesis is closely associated with fibril formation of the amyloid-β peptide (Aβ). The surface of AD-associated amyloid-β peptide (Aβ) fibrils has been suggested to act as a catalyzer for the self-replication of aggregation mass and generation of toxic oligomers. Molecular chaperones, such as the BRICHOS protein domain, were shown to bind to amyloid fibrils and break this autocatalytic cycle and exhibited positive effects in treatment studies of AD mouse models. Here, we identify structural properties of the catalytic site on the fibril surface, which are efficiently sensed by Bri2 BRICHOS. Applying solid-state NMR with 1H-detection and dynamic nuclear polarization combined with electron microscopy methods, we localized the catalytic site on the Aβ42 fibril structure. Remarkably, while only low amounts of BRICHOS binds to Aβ42 fibrils, fibril-catalyzed nucleation processes are effectively prevented suggesting that the identified structurally modulated sites act as "aggregation hotspots" on the Aβ42 fibril surface, which can specifically be blocked by BRICHOS. Hence, these findings provide a general understanding how toxic nucleation events occurring on the surface amyloid fibrils can be targeted by designer molecular chaperones.

O-128 Short talk
The contribution of Short Linear Motifs (SLiMs) to the mechanostability of mechanosensitive proteins
Ismahene Mesbah[1,2,3,4], Felix Rico[1,2,3,5,6], Marcos Sotomayor[7], Bianca Hebermann[1,3,4,5]
[1] Aix Marseille Université, France [2] INSERM, France [3] Turing Center for living systems (CENTURI) [4] Institut de la Biologie du Développement de Marseille IBDM [5] CNRS [6] Laboratoire Adhesion Inflammation LAI [7] Ohio State University

Mechanical forces are involved in a variety of biological processes, including muscle function. Proteins are the main participants in these events as they fold into specific structures to use mechanical force at cellular and molecular level. Studying the relationship between structure and mechanics of proteins is important to understanding protein function. It is known that β-sheet proteins are more mechanically stable than α-helix proteins, but we are still not able to predict unfolding forces from protein folds. Atomic force microscopy (AFM) permits to measure unfolding forces and computational biology allows predicting structural folds and their functions. The main objective of the project is to find correlations between protein folds, sequence, and structural motifs with their mechanical stability. We created MechanoProtein database which stores information about mechanical proteins such as their unfolding pattern and forces. Comparing similar protein folds using distance matrix allows extracting sequence alignments, from which sequence patterns can be observed, based on structural features. These are called Short Linear Motifs (SLiMs). We found [NE][LI][KQR]V, a SLiM located in the last beta sheet involved in the unfolding of titin I27. This SLiM is also found in ExtraCellular domain of Cadherin-23□, InterCellular Adhesion Molecule and Vascular Cellular Molecule1, all mechanosensitive proteins. To understand the role of each amino acid within the motif, we use Steered Molecular Dynamics (SMD) simulations. SMD simulations mimic AFM experiments which enables us to have an atomistic description of the unfolding process. Results show that SLiM amino acids are as important for mechanical stability.

O-129 Short talk
Surveying Chaperone Action on Protein Folding Landscape Inside Living Cells
Sara Ribeiro[1,2], Nirnay Samanta[1,2], David Gnutt[1,2,3], Tom Dixon[4], Zamira Fetahaj[3], Albert Avetisyan[1,2], Gawain McColl[5], Danny Hatters[6], Alex Dickson[4], Simon Ebbinghaus[1,2,3]
[1] TU Braunschweig, Braunschweig, Germany [2] Braunschweig Integrated Centre of Systems Biology (BRICS), Braunschweig, Germany [3] Ruhr University, Bochum, Germany [4] Michigan State University, East Lansing, USA [5] University of Melbourne, Parkville, Australia [6] University of Melbourne, Parkville, Australia

Protein folding inside the cells is assisted by molecular chaperones. These machines bind promiscuously to clients in multiple regions as well as to different conformations, including unfolded, partially folded, or native-like states. The functional consequences of such heterogeneous work on the folding energy landscape resolves around increased native-state stability, faster folding and decreased aggregation. Nevertheless, the majority of this knowledge comes from in vitro studies where chaperone-substrate complex formation is not limited by competitive interactions alongside with the steric constrains. Here, we establish the existing mechanistic action of chaperones inside living cells and sustain the known dependency of such action on the substrate folding stability and rate. We show that the heat shock protein (Hsp70) and heat shock cognate (Hsp70) chaperones shape the folding energy landscape of a set of barnase variants by acting as holdases, preemptive holdases and foldases, hence reinforcing the functional plasticity of these machines on yielding proteome homeostasis.

Biophysics of biological barriers

Session chairs: Jesús Pérez Gil, Complutense University of Madrid & Emma Sparr, Lund University

O-130 Invited speaker
Lung surfactant at the respiratory air-liquid interface: from biophysical properties to translational medicine
Chiara Autilio[1]
[1] "San Carlo" Hospital, Italy

Lung surfactant is a multifunctional and complex mixture of lipids and proteins, which covers the alveolar air-liquid interface and is essential to stabilise mammalian alveoli upon breathing since lung elastance mostly depends on its activity. This versatile surface-active material is synthetised by the Alveolar Type II cells and secreted into the alveolar lining fluid from highly packed membranous organelles, called lamellar bodies. Once out of the cell, surfactant organises itself in a complex network of membranes between the epithelial layer and the air-liquid interface to accomplish its biophysical role of reducing drastically surface tension while also serving as first barrier against pathogens and nanoparticles. This is possible thanks to its precise lipid and protein composition, mainly characterised by disaturated phospholipids, anionic lipids, cholesterol, two hydrophilic proteins, SP-A and SP-D, with immunomodulatory roles and two highly hydrophobic proteins, SP-B and SP-C, involved in lipid adsorption, reorganisation and structural stability under breathing dynamics. There is increasing evidence of how impairment of surfactant composition and its biophysical properties at the air-liquid interface may directly impact on respiratory pathology, and different strategies pursuing its restoration are reaching the medical practice. For instance, delving into the lung surfactant compositional changes at the air-liquid interface under both pathological and physiological conditions is helping to elucidate the etiopathogenesis of different alveolar diseases. On the other hand, the essential role of surfactant as an interfacial barrier can be used to optimize therapeutic carrier actions to deliver inhaled drugs.

O-131 Invited speaker
Uncovering the Nanoscopic Molecular Pathway and Mechanism of Penetration Enhancement by Lipid-Based Nanoparticles in Human Skin
Jonathan Brewer[1], Jes Dreier[1], Irina Iachina[1]
[1]University of Southern Denmark

Understanding the barrier properties of human skin is crucial for skin pathology, drug delivery, and absorption modeling. However, the nanoscopic molecular pathways through human skin, especially for molecules passing the intercellular lipid bilayers in the stratum corneum (SC), are not fully established. This study uses STED and FRET to investigate molecular pathways through the skin's main barrier, the stratum corneum, at a nanoscopic level for lipophilic and water-soluble molecules. The study's findings suggest that the penetration of lipophilic molecules through the SC occurs by traversing the intercellular lipid bilayers that separate the corneocytes in the SC. Conversely, for more water-soluble molecules, the preferred route of penetration involves taking the transcellular route through the corneocytes and intercellular lipid bilayers by interacting with the polar head groups of lipid molecules within the bilayers. Lipid nanoparticles enhance penetration, but the mechanism is unclear. Recent studies found that lipid nanoparticles burst on the skin surface instead of penetrating it unbroken. This study used Laurdan GP analysis to investigate the penetration enhancement of ultra-deformable and ridged liposomes. Results show that ultra-deformable liposomes have a penetration-enhancing effect and cause disordering of stratum corneum lipids, suggesting destabilization of lipid organization as the mechanism behind lipid nanoparticle penetration enhancement.

O-132 Short talk
Peptide coating boosts membrane interactions and antimicrobial effects of photocatalytic titanium dioxide nanoparticles
Lucrezia Caselli[1,2], Elisa Parra-Ortiz[1], Samantha Micciulla[3], Thomas Saerbeck[3], Maximilian Skoda[4], Martin Malmsten[1,2]
[1] University of Copenhagen, Copenhagen, Denmark [2] University of Lund, Lund, Sweden [3] Institut Laue–Langevin, CS 20156, 38042 Grenoble Cedex 9, France [4] Rutherford Appleton Laboratory, Harwell, Oxfordshire, UK

Photocatalytic nanoparticles offer potent antimicrobial effects under illumination by generating reactive oxygen species (ROS), that degrade bacterial membrane components, such as phospholipids and lipopolysaccharides1-4. Such nanoparticles may, however, also degrade human cell membranes and trigger toxicity. To develop nanoparticle-based antimicrobial therapies, selectivity between bacterial and human cells is thus crucial. Given the excellent selectivity of antimicrobial peptides (AMPs) towards bacteria, we hypothesized that these may offer opportunities to "target" coated nanoparticles to bacterial membranes. Investigating this, photocatalytic TiO2 nanoparticles (TiO2NPs) were coated with the cationic antimicrobial peptide LL-37. The interaction of peptide-coated TiO2NPs with model lipid bilayers, mimicking either bacterial or human cell membranes, was investigated with neutron reflectometry, quartz crystal microbalance, fluorescence spectroscopy, and light scattering. The results were correlated to those on antimicrobial effects and cell toxicity. This study showed that the peptide coating promoted membrane binding of TiO2NPs to anionic membranes void of cholesterol (mimicking bacterial membranes). As a result of this, oxidative membrane degradation during UV illumination was promoted, resulting in increased hydration, lipid removal, membrane thinning, and solubilization. Analogously, peptide coating resulted in enhanced antimicrobial effects of TiO2NPs for Gram-negative Escherichia coli. These findings illustrate that peptide coating offers opportunities for increasing the selectivity of photocatalytic nanoparticles, possibly leading to the development of safer antimicrobial therapies.

O-133 Short talk
Exploring the Interactions of Topical Ophthalmologic Drugs with a Tear Film Model through a Hybrid Experimental-
Computational Approach
Lukasz Cwiklik[1]
[1] J. Heyrovsky Institute of Physical Chemistry, Czech Republic

Numerous topical ophthalmologic drugs are delivered with micellar-type formulations, but advanced approach was proposed involving complex oil-in-water nanoemulsions. Our research focuses on advanced drug delivery methods for ophthalmologic drugs, specifically using nonpolar lipid nanodroplets coated in cationic surfactants that create oil-in-water nanoemulsions. We investigate the behavior of these drug carriers using both experimental and computational techniques. To simulate the human tear film in experiments, we developed a unique acellular in vitro system that incorporates tear inflow and evaporation, as well as simulated eyelid-blinking, allowing us to analyze the response of the film model to various topical ophthalmologic drugs. With the computational approach, we employ coarse-grain molecular dynamics simulations to examine the interactions between drug delivery vehicles and an in silico tear film model, providing molecular-level insight into the interactions of drug formulations with tear film components. By combining both methods, we can characterize the behavior of various topical ophthalmologic formulations and use this information to guide the development of future therapies. Our recent study focuses on novel formulations of Latanoprost, a prostaglandin analog drug used to treat glaucoma, an eye disorder that slowly damages the optic nerve and can eventually cause blindness.

O-134 Short talk
Deciphering the role of nuclear envelope lipids in human healthy aging
Maria J. Sarmento[1], Petra Kohutova[2], Filomena A. Carvalho[1], Martin Hof[2], Nuno C. Santos[1]
[1] Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal [2] J. Heyrovský Institute of Physical Chemistry of the Czech Academy of Sciences, Prague, Czech Republic

In human cells, nuclear structure is safeguarded by the nuclear envelope (NE), in particular the nuclear lamina (NL). The NL is mainly composed of lamins that assemble into filaments and bind both heterochromatin and the inner nuclear membrane. During human physiological aging, cell nuclei tend to lose structural and mechanical integrity. However, how this happens is not yet clear. Here, we hypothesize that NE lipid composition changes with age progression, actively altering lamins association with the NE. To explore this idea, we first developed a flow cytometry-based methodology to isolate nuclei from human primary skin fibroblasts of individuals of different age. Nuclei were characterized using image flow cytometry, confocal microscopy, atomic force microscopy (AFM) and transmission electron microscopy (TEM). Preliminary lipidomics analysis of these nuclei revealed that, with aging, PC and PE ether lipid species consistently decrease. Since bulk membrane properties might affect how lamins interact with the NE, we then designed membrane models containing different ether lipids, and characterized them using time-dependent fluorescent shifts (TDFS). Our results show that, in the presence of ether lipids, lipid motility increases while the microenvironment becomes more polar. To understand if these changes are enough to result in altered lamin binding, we are now using a fluorescein-labelled peptide corresponding to the farnesylated C-terminal of prelamin A. Interaction with our age-tuned lipid models is being studied by fluorescence cross-correlation spectroscopy (FCCS). Overall, this will get us closer to understand an additional reason why cells become more prone to disease with age progression.

O-135 Short talk
Jellied vesicles: probing the interactions between cell-derived extracellular vesicles and matrix materials
Nicky Tam[1], Rumiana Dimova[1], Amaia Cipitria[1,2,3]
[1] Max Planck Institute of Colloids and Interfaces, Potsdam, Germany [2] Biodonostia, San Sebastián, Spain [3] IKERBASQUE, Basque Foundation for Science, Bilbao, Spain

Extracellular vesicles (EVs) are membrane-bound structures released by cells that allow for cell-to-cell trafficking of various cargo. They are a prominent research topic for their roles in immune modulation, wound healing, and cancer progression. Despite their ubiquity in biological fluids, it is not understood how the diffusion of EVs is affected by diverse tissue environments. To study how EVs interact with extracellular matrix (ECM) materials, we use epifluorescence microscopy combined with single particle tracking on EVs derived from a breast cancer cell line in reconstituted ECM hydrogels. Preliminary results show that a significant portion of cell-derived EVs become immobilized in type I collagen hydrogels, while similarly-sized synthetic liposomes remain entirely mobile. We also consider the possibility of EVs being involved in the formation of new ECM. Rheology of collagen I gels formed in the presence of EVs and calcium ions shows an accelerated growth phase in gelation kinetics compared to gels formed with EVs or calcium alone. This suggests a possible role for EVs in collagen crosslinking involving calcium-dependent machinery. Confocal reflectance microscopy of gels provides further structural information. Distribution patterns of EVs in tissues and the complexity of extracellular environments suggest an interplay of molecular interactions that control EV diffusion. By better understanding how EVs interact with ECM, we hope to find new ways to control how vesicles and nanoparticles are distributed in tissues, which could lead to improved targeted delivery systems for nanotherapeutics.

Self-organised and biomimetic systems

Session chairs: Björn Högberg, Karolinska Institutet & Hendrik Dietz, Technische Universität München

O-136 Invited speaker
Mapping and targetting membrane protein nanoenvironments
Ana Teixeira [1]
1 Karolinska institutet, Sweden

O-137 Invited speaker
Engineering a synthetic model cell with DNA nanotechnology
Kerstin Göpfrich[2] Kevin Jahnke[1], Yannik Dreher[1], Mai P. Tran[1,2], Tobias Abele[1,2], Tobias Walther[1,2], Maja Illig[1,2]
[1] Max Planck Institute for Medical Research, Germany [2] Heidelberg University, Germany

Can we construct a cell from non-living matter? In search for answers, bottom-up synthetic biology has successfully encapsulated functional sets of biomolecules inside lipid vesicles, yet a "living" synthetic cell remains unattained. Instead of relying exclusively on biological building blocks, the integration of new tools can be a shortcut towards the assembly of active and eventually fully functional synthetic cells. This is especially apparent when considering recent advances in DNA nanotechnology and 3D laser printing. DNA nanotechnology allowed us to engineer our own molecular hardware for synthetic cells. Recently, we engineered functional DNA-based mimics of a cytoskeleton. These cytoskeletons are capable of stimuli-responsive reversible assembly, cargo transport, mechanochemical signal transduction and can deform giant unilamellar lipid vesicles (GUVs) from within. We further demonstrate the division of GUVs based on phase separation or spontaneous curvature increase and osmosis rather than the biological building blocks of a cell's division machinery. We derive a parameter-free analytical model which makes quantitative predictions that we verify experimentally. The osmolarity increase can be triggered by enzymatic reactions or by light-triggered release of caged compounds. Ultimately, by coupling GUV division to their informational content and their function, we aim for a prototype of a synthetic cell capable of evolution.

O-138 Short talk
Notch engagement by Jag1 nanoscale clusters indicates a force-independent mode of activation
Ioanna Smyrlaki[1], Ferenc Fördös[1], Iris Rocamonde Lago[1], Yang Wang[1], Antonio Lentini[1], Vincent Luca[2], Björn Reinius[1], Ana Teixeira[1], Björn Högberg[1]
[1] Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden [2] Department of Drug Discovery, Moffitt Cancer Center, Tampa, FL, USA

The Notch signaling pathway is a highly conserved signaling pathway that plays a critical role in cell differentiation and development. The current model of Notch receptor activation, involves a pulling force-induced conformational change at the negative regulatory region of the receptor, leading to the subsequent recruitment of ADAM metalloproteases and a cleavage cascade that releases the Notch intracellular domain. In this study, we investigated the Notch activation through the formation of soluble, multivalent ligand-receptor complexes. We evaluated the effects of ligand valency on Notch activation by treating neuroepithelial stem-like (lt-NES) cells with spatially defined, molecularly precise ligand nanopatterns on DNA origami nanostructures. Our findings demonstrate that multivalent clusters of the Jag1 ligand can activate Notch receptors in a force-independent manner, even when some Jag1 proteins are replaced by other binders that do not target Notch. These findings reveal a distinct mode of Notch activation where Jag1 ligands are able to activate Notch receptors upon prolonged binding, which may have important implications for our understanding of Notch signaling and the development of soluble Notch agonists.

O-139 Short talk
Influence of Lipid Composition and Peptide Conjugation Strategies on Controlled Release from Liposome-Based Delivery
Systems
Johanna Utterström[1], Alexandra Iversen[1], Hanna M. G. Barriga[2], Margaret N. Holme[2], Robert Selegård[1], Molly M. Stevens[2,3], Daniel Aili[1]
[1] Laboratory of Molecular Materials, Division of Biophysics and Bioengineering, Department of Physics, Chemistry and Biology, Linköping, Sweden [2] Karolinska Institutet, Stockholm, Sweden [3] Imperial College London, London, U.K.

Liposome-based drug delivery systems (DDS) can reduce both the toxicity of encapsulated drugs and improve their pharmacokinetics. The possibilities to modulate liposomal drug release can further enhance the efficacy of liposomal drug formulations. Lipid membrane permeability can be tuned using specifically designed bioresponsive membrane-active peptides. However, achieving explicit control over peptide-lipid interactions is necessary. Here, we present a systematic investigation of how lipid composition and peptide conjugation strategies influence the release of compounds from vesicles, using a de novo designed membrane-active peptide (MAP). We found that conjugating a cysteine-containing MAP to maleimide headgroup functionalized lipids using a maleimide-thiol Michael addition resulted in faster release kinetics than conjugating an identical azide-functionalized MAP using a strain-promoted azide-alkyne cycloaddition reaction. The release kinetics was also heavily influenced by the cholesterol content. Increasing the concentration of cholesterol resulted in faster and more extensive vesicle content release, regardless of the conjugation strategy. Interestingly, peptide conjugation to cholesterol-rich liposomes also triggered lipid phase separation, which likely contributed to the observed increase in lipid membrane permeability. The ability to control lipid membrane permeability through specific and tunable peptide-mediated interactions can facilitate the development of bioresponsive drug delivery systems.

O-140 Short talk
Lipid-water-ion interactions determine cell membrane structure and dynamics
Lukasz Piatkowski[1], Madhurima Chattopadhyay[1], Emilia Krok[1], Hanna Orlikowska-Rzeznik[1], Petra Schwille[2], Henri Franquelim[2]
[1] Poznan University of Technology, Poland [2] Max Planck Institute for Biochemistry, Germany

Self-assembly of biomembranes results from the intricate interactions between the lipids' head groups, water and ions, which altogether modulate membrane architecture, lipid diffusion, and chemical activity. We have recently developed a methodology that allows for precise control of the hydration state of cell membranes and the routine studies of their structure and dynamics. We reveal that biomimetic membranes can survive even in very harsh dehydration conditions. The overall structure of the phase-separated membranes is largely insensitive to the changes in the hydration state. The nanoscale imaging using AFM, however, reveals enhanced mixing of lipids forming different phases, decrease of the hydrophobic mismatch and of the lipid domain line tension. Intriguingly, lipid mobility is drastically affected by dehydration, showing a 6-fold decrease in lateral diffusion. Furthermore, we demonstrate that the cooperativity between water and Na+ ions is crucial to maintain lipid mobility upon the removal of the outer hydration layer of the lipid membrane. We unravel that Na+ ions (and similarly K+ ions) strengthen the water hydration shell around the lipid phosphocholine headgroup and thus prevent its breaking upon removal of bulk water. Interestingly, Ca2+ and Mg2
+ do not show this effect. Altogether we provide a detailed molecular-level picture of ion specific dependence of lipid mobility and membrane hydration properties as well as deeper insights into the fundamental cellular processes that involve local and transient dehydration, such as during cell–cell fusion events.

O-141 Short talk
Tetraspanin 4 mediated migrasome formation mechanism
Raviv Dharan[1], Yuwei Huang, Shahar Goren, Michael M. Kozlov, Li Yu, Raya Sorkin
[1] Tel-Aviv University, Israel

Migrasomes are recently discovered signaling organelles, enriched with tetraspanin proteins. They originate by local swelling of retraction fibers, the cylindrical protrusions of cell membranes that form as a result of cell migration. Recently, evidence emerged showing that migrasomes play essential roles in fundamental cellular processes such transfer of mRNA and proteins, organ morphogenesis, and mitochondria quality control. Previously, it was established that tetraspanin proteins are crucial for migrasome formation by forming macrodomains in the migrasome membrane, while the physical forces driving local swelling of the retraction fibers originate from membrane tension and bending rigidity. Yet, it remained unknown how and in which time sequence these factors are involved in migrasome nucleation, growth, and stabilization, and what are the possible intermediate stages of migrasome biogenesis. Here we reveal that tetraspanin 4 is a membrane curvature sensor, which mediates a two-stage process of migrasome formation. At the first stage, membrane swellings form along retraction fibers. At the second stage, tetraspanin 4 migrates toward and onto these swellings, which grow to migrasomes of several microns in size. This tetraspanin 4 recruitment to the swellings is essential for migrasome growth and stabilization. We demonstrate this mechanism by imaging migrasome generation in live cells and validate it by recreating the conditions leading to migrasome-like vesicle generation in a biomimetic model system. Based on these findings we propose that the major role of tetraspanin proteins is in stabilizing the migrasome structure while the migrasome nucleation and initial growth stages can be driven by membrane mechanical stresses.

Novel methods for cell biophysics

Session chairs: Jonas Tegenfeldt, Lund University & Sarah Köster, Georg-August-Universität Göttingen

O-142 Invited speaker
iSCAT: from detection of very small proteins to characterisation of extracellular vesicles and 3D imaging of cellular nanostrcutures
Vahid Sandoghdar[1]
[1] Max Planck Institute for the Science of Light, Germany

Fundamental limitations of fluorescence image have motivated many groups to develop fluorescence-free methods. Among various contrast mechanisms, scattering offers unique opportunities. About two decades ago, we showed that single gold nanoparticles as small as 5 nm could be detected via interferometric detection of their scattering, coined iSCAT. Since then, it has been shown that unlabeled nano-objects such as viruses and proteins as small as 10kDa can be detected, weighed, counted and tracked. We will discuss the newest achievements of iSCAT in characterizing unlabelled proteins, extracellular vesicles and cellular secretomes. Furthermore, I will present our results on three-dimensional label-free imaging of cellular events such as the endoplasmic reticulum and microtubule dynamics as well as virus diffusion via confocal iSCAT microscopy. If time permits, I also plan to present an efficient method for delivering nanoparticles and small molecules to well-defined positions on a cell.

O-143 Invited speaker
Simultaneous nanorheology and nanothermometry using intracellular diamond quantum sensors
Helena Knowles[1]
[1] University of Cambridge, UK

Next-generation biological sensors and diagnostic tools require high sensitivity and spatial resolution to be able to identify emergent biological behaviour. Correlating multiple interdependent parameters at the nanoscale could be particularly helpful for uncovering details of cellular response to external perturbations. Temperature and viscosity are key parameters of interest that relate to cellular energetics and metabolism, morphological changes, cell division and active transport. Yet, even nanoscale thermometry alone remains a challenge. Diamond nanocrystals containing nitrogen-vacancy colour centres can harness quantum phenomena to perform a variety of sensing tasks such as measuring temperature, viscosity and external magnetic and electric field, at the nanoscale inside live cells. These quantum sensors can operate without suffering from bleaching and are unaffected by any changes in local pH and local refractive index. The sensor is read out through a combination of optical and microwave radiation, which makes its signal robust to fluctuations in background fluorescence. In this talk, I will present our latest results on performing multimodal nanoscale quantum sensing in living cells. We extract simultaneously information about the nanoscale temperature environment, the thermal and stochastic forces acting on the nanodiamond, and the viscoelastic properties of its environment.

O-144 Short talk
Temporal monitoring of size, mass, and morphology of weakly interacting systems
Daniel Midtvedt[1], Erik Olsén[2], Benjamin Midtvedt[1], Fredrik Eklund[2], Karsten Weis[3], Berenice Garcia[1], Fredrik Skärberg[1], Giovanni Volpe[1], Fredrik Höök[2]
[1] Göteborgs Universitet, Sweden [2] Chalmers University of Technology [3] ETH Zürich

Over the past five years, our group has developed tools for characterization of the physical properties of individual nanoparticles using interferometric microscopy. Using deep learning enhanced holographic imaging, we recently demonstrated that size and mass of individual suspended nanoparticles can be quantified with subsecond temporal resolution, with precision rivaling transmission electron microscopy. Taking this approach, we can quantify temporal fluctuations of size, mass, and morphology of individual biomolecular complexes of submicron size, and relate these measurable parameters to the interaction parameters between the constituents of the complexes. In this talk, I will discuss some of our recent results regarding quantification of temporal fluctuations in submicron biomolecular condensates and some possible implications for the formation mechanisms of such condensates, and discuss how quantitative imaging coupled with deep learning powered analysis may propel our understanding of biomolecular condensates.

O-145 Short talk
Novel methodology to measure rotational diffusivity in cells with fluorescence photo-switching
Guillem Marín-Aguilera[1], Andrea Volpato[1], Dirk Ollech[1], Jonatan Alvelid[1], Ilaria Testa[1]
[1] KTH Royal Institute of Technology, Sweden

Rotational diffusivity of biomolecular complexes are commonly probed with time-resolved fluorescent anisotropy (TR-FA), which is limited to rotational diffusion processes that happen within the fluorescent probe's lifetime (1-5 ns), which also limits the method's applicability to small-sized molecules (kDa). Larger biomolecules and their complexes ranging 100kDa-MDa are simply too slow to be probed during the fluorescent lifetime relaxation time window. Recently, Volpato et al. (2022) introduced selective time-resolved anisotropy with reversibly switchable states (STARSS), a novel methodology that extends the window of observation for time-resolved fluorescent anisotropy to arbitrary longer times. Photo-switching is used to select a subset of molecules with annotated orientation, which can be followed over time. Here, we aim to generalize the concept by exploring other light-induced transitions in common fluorescent probes, particularly, the formation of triplet states via inter-system crossing. Screening among different fluorescent probes, we characterize the formation and relaxation kinetics of such dark states as well as identify the best set of environmental conditions to maximize the anisotropy signal. Using fluorescent dyes with efficient dark state formation we observe anisotropy modulations with correlation times of a few μs, which enables bridging the gap between traditional TR-FA and STARSS.

O-146 Short talk
Fast super-resolution single-molecule localization microscopy using exchangeable fluorescent probes
Soohyen Jang[1,2], Kaarjel Narayanasamy[1,3], Alon Saguy[4], Johanna Rahm[1], Julian Kompa [5], Yoav Shechtman[4], Julien Hiblot[5], Kai Johnsson[5], Mike Heilemenn[1,2,3]
[1] Goethe University, Germany [2] IMPRS on Cellular Biophysics [3] Heidelberg University [4] Technion – Israel Institute of Technology [5] Max Planck Institute for Medical Research

Single-molecule localization microscopy (SMLM) is a set of microscopy techniques that overcomes the diffraction limit of light. One SMLM technique in particular, point accumulation in nanoscale topography (PAINT), achieves nanometer resolution by localizing fluorophores that transiently bind to the target molecules. However, PAINT and its related technique, DNA-PAINT, both suffer from slow imaging speeds because fluorophores need to be sparsely distributed in space and time. In addition, the need for labeling targets using antibodies limits most SMLM techniques to fixed cells. To overcome slow imaging speed, various approaches have been studied including designing faster binding probes or improving image analysis tools. One that stands out is the application of neural networks (NNs) in image analysis to predict single-molecule localizations from high-density data which has overlapping signal that cannot be localized using conventional image analysis tools. Here, we present SMLM with short acquisition time by combining the NN DeepSTORM with PAINT fluorophore labels to achieve a constant emitter density over time. Furthermore, using exchangeable HaloTag ligands (xHTLs), dynamics within live cells are accessible with increased temporal resolution than for conventional SMLM.

Time-resolved structural biology

Session chairs: Arwen Pearson, University of Hamburg & Gisela Brändén, University of Gothenburg

O-147 Invited speaker
Microsecond Time-Resolved Cryo-EM
Ulrich Lorenz[1]
[1] École Polytechnique Fédérale de Lausanne, Switzerland

Proteins provide the machinery of life. Yet, our understanding of protein function has remained fundamentally incomplete, since it has largely remained elusive to watch proteins perform their tasks. This requires not only a near-atomic spatial resolution, but also a time resolution of microseconds. My group has recently introduced microsecond time-resolved cryo-EM, a novel technique that enables such fast observations. Our method involves melting a cryo sample with a laser beam to allow dynamics to occur, before rapidly revitrifying it to trap particles in their transient configurations, in which they are subsequently imaged. We have shown that our experiments provide a time resolution of 5 µs or better and have demonstrated that near-atomic resolution reconstructions can be obtained from revitrified cryo samples. The revitrification process leaves the protein structure unchanged. It may however provide a new tool to overcome issues with preferred orientation, which still cause many cryo-EM projects to fail. Finally, I will present microsecond time-resolved pH jump experiment in which we observe the capsid dynamics of CCMV, an icosahedral plant virus.

O-148 Invited speaker
Multiscale time-resolved spectroscopy and serial crystallography for studies of protein dynamics
Henrike Müller-Werkmeister[1]
[1] University of Potsdam, Germany

Time-resolved serial crystallography (TR-SX) is providing detailed structural and dynamic information on protein function. Not only light-sensitive systems can be studied, but the use of photocaged compounds, ideally with ultrafast substrate release and photophysical properties compatible with protein studies, enables detailed investigation of enzymatic reactions. The methods developed for serial crystallography, in particular serial sample delivery approaches, can now be applied also in time-resolved spectroscopy. This allows to perform ultrafast transient absorption or ultrafast IR spectroscopy as true multiscale experiment from femtoseconds onwards. I will review work on the enzyme defluorinase, spectroscopic studies on photocages and the requirements for using them in TR-SX studies and our first results towards integrated time-resolved spectroscopic experiments on protein crystals.

O-149 Short talk
Functional cycle of the human Hsp70 chaperone BiP at atomic resolution
Guillaume Mas[1], Sebastian Hiller[1]
[1] Biozentrum - Universisty of Basel, Switzerland

Hsp70 chaperones are molecular machines essential to all kingdoms of live. Their ATP dependent functional cycle is crucial to their role as central client processing hubs interacting with multiple co-factors. Segments of the functional cycle have been studied by structural and biophysics methods, but the connections between these segments and the interplay with co-chaperones are only poorly understood. Here we show how the superior power of methyl NMR spectroscopy combined with an ATP regeneration system can be harnessed to probe the functional cycle of the human Hsp70 BiP at unprecedented spatial and temporal resolution. Determination of the kinetic reaction rate constants under turnover conditions results in a quantitative description of the underlying non-equilibrium thermodynamic energy landscape. The data resolve that BiP undergoes a unique five-state functional cycle that includes two previously unrecognized high-energy conformations. Analysis of NMR parameters on the background of published structural data resolves for the first time the clockwork mechanism underlying Hsp70 function. One of the novel high-energy conformations retains ADP deep-locked, serving as a timer of the functional cycle, while the second starkly alters ATP-hydrolysis. We also illustrate how our unique setup allows studies of the interplay between diverse co-chaperones at the atomic level by reaction-step-specific enhancement factors and determining state-specific interaction interfaces. The technology sets a paradigm for the time-resolved studies of dynamic networks of molecular machines in general and a template for Hsp70 functional cycles in all kingdoms of life.

O-150 Short talk
Rhodopsin Activation Monitored by Single-Shot IR Spectroscopy
Luiz Schubert[1], Pit Langner[1], Franz Bartl[2], Joachim Heberle[1]
[1] Experimental Molecular Biophysics, Department of Physics, Freie Universität Berlin, Berlin, Germany [2] Biophysical Chemistry Research Group, Humboldt-Universität zu Berlin, Berlin, Germany

Time-resolved IR-spectroscopy is a label-free technique to study structure-function relationships in proteins. The established method of step-scan FTIR spectroscopy yields spectrally broad information (>3000 cm-1) with sufficiently high time resolution (up to ns) but strictly requires reversible processes and stable samples making it only suited for a limited number of systems, such as bacteriorhodopsin. Although setups based on tunable quantum cascade lasers (QCL) are capable of collecting kinetic information with ns time resolution by a single acquisition, the spectral information is limited to a single frequency. Contrary, QCL-based dual comb spectroscopy (DCS) allows for a simultaneous broadband (~60 cm-1) observation of reaction kinetics with µs time resolution. Recently, we used QCL-based IR spectroscopy to study protein conformational and protonation dynamics of the well-characterized membrane protein bacteriorhodopsin, where we discussed the possibility of probing non-repetitive protein dynamics. In this study, we apply these technologies to the study of vertebrate rhodopsin, which irreversibly bleaches after photoactivation. We observe protein conformational changes associated with the formation of the active Meta-II state at room temperature in a time-resolved manner, which is not possible with conventional FTIR methods. This proves QCL-based methods as powerful tools to extend the application of time-resolved mid-IR spectroscopy from cyclic photoreceptors to non-repetitive protein reactions.

O-151 Short talk
Structural and energetic characterizations of the conformational landscapes in ligand gated ion channels using adaptive
sampling and Markov state modeling
Nandan Haloi[1], Rebecca Howard[2], Erik Lindahl[1,2]
[1] KTH Royal Institute of Technology, Sweden [2] Stockholm University

Ligand-gated ion channels (LGICs) are central receptors of electrochemical signaling in cells across evolution. These channels open ion-selective pores through the cell membrane in response to neurotransmitter release and transit to a desensitized/closed state in the presence of neurotransmitters, before resetting to their initial, structurally distinct resting/closed state. A diverse set of allosteric modulators, including neurosteroids, anesthetics, and lipids modulate their functions in myriad different ways, suggesting a complex conformational landscape of these protein functions. Here, we used a molecular dynamics simulations based goal-oriented adaptive sampling method, named fluctuation amplification of specific traits (FAST) in combination with Markov state modeling to characterize the energetics of the opening of the pore and allosteric/cryptic pockets. Though functional evidence of these states is present in the literature, structural characterization with experimental techniques such as cryo-EM was not feasible before, possibly due to the transient nature of these states. Given the high pharmaceutical relevance of LGICs in diseases such as depression and epilepsy, exploring uncharted territories in the conformational landscape of these proteins by computational methods can provide an unprecedented ability to regulate biomolecular function by designing state-dependent drugs.

O-152 Short talk
Timescales of Cell Membrane Fusion Mediated by SARS-CoV2 Spike Protein and its Receptor ACE2
Sebastian Jaksch[1], Rosina Ehmann[2], Joachim Bugert[2], Dominic Hayward[3], Henrich Frielinghaus[3], Olaf Holderer[3], Purushottam Dubey[3], Marie-Sousai Appavou[3], Anna Sokolova[4], Sylvain Prevost[5], Piotr Zolnierczuk[6], Bela Farago[5]
[1] European Spallation Source, Sweden [2] Institut für Mikrobiologie der Bundeswehr [3] Juelich Centre for Neutron Science [4] Australian Neutron and Technology Organisation [5] Institut Laue Langevin [6] Oak Ridge National Laboratory

In this contribution we describe the investigation of the SARS-CoV2 membrane fusion timescale by means of small-angle neutron scattering (SANS) using hydrogen/deuterium contrast variation. After the successful production of virus-like vesicles and human-host-cell-like vesicles we were able to follow the fusion of the respective vesicles in real-time. This was done using deuterated and protonated phospholipids in the vesicles in a neutron-contrast matched solvent. The vesicles were identical apart from either the presence or absence of the SARS-CoV2 spike protein. The human-host-cell-like vesicles were carrying an ACE2 receptor protein in all cases. In case of the absence of the spike protein a fusion over several hours was observed in agreement with literature, with a time constant of 4.5 h. In comparison, there was not time-evolution, but immediate fusion of the vesicles when the spike protein was present. Those two figures, fusion over several hours and fusion below 10 s corresponding to the absence or presence of the spike protein allow an upper-limit estimate for the fusion times of virus-like vesicles with the SARS-CoV2 spike protein of 10 s. This very fast fusion, when compared to the case without spike protein it is a factor of 2500, can also help to explain why infection with SARS-CoV2 can be so effective and fast. Studying spike protein variants using our method may explain differences in transmissibility between SARS-CoV2 strains. In addition, the model developed here can potentially be applied to any enveloped virus.

Supporting Biophysics - into the future!

Session chairs: Göran Karlsson, University of Gothenburg, Sweden

O-153 Short talk
European Research Council – Funding for frontier research in Europe
Janne Salo[1]
[1] European Research Council Executive Agency

The European Research Council (ERC) is a research funding body established by the European Commission in 2007 to fund individual scientists to carry out research at the frontiers of knowledge. The ERC provides attractive, long-term grants to pursue ground-breaking, high-risk/high-gain research in any field. The ERC grants are selected using scientific excellence as the sole criterion. This session provides an overview of all ERC grant schemes and the proposal evaluation process with a focus on the scientific aspects. The session will also be joined by an ERC grant holder who will share their experience from the application process.

O-154 Short talk
Instruct-ERIC: Current and Future Approaches and Challenges
Harald Schwalbe[1]
[1] Goethe University Frankfurt, Germany

New and notable

Session chairs: Robert Gilbert, Oxford University & Maria Sunnerhagen, Linköping University

Arranged in collaboration with EBJ

O-156 Short talk
Ancestral sequence reconstruction exploration of the mitochondrial cristae junction
Luke H. Chao[1,2],
[1] Harvard Medical School, USA, [2] Massachusetts General Hospital, USA

Cristae are characteristic inner membrane invaginations in mitochondria that house the respiratory machinery. Cristae junctions are neck-like membrane structures that connect cristae to the boundary membrane region of the inner-membrane. Cristae integrity is essential for energy production, metabolism, and organelle function. The mitochondrial contact site and cristae organizing system (MICOS) stabilizes cristae junctions through its core protein Mic60. Mic60 is conserved across eukaryotes, with homologues found in alpha-protebacteria, the proposed extant cousin of the mitochondrial progenitor. Mitochondrial cristae are hypothesized to have evolved from alpha-proteobacterial intracytoplasmic membranes. The conserved functional and structural roles for Mic60 in cristae and/or intracytoplasmic membrane biogenesis have remained unknown. Here, we present functional and phylogenetic dissection of Mic60's most conserved domain, the C-terminal mitofilin domain, from alpha-proteobacterial to human and yeast. Using reconstructed and resurrected ancestral sequences dating back ~1.2 billion years in evolutionary time, we demonstrate that Mic60's function in mitochondrial respiration diverged between the last vertebrate common ancestor and modern humans. This suggests that yeast Mic60 reflects a more ancestral respiratory function, whereas vertebrate Mic60 mitofilin domain function diverged and diversified from the common ancestor. Our results provide insight into the evolution of different MICOS complexes in mitochondria, and push the capabilities of ancestral sequence reconstruction to investigate evolutionary relationships.

O-157 Short talk
A model system reveals how different linker histones direct chromatin to liquid-like or fibre-like condensed states
Katherine Stott[1], Matthew Watson[1], Dilyara Sabirova[1], Megan Hardy[1], Ebru Destan[1] [1] University of Cambridge, United Kingdom

In all organisms, metre-length DNA is packaged to a micrometre scale through charge neutralisation by basic polymers. In eukaryotes, the main condensing polymers are the histone proteins and the resulting protein/DNA complex is called chromatin. Chromatin organisation is hierarchical: the basic unit is the nucleosome, arrays of which form "beads on a string" that make up a 10 nm fibre. The second stage of condensation is achieved through binding of a different set of histones, the linker histones, which ultimately dictates DNA accessibility. This stage is more enigmatic and results in condensed states that range from dynamic and liquid-like to the more traditional textbook 30-nm fibres, depending on many factors, including the type of linker histone. The growing appreciation of dynamics in chromatin packaging has paralleled developments by us and others in our "bottom-up" understanding of the linker histone proteins themselves. We have developed an in-vitro model system of linker histone and linker DNA, which although very minimal, displays surprisingly complex behaviour, and is sufficient to model the known states of H1-condensed chromatin: disordered complexes ("open" chromatin), dense liquid-like assemblies (dynamic condensates) and higher-order structures (organised 30-nm fibres). A crucial advantage of such a simple model is that it allows the study of the various states by NMR, CD and scattering methods. Moreover, it allows capture of the thermodynamics underpinning the transitions between states through calorimetry. With these methods we can rationalise the distinct condensing properties of linker histone variants across species that are encoded by their amino acid sequence.

O-158 Short talk
Amyloids of alpha-synuclein catalyze chemical reactions
Pernilla Wittung-Stafshede[1]
[1] Chalmers University of Technology, Sweden

Aggregation of alpha-synuclein into amyloid fibers results in Lewy body deposits and neuronal degeneration in Parkinson's disease patients. As the cell environment is highly crowded, interactions with a range of biomolecules may modulate alpha synuclein amyloid formation and interact with resulting amyloids. I will here describe the surprising observation that alpha-synuclein amyloids act like enzymes: they catalyze biologically relevant chemical reactions. In vitro biochemical experiments with model compounds reveal that the amyloid fibers, but not the monomers, of alpha-synuclein catalyze hydrolysis of ester and phosphoester bonds. When His50 is replaced with Ala in alpha-synuclein, the phosphatase but not the esterase activity is diminished. Truncation of the protein's C-terminus had no effect on amyloid catalytic efficiency. To search for putative metabolite reactions in vivo, we incubated alpha-synuclein amyloids with neuronal cell lysate devoid of proteins. Targeted metabolomics analysis of such samples unraveled distinct changes in several metabolite levels upon amyloid incubation. Catalytic activity of alpha-synuclein amyloids may be a new gain-of-function that plays a role in Parkinson's disease progression. In fact, weak enzymatic activity may be a general feature of most amyloid systems not yet explored.

O-159 Short talk
Massively multiplexed single-molecule fluorescence microscopy
Sebastian Deindl[1], Javier Aguirre Rivera, Guanzhong Mao, Anton Sabantsev, Qinhan Hou, Magnus Lindell
[1] Uppsala University, Sweden

O-160 Short talk
Evolution at play - from primordial circadian oscillators to AI-assisted prediction of conformational substates
Dorothee Kern[1]
[1] Department of Biochemistry and Howard Hughes Medical Institute, Brandeis University, Waltham, MA, USA

Circadian clocks, biochemical oscillators controlled by the day and night cycle on earth, play a central role in many biological processes. I will describe the mechanism underlying the earliest form of such an oscillator comprised of two proteins, KaiC and KaiB, creating an oscillatory phosphorylation of the central protein KaiC that possesses both kinase and phosphatase activity. Using a combination of X-ray crystallography and cryo EM, we find a new dodecameric fold for KaiC, in which two hexamers are held together by a coiled-coil bundle of 12 helices. This interaction is formed by the carboxy-terminal extension of KaiC and serves as an ancient allosteric regulatory moiety that is later superseded by KaiA. A coiled-coil register shift between daytime and night-time conformations is connected to phosphorylation sites through a long-range allosteric network that spans over 140 Å. Our results shed light into the evolution of the more sophisticated circadian rhythms that are independent of the day and night cycle. As dessert, I describe a new method we developed to expands AlphaFold2 's capability by predicting *conformational substates* that are essential for biological function. The key for this breakthrough is using the knowledge of evolution again.

O-161 Short talk
Intuitively extracting structural dynamics from macromolecular disorder
Nicholas Pearce[1], Piet Gros[2]
[1] Linköping University, Sweden [2] University of Utrecht

Displacement parameters (B-factors) play a crucial role in macromolecular structure determination, yet are rarely used for biological interpretation. This is somewhat egregious, since these are the experimental parameters that account for the local flexibility of conformational states. The absence of approaches for extracting flexibility from experimental structures has meant this is largely left to computational approaches such as molecular dynamics. We have recently developed a new approach for extracting molecular, domain, secondary structure, and atomic motions from the disorder in macromolecular structures, which is available as an open-source tool. This method makes both large-scale molecular and small-scale atomic disorder intuitively understandable, and allows a widespread reinterpretation of experimental macromolecular structures. We demonstrate the method by applying it to SARS-CoV-2 structures, where we characterize the flexibility of the binding site of the main protease, and clearly reveal the collective motions of the receptor binding domains of the spike glycoprotein that correspond to transitions from closed to open states. Lastly, we demonstrate how flexibility can link structure to function, through an analysis of the cryo-EM structure of STEAP4, an iron reductase. We find that analysis of experimental disorder points the way to closing the gap between the static structures of crystallography and cryo-electron microscopy, and the dynamic ensembles obtained from molecular dynamics simulations.

Breakthrough methods in molecular biophysics

Session chairs: Isabel Alves & Tomasz Kobiela

O-162 Invited speaker
Tau envelopes regulate access to microtubules
Zdenek Lansky[1]
[1] Institute of Biotechnology, Czech Academy of Sciences, Czech Republic

Tau is an axon specific microtubule-associated protein implicated in a number of pathologies, collectively termed tauopathies. On the microtubule surface tau molecules can cooperatively assemble into a cohesive layer termed envelope. Tau envelopes regulate the action of other microtubule-associated proteins, such as the motility of molecular motors, and protect the microtubule against degradation by microtubule-severing enzymes. I will discuss how is the formation and dynamics of tau envelopes regulated the structure of the underlying microtubule lattice, tubulin post-translational modifications and tau phosphorylation.

O-163 Invited speaker
Label-free Microscopy for Investigation of Biomolecular Interactions at Single Molecule Level
Barbora Spackova[1]
[1] Department of Physics, Chalmers University of Technology, Sweden

Nanofluidic Scattering Microscopy (NSM) is a cutting-edge technique based on dark-field optical microscopy that enables direct observation of individual biomolecules in their native state as they freely diffuse in solution. A significant advantage of NSM lies in its ability to monitor and quantify the behavior of individual biomolecules in real-time, without introducing any perturbations due to labeling, surface attachment, or chemical modification. NSM can also provide continuous measurements of molecular weight, size, and conformation for each imaged molecule, allowing for the determination of individual population distributions in heterogeneous samples, monitoring of dynamic biomolecular changes, and detection of molecular binding and unbinding events. In this talk, we will highlight recent advancements in NSM and its applications for investigating biomolecular interactions. We will demonstrate how single-molecule imaging unveils intricate details of protein interactions in their native state, including assessment of binding affinity and the determination of ligand association and dissociation rate constants. Looking forward, the ongoing progress and utilization of NSM hold the potential to significantly impact our understanding of biological processes and enable new opportunities for therapeutic development.

O-164 Invited speaker
Dynamics of the prepore-to-pore transition of a Tc toxin
Enrica Bordignon[1], Svetlana Kucher[1], Peter Njenga Ng'ang'a[2], Julian Folz[3], Daniel Roderer[2], Ralf Kühnemuth[3], Claus A.M. Seidel[3], Stefan Raunser[2]
[1] University of Geneva, Switzerland [2] Max Planck Institute of Molecular Physiology, Dortmund, Germany [3] Heinrich-Heine University of Düsseldorf, Germany

Tc toxins are 1.7 MDa protein complexes found in insect and human pathogenic bacteria. They consist of a pentameric TcA subunit, and two smaller subunits, TcB and TcC, which encapsulate the toxic enzyme. Tc uses a syringe-like mechanism to penetrate the membrane of the host's cells and translocate a deadly enzyme into the cytosol through an open channel in the so- called pore state. Toxins primarily attach to glycans on host cells, and in the late endosome transform into the pore state at acidic pH, but can also be activated by high pH directly in the midgut of the insects. The prepore and pore states have been structurally studied, however the initiation of the process, the sequence of events to form the pore state and the rate of the steps involved have remained elusive. Here, combining cryo-EM with Electron Paramagnetic Resonance spectroscopy and single molecule fluorescence spectroscopy, we characterize in detail the prepore-to-pore transition pathway induced in vitro by high pH. The kinetics of the formation of the intermediate states could be determined by EPR and fluorescence methods. The EPR analysis showed that liposomes, binding of receptors and site-specific mutagenesis shorten the transition time from prepore to pore by facilitating shell destabilization. The possibility of targeted shell destabilization, even in the absence of receptors, is relevant for the future application of Tc toxins as biomedical devices or biopesticides.

O-165 Short talk
Measuring force- and orientation- dependence of F-actin-binding mechanotransductor proteins with an ultra-fast optical trapping assay
Giulio Bianchi [1,2], Lucia Gardini[1,3], Anatolii V. Kashchuk[1,2], Giulia Senesi[1], Viktoriia Kashchuk[2], Marco Capitanio[1,2]
[1] LENS, European Laboratory for Non-linear Spectroscopy, Italy [2] Dept. of Physics and Astronomy, University of Florence [3] National Institute of Optics – National Research Council

In cells, a vast array of actin-binding proteins regulates the cytoskeleton structure, impinging on cell motility and morphogenesis. In particular, adhesion proteins interact with the actin cytoskeleton in a complex way that is amenable to mechanical stress. Recently, optical tweezers have been applied to study the interaction between adhesion proteins and actin to unveil their mechanoregulation. Such studies, however, face challenges related to the broad time scale of those interactions, ranging from weak sub-ms interactions up to stable interactions on the order of tens of seconds. Moreover, since actin filament orientation in cells plays a critical role, such interactions are usually directionally asymmetric, but it is far from trivial to detect the orientation of actin filaments in optical trapping experiments. Here, we propose an experimental assay to overcome these challenges. The method exploits ultrafast force-clamp spectroscopy, a technique with sub-millisecond and sub-nanometer resolution based on laser tweezers, combined with an experimental arrangement (termed oriented dumbbell) that allow us to suspend an actin filament with known polarity between two trapped microspheres. In the oriented dumbbell, one green-fluorescent bead is functionalized with gelsolin to bind F-actin to the barbed end, while a red-fluorescent bead is functionalized with neutravidin or α-actinin to bind the other end of the actin filament. This new method paves the way to study the mechanoregulation of actin-binding proteins at the single molecule level in an in-vitro reconstituted system that correctly probes the variability in time scale and directionality typical of the in-vivo system.

O-166 Short talk
The role of biosensor-based interaction kinetic analysis for life science research and drug discovery – from fragments to PROTACs
Helena Danielson[1]
[1] Uppsala university, Sweden

Biosensor-based kinetic analysis of molecular interactions has exposed the complexity of biomolecular interaction mechanisms and the importance of association and dissociation kinetics for drug action. It has changed our fundamental understanding of molecular interactions and transformed drug discovery. Surface plasmon resonance (SPR)-based biosensors pioneered this field and are well-established for detection of both weak and strong interactions, as well as all types of biomacromolecules and low molecular weight analytes (fragments). Other types of biosensors have since emerged. They differ in detection principles, technical features, experimental design and methods for data analysis. This influences their sensitivity, sample consumption, throughput, and suitability for a certain project. Biosensor data can be very revealing, often showing that interactions detected by other methods suffer from limited solubility or mechanistic complexities, and that the interactions are more complex or have different characteristics than anticipated. Less recognised is the capability to detect ligand-induced conformational changes. SPR-based biosensors can indicate such dynamic effects while surface acoustic wave (SAW) and second harmonic generation (SHG) based biosensors can both directly sense conformational changes. Grating coupled interferometry (GCI) based biosensors can quantify kinetics for rapid interactions, typically seen for fragments, while switchSENSE biosensors allow direct detection of ternary complex formation. This is expected to be useful for the discovery of PROTACs and other proximity inducing agents. The use of biosensors for detailed analysis of molecular interactions will be illustrated, showing the kinetic and structural dynamic information that can be obtained and new applications in life science and drug discovery projects.

O-167 Short talk
Single particle profiler for measuring content and properties of nano-sized bioparticles
Taras Sych[1], Jan Schlegel[1], Hanna Barriga[2], Miina Ojansivu[2], Leo Hanke[2], Florian Weber[1,4], Beklem Bostancioglu[5], Kariem Ezzat[5], Herbert Stangl[6], Birgit Plochberger[7], Jurga Laurencikiene[2], Samir El Andaloussi[5], Daniel Fürth[9], Molly Stevens[10], Erdinc Sezgin[1]
[1] Karolinska Institutet, Solna, Sweden [2] Karolinska Institutet, Stockholm, Sweden [4] Upper Austria University of Applied Sciences, Austria [5] Karolinska Institutet, Huddinge, Sweden [6] Medical University of Vienna, Vienna, Austria [7] Johannes Kepler University Linz, Linz, Austria [9] Uppsala Universitet, Uppsala, Sweden [10] Imperial College London, London, UK.

It is technically challenging to study content and properties of nanoscale bioparticles such as lipoproteins, lipid nanoparticles, extracellular vesicles (EVs) and viruses in a high-throughput manner. We developed a high-throughput analysis method, called single particle profiler (SPP), based on the fluorescence signal from thousands of individual particles. This method provides single-particle information on particle content, biophysical properties and diffusion of particles, simultaneously. We evaluated the performance of SPP using artificial liposomes of varying lipid compositions and applied this method to measure the RNA encapsulation efficiency of lipid nanoparticles and binding efficiency of different antibodies to SARS-COV-2 virus-like particles. Furthermore, using environmental sensitive lipophilic markers we studied the biophysical heterogeneity of artificial liposomes, assessed the quality of extracellular vesicles and virus-like particles preparations as well as explored lipid organization of lipoproteins in health and disease.

50 Years of Pure and Applied Biophysics in Italy

Session chairs - Alberto Diaspro & Velia Minicozzi

O-168 Invited speaker
Investigating genome plasticity at the nanoscale.
Irene Farabella[1], Guy Nire, Dean Lee[1], Marc-A Marti-Renom[2,3,4,5], Ting-C Wu[1,6]
[1] Harvard Medical School, Boston, Massachusetts, USA [2] Centre for Genomic Regulation, Barcelona, Spain. [3] Universitat Pompeu Fabra, Barcelona, Spain [4] ICREA, Barcelona, Spain [5] CNAG-CRG, Centre for Genomic Regulation, Barcelona Institute of Science and Technology, Barcelona, Spain
[6] Harvard University, Boston, Massachusetts, USA.

Recent advances in image-based 3D genomics techniques have enabled single-cell omics analysis in a spatially resolved manner in intact cells. One such approach is sequential-OligoSTORM (seq-OligoSTORM), which couples the use of Oligopaints probes to achieve super-resolution via single molecule localisation microscopy. Coupling seq-OligoSTORM with our suite of modelling and analysis tools, we visualised and quantified structures ranging in size from tens of kilobases to over one megabase, detailing the variation in chromatin arrangement at the nanoscale in single nuclei. Focusing on chromosomal regions at the megabase scale, we identified chromatin arrangements that can predict whether regions correspond to active (A-type) or inactive (B-type) compartments. This outcome suggests that variation in patterns of chromatin folding may ultimately reflect specific signatures.

O-169 Short talk
Functional characterisation of electron currents in plant vacuoles: direct recordings of cytochrome b561A activity.
Armando Carpaneto[1,2], Antonella Gradogna[1], Laura Lagostena[1], Sara Beltrami[3], Edoardo Tosato[3], Cristiana Picco[1], Joachim Scholz-Starke[1], Francesca Sparla[3], Paolo Trost[3]
[1] Institute of Biophysics - CNR, Genova, Italy [2] Department of Earth, Environment and Life Sciences (DISTAV) - University of Genoa, Genova, Italy [3] Department of Pharmacy and Biotechnology (FaBiT) - University of Bologna, Bologna, Italy

Ascorbate (Asc) is a versatile antioxidant of plant cells, whose redox activity depends on the ratio with its one-electron oxidation product monodehydroascorbate (MDHA). The cytoplasm contains high concentrations of Asc and soluble enzymes that can regenerate Asc from its oxidized forms. The vacuole, the largest compartment in mature cells, also contains Asc, but no soluble Asc-regenerating enzymes. In this work (doi: 10.1111/nph.18823) we show that an electron transporter, which works as a reversible, Asc-dependent transmembrane MDHA oxidoreductase, is localised on the membrane of vacuoles from Arabidopsis mesophyll cells. Electron currents, detected 39 years after the first patch-clamp potassium current recordings in plant cells, were measured on isolated vacuoles and found to depend on the availability of Asc (electron donor) and ferricyanide or MDHA (electron acceptors) on opposite sides of the membrane. Electron currents were mediated by the tonoplast redox protein cytochrome b561 isoform A (CYB561A). The apparent affinities for Asc of the luminal (4.5 mM) and cytoplasmic sites (51 mM) reflected the physiological Asc concentrations in these compartments. In mutant plants with impaired CYB561A expression there were no detectable trans-tonoplast electron currents; under high-light stress, mutants showed strong accumulation of leaf anthocyanins, suggesting a CYB561A redox-modulation on the typical anthocyanin response to excessive illumination. Experimental data could be described by an extension of the Michaelis–Menten equation, derived from a simplified kinetic model, shedding light on the complex relationships among the electron acceptor and electron donor in controlling CYB561A activity.

O-170 Short talk
Establishing model membrane platforms to understand the role of lipids in the regulation of plasma membrane activity
Elena Ferraguzzi[1,2], Carolina Paba[1,2], Pietro Parisse[3], Loredana Casalis[2]
[1] University of Trieste, Italy [2] Elettra Sincrotrone Trieste, Italy [3] CNR-IOM Istituto Officina dei Materiali, Italy

Model systems of eukaryotic cellular membranes as supported lipid bilayers (SLBs) are employed here for mimicking an idealized eukaryotic cellular membrane bearing a certain degree of complexity. The final aim of our work is to establish a biophysical approach to understand the role of different molecular players in the regulation of plasma membrane activity. Our first model systems consist of a SLB with 4 lipid components: DOPC, DSPC, sphingomyelin (SM) at stoichiometric ratio of (35:20:12), and cholesterol at different molar concentrations, namely χ=0.05, 0.25 and 0.33. These lipids phase-separate forming ordered transient phases, called lipid rafts, embedded in gel-like phases. SLBs have been investigated through atomic force microscopy to get insights into the role of cholesterol in the modulation of lipid rafts. We observed that raft domain height decreases as cholesterol concentration increases, while the lateral dimension of lipid rafts increases in size, pointing to higher raft fluidity. To achieve more insights into the fluidity of our systems, we investigated the variation of the surface plasmon resonance peak (SPR) of Citrate@AuNPs adsorbed on liposomes matching the concentration of SLBs. The aggregation of the particles was shown to be promoted by fluid-phase membranes. Our results show a wide fluidity variation as the cholesterol concentration increases, with liposomes composed of χ=0.33 being the softest ones. To increase the complexity of the model, we are challenging our SLB with the reconstitution of transmembrane proteins, specifically, Caveolin 1, forming partially suspended pore-spanning membranes.

O-171 Short talk
Bridging the gap between biophysical and microbiological studies of membrane-active host defense peptides: thermodynamics and kinetics of interaction with live bacterial cells.
Lorenzo Stella[1], Cassandra Troiano[1], Maria Rosa Loffredo[2], Emiliano Franci[1], Bruno Casciaro[2], Chiara Fulci[1], Yoonkyung Park[3], Maria Luisa Mangoni[2], Stefano Gianni[2]
[1] University of Rome Tor Vergata, Italy [2] Sapienza University of Rome [3] Chosun University

Host defense peptides (HDPs) are crucial effectors in innate immunity that usually kill bacteria by perturbing their cellular membranes and are promising drug candidates against antibiotic-resistant microbes. Biophysical studies of HDP interaction with artificial membranes were pivotal in developing models of the mechanisms of pore formation. However, quantitative data on the behavior of HDPs in real cells are scant. We developed spectroscopic assays allowing the thermodynamic and kinetic characterization of peptide interaction with live bacterial cells, showing that a representative HDP (PMAP-23) binds to cell membranes in less than one second, and that millions of molecules accumulate on the surface of a single cells, before pore formation occurs. Bacterial killing then takes place in a few minutes, and peptides translocate to the cell interior, where they associate to cytosolic components. This sequestration of peptide molecules by dead cells can protect the remaining bacteria from HDP activity. Based on cell-binding results, we predicted and observed a specific trend for the cell-density dependence of HDP activity. As a consequence, HDP activity and selectivity depend on the concentrations of target and host cells, and the commonly used activity and selectivity determinations performed at fixed, standardized cell densities, are questionable. In addition, active HDP concentrations are always in the micromolar range, even in the presence of low bacterial counts. Overall, our findings clarified some key aspects of HDP behavior but also led to several new questions, which will be addressed during the presentation.

O-172 Short talk
Extracellular vesicles based technologies for next-generation drug-delivery
Mauro Manno[1], Angela Paterna[1], Estella Rao[1], Giorgia Adamo[2], Sabrina Picciotto[2], Paola Gargano[2], Valeria Longo[2], Noemi Aloi[2], Daniele Romancino[2], Samuele Raccosta[1], Paolo Colombo[2], Antonella Bongiovanni[2]
[1] Institute of Biophysics (IBF), National Research Council (CNR) of Italy, Palermo, 90146, Italy [2] Institute for Biomedical Research and Innovation (IRIB), National Research Council (CNR) of Italy, Palermo, 90146, Italy

Introduction. Extracellular Vesicles (EVs) are nanometer-sized particles secreted from different cell types and operating as cell-cell communicators. Due to their bioavailability and mild immunogenicity, they are promising natural drug delivery systems. Our group has pioneered the renewable production of (nano)algosomes, an EVs subtype derived from a sustainable bio-source, microalgae, and is focused on their validation as nanovehicles with high therapeutical and cosmeceutical potential. Methods. Our methodologies are based on (i) the GMP-compliant bioprocesses for algosome production and isolation; (ii) the control of EVs biophysical/biochemical/biological properties, including their structural and nanomechanical features, their biocompatibility and bioactivity; (iii) the exogenous loading of bioactive compounds by different methods such as sonication, electroporation, extrusion. Results. We have engineered algosomes by membrane labelling and loading of biomolecules, such as chemotherapic drugs, nucleic acids, and proteins; in particular, we showed how EVs may load and mask a recombinant allergen, paving new roads for immunotherapy. Conclusion. We have implemented a disruptive platform for the sustainable and cost-effective production, quality control and engineering of microalgae-derived EVs to overcome the current challenges of drug delivery approaches.

O-173 Short talk
Nuclear cell mechanics
Sajedeh Kerdegari[1], Virginia Bazzurro [1], Alessandra Anna Passeri[3], Chiara Argentati [4], Giuseppe Ciccone [5], Silvia Caponi [6], Maurizio Mattarelli [3], Sabata Martino [4], Massimo Vassalli [5], Alberto Diaspro[1], Claudio Canale[1]
[1] Department of Physics, University of Genova, Italy [3] Department of Physics and Geology, University of Perugia, Perugia, Italy [4] Department of Chemistry, Biology, and Biotechnology, Perugia, Italy [5] James Watt School of Engineering, University of Glasgow, Glasgow, UK [6] Istituto Officina dei Materiali del CNR, (CNR-IOM) unità di Perugia, Italy

The mechanical properties of cells affect important factors of cellular function, including shape, motility, differentiation, and division. Cell mechanics is a novel label-free biomarker indicating cell states and pathological changes. However, cells have a complex structure, and their internal components play a role in the total mechanics; among all, the nucleus is the biggest one, surrounded by the nuclear envelope that provides mechanical support to it. Mutations in the proteins of this envelope can cause a range of illnesses known as laminopathies, including progeria, diabetes, and cancer. Therefore, studying nuclear mechanics is of great importance for comprehending physio-pathological mechanisms and for early diagnosis. It is preferred to examine the cell nucleus near its physiological environment inside the cell, but AFM probe, as a conventional method of measuring stiffness, will unavoidably contact the upper cellular components that may potentially affect the measured nuclear modulus of elasticity. To address this problem, a novel non-contact label-free method, Brillouin Microscopy, which uses light-matter interaction to measure the elasticity, is employed. Subsequently, the nucleus was extracted and studied in isolation by the two techniques once more. Integrating the information of Young's modulus of AFM and longitudinal modulus of BM helps to shed light on the nuclear mechanics and mismatch of the reported results so far. Our findings show that the nucleus exhibits a lower stiffness in an isolated form, which can be attributed to the change in the surrounding microenvironment and loss of physical connections with the cytoskeleton.

O-174 Short talk
Effect of Cell Membrane Tension Dynamics on Piezo1: a combined FluidFM and FLIM-Flipper Study
Tomaso Zambelli[1], Ines Lüchtefeld[1], Elaheh Zare-Eelanjegh[1], Massimo Vassalli
[1] ETH Zurich, Switzerland

The influence of membrane tension on the cell mechanosensitive response is still a challenging open question. Here, we demonstrated the potential of combining FluidFM stimulation with fluorescent calcium and tension imaging (FLIM-Flipper) to investigate the effect of tension dynamics on cellular mechanosensitivity. The FluidFM, a force-controlled pipette, enables to control and tune the pretension during pipette-cell contact, to induce the cell-wide mechanosensitive response in combination with calcium imaging, as well as to quantify the spatial and temporal distribution of membrane tension without disruption in combination with FLIM-Flipper. In contrast to patch-clamp, FluidFM measurements can be performed at physiological temperatures influencing both the mechanical and biochemical components of the cell. The ability to tailor both pressure and force independently allows the investigation of the cross-talk between cytoskeleton and membrane mechanics. In our studies, we observed a localized increase in tension upon stimulation of the cell membrane, potentially activating a limited number of mechanosensitive ion channels that allow for calcium influx from the stimulation site. The method presented here will be of value for the growing research field assessing the spatiotemporal dynamics of cell membrane tension and its role in regulating processes including mechanosensation, cell shape and migration, membrane fusion and fission.

Poster Presentations
– Session 1 –

P-1
Structural characterization of PGRP-LC amyloid fibrils using solid-state NMR
Muhammed Bilal ABDUL SHUKKOOR[1], Sujeesh kizhakkekudi SUKUMARAN[1], Mélanie BERBON[1], Axelle GRÉLARD[1], Alons LENDS[1], Brice KAUFFMANN[2], Estelle MORVAN[2], Birgit HABENSTEIN[1], Virginie COUSTOU[3], Sven SAUPE[3], Antoine LOQUET[1]
[1] IECB, CBMN CNRS, UMR 5248, Université de Bordeaux, France, [2] IECB, CNRS, UAR3033, Université de Bordeaux, France, [3] IBGC, CNRS, Université de Bordeaux, 33077 Bordeaux, France

Many proteins that function as monomers can undergo conformational transitions and self-assemble into supramolecular assemblies to serve different purposes. Amyloid formation is associated with several neurodegenerative diseases and also has been implicated in performing specific biological functions in fungi, mammals, and bacteria. In our study, we use a combination of electron microscopy, atomic force microscopy, X-ray diffraction, and solid-state NMR spectroscopy to investigate and obtain high-resolution structural information on Peptidoglycan Receptor Protein- LC (PGRP-LC) amyloid protein. Programmed cell death (PCD) plays a vital role in the development, homeostasis, and both control and progression of a multitude of diseases. Apoptosis and necroptosis are examples of PCD processes. RIP homotypic interaction motif (RHIM)-like amyloid motifs were recently identified in the Imd pathway controlling anti-bacterial defense pathways in insects. Our goal is to understand the molecular mechanism of the Imd pathway from Drosophila melanogaster via the formation of amyloid structures. We use the recombinant bacterial expression in E. coli to overexpress and purify amyloid motifs. Then, dipolar coupling-based NMR experiments are used to get information about the rigid part of the protein, and J-coupling-based NMR experiments to obtain information on the dynamic part of the proteins. We aim to provide a high-resolution structural characterization of Drosophila amyloid structures formed by the protein PGRP-LC. Our results provide preliminary structural insights into the fibrillar assembly of PGRP-LC amyloid fibrils and also the characterization of the structural evolutionary diversification of amyloid motifs across different kingdoms.

P-2
Ferritin-coated SPIONs as a smart magnetic nanocarrier towards cancer cells
Luisa Affatigato[1], Mariano Licciardi[1], Alessandra Bonamore[2], Alessio Incocciati[2], Alberto Boffi[2], Vito Foderà[3], Marco van de Weert[3], Valeria Militello[1]
[1] University of Palermo, Italy, [2] Sapienza University of Rome, Italy, [3] University of Copenhagen, Denmark

The versatility of the Superparamagnetic Iron Oxide Nanoparticles (SPIONs) allows the production of theranostic and multifunctional devices that can be used for simultaneous drug delivery and imaging. For the first time, ferritin coated SPIONs were investigated as nanotools for site targeted theranostic applications. We decided to use the Humanized Archaeoglobus fulgidus ferritin (HumAfFt) as a coating material for 10 nm SPIONs, in order to produce a new magnetic nanocarrier able to discriminate cancer cells from normal cells and maintain the potential theranostic properties of SPIONs. HumAfFt is an engineered ferritin characterized by the peculiar salt-triggered assembly-disassembly of the hyperthermophile Archaeoglobus fulgidus ferritin and which is successfully endowed with the human H homopolymer recognition sequence by the transferrin receptor, overexpressed in cancer cells in response to the increased demand of iron. The new complex HumAfFt-SPIONs were exhaustively characterized in terms of size, morphology, composition, and cytotoxicity by using different biophysical and biological techniques (DLS, ATR-FTIR, CD, TGA, EDX, AFM and SEM). The imaging properties of the prepared HumAfFt-SPIONs were further evaluated using magnetic resonance imaging (MRI). HumAfFt-SPIONs exhibited the ability to act as efficient contrast agents in conventional MRI, providing a potential nanoplatform for tumor diagnosis.

P-3
The Role of Disaccharides in Stabilising Proteins and Inhibiting Fibril Formation
Kajsa Ahlgren[1], Jan Swenson
[1] Chalmers University of Technology, Sweden

The small globular enzyme, human lysozyme, is associated with hereditary amyloidosis, which is a condition in which normally soluble proteins form insoluble fibrillar structures due to abnormal folding. Therefore, it is essential to study the stability of the protein and which additives may affect both its stability and propensity to form fibrillar structures. Hen egg white lysozyme (HEWL) have similar physiological properties compared to human lysozyme and has therefore been used as model protein. Disaccharides have been shown to possess stabilising and anti-aggregating properties on proteins, however, a deeper understanding as to why, remains unknown. In this paper, DSC was used to analyse the stability of HEWL in both absence and presence of trehalose and sucrose in acidic conditions (pH 2 and pH 3.5). A clear increase of the denaturation temperature, Tden, was observed as either disaccharide was added to the system. Despite the structural similarities of the two disaccharides, surprisingly sucrose increased Tden slightly more than trehalose at both pH 2 and pH 3.5. The structural arrangement of the systems was examined using SAXS. These results showed that a high amount of fibrillar structures could only be found in the sample without sugar at the most acidic condition. Further, that both trehalose and sucrose inhibit fibril formation although sucrose exhibit slightly superior stabilizing and inhibitory properties. These results were further confirmed by AFM. By comparing the two disaccharides stabilising and anti-aggregating effect, a greater understanding of how sucrose and trehalose can be used to prevent fibril formation has been obtained.

P-4
Solution structural, dynamic, and allosteric properties of Ubiquitin-Specific Protease 14
Johannes Salomonsson[1], Björn Wallner[1], Linda Sjöstrand[1], Arvid Eskilsson[1], Dean Derbyshire[1], Padraig D'Arcy[1], Alexandra Ahlner[1], Maria Sunnerhagen[1]
[1] Linköping University, Sweden

The deubiquitinating enzyme ubiquitin-specific protease 14 (USP14) is a dual domain protein that plays a regulatory role in proteasomal degradation. USP14 consists of a conserved USP domain and a ubiquitin-like (Ubl) domain separated by a linker and adopts an extended confirmation in complex with the proteasome. In-depth structural analysis by several crystal structures has helped identify critical regions for USP deubiquitination. While these include several loop structures, such as blocking loops BL1 and BL2, involved in ubiquitin (Ub) binding, the dynamic properties of these essential regions of the USP14 have not yet been described and no structure of full-length USP14, has yet been presented. To increase our understanding of the structural and dynamic properties of USP14 we have studied the domains separate as well as in the full-length protein with a combination of nuclear magnetic resonance (NMR) spectroscopy, small angle X-ray scattering (SAXS), molecular modeling and other biophysical techniques. Our results indicate that the spatial envelope of full-length USP14 is biased towards its proteasome-bound structure, which may have functional implications for proteasome regulation. By NMR, we show that the effects of the mutations in the USP domain designed to affect Ub binding is both local and long-range and involve a closely knit network of residues including the active site region and extending to include the proteasome interaction surface. Based on this analysis, we propose the existence of allosteric networks within USP14 in solution, linking Ub binding with the active site as well as the proteasome interaction surface.

POSTER # 1

Poster Presentations
– Session 1 –

P-5
Ionic Conductivity in Engineered Protein Thin Films
Lisa Almonte[1], Juan David Cortés1[1]
[1] Applied Physics Department & IUMA, University of Alicante, 03690, Alicante, Spain

Protein-based conductive materials are promising for next-generation bioelectronics due to their versatility, biocompatibility, and biodegradability. Protein films composed of engineered consensus tetratricopeptide repeat (CTPR) – proteins with a high content of negatively charged amino acids – have been demonstrated to be ionic conductors, with the dominant mechanism being protonic conduction. Proton transport within proteins is of interest for designing biocompatible energy storage devices or intelligent devices such as biosensors; raising the interest on improving protein film conductivities that can make them suitable for applications. In this work, we present an electrical characterization of thin films (≈ 100-200 nm thickness) prepared using Engineered CTPR protein variants modified by different rates of glutamic acid substitution. From impedance spectroscopy measurements of CTPR films drop-casted on interdigitated electrodes, we find an increase of ionic conductivity with increasing the substitution rate, which suggests an increase in protonic carrier concentration. Additionally, we investigate the impact of NaCl doping on the ionic transport of the films. Finally, we explore the variations of the mechanical properties of the films with the amino acid substitution. Our results suggest that engineered proteins, such as modified CTPR scaffolds, can generate appropriate materials for bioelectronics applications.

P-6
All residues considered: Target recognition and selectivity determinants in sortase enzymes
Jeanine Amacher[1], Isabel Piper[1], D. Alex Johnson[1], Brandon Vogel[1], Sophie Jackson[1], Justin Svendsen[1], Jadon Blount[1], Hanna Kodama[1], Darren Lee[1], Katy Lindblom[1], Jay McCarty[1], John Antos[1]
[1] Western Washington University, USA

Sortases are cysteine transpeptidases that gram-positive bacteria use to covalently attach proteins to their cell wall in order to assemble pili, display virulence factors, or for other uses. The ability to cleave a signal sequence and subsequently attach two peptides via a covalent bond make sortases an attractive tool for protein engineering efforts. Sortase A from Staphylococcus aureus (saSrtA) is the gold standard for in vitro sortase-mediated ligation experiments. SaSrtA is also incredibly selective, recognizing the cell wall sorting signal (CWSS), the pentapeptide sequence LPXTG. This is in contrast to Class A sortases from Streptococcus organisms, which can recognize several amino acids at the P1' (or Gly in LPXTG) position. We previously used natural sequence variation, biochemical assays, and structural analyses of published structures to describe P1' selectivity determinants mediated by loop residues in these enzymes. However, knowledge of the stereochemical details of peptide recognition by Class A sortases remains limited. Therefore, we solved the structures of Streptococcus pyogenes SrtA (spySrtA) bound to LPATA and LPATS peptides. In addition, we synthesized a mimetic of the endogenous Class A sortase ligand, lipid II, and determined a high-resolution crystal structure bound to spySrtA. Our structures reveal a number of specific non-covalent interactions along the peptide-binding cleft, including outside the canonical CWSS sequence. We ran ~1 microsecond molecular dynamics simulations that confirm minor changes in overall flexibility and conformation between the apo and peptide-bound spySrtA states. Overall, we provide a descriptive view of how Class A sortases recognize and interact with cellular targets.

P-7
Structural and biophysical characterization of an alternative IL-37 receptor complex
Julie Andries[1,2], Danielle Clancy[1,2], Savvas Savvides[1,2]
[1] Unit for Structural Biology, Department of Biochemistry and Microbiology, Ghent, Belgium, [2] VIB-UGent Center for Inflammation Research, Ghent, Belgium

Interleukin-37, a member of the IL-1 cytokine family, has been described as an anti- inflammatory cytokine which acts to suppress innate immunity. Previous literature suggested that IL-37 engages IL-18Rα and SIGIRR to exert its anti-inflammatory functions. In addition, the soluble decoy receptor IL-18 binding protein was also proposed as an interaction partner of IL-37. Here, we confirmed an alternative IL-37 receptor complex by applying biophysical techniques such as bio-layer interferometry and multi-angle laser light scattering. In addition, we aim to determine the structure of both IL-37 and IL-36γ in complex with their receptors using single particle cryo-electron microscopy. Cryo-grid screening and optimization of IL-36γ:IL -36R: IL-1RAcP is ongoing to set the stage for 3D structure determination to near-atomic resolution and is expected to yield important insights into the assembly and regulation of this cytokine-receptor complex. Owing to the recent discovery of new interaction partners of IL-37, important challenges remain to characterize the biochemical, biophysical and functional interactions of IL-37 with its binding partners. Collectively, we foresee that these approaches will greatly contribute to a better understanding of IL-37/IL-36 biology, and may open opportunities for the development of novel therapeutics targeting IL-1 cytokines and receptors.

P-8
Electron transfer studies between redox partners provide insight into the CbcL dependent pathway in Geobacter sulfurreducens
Jorge M. A. Antunes[1,2], Carlos A. Salgueiro[1,2], Leonor Morgado[1,2]
[1] Associate Lab i4HB - Institute for Health and Bioeconomy, [2] UCIBIO, NOVA School of Science and Technology, Universidade NOVA de Lisboa, 2819-516 Caparica, Portugal

The respiratory mechanisms of Geobacter bacteria that use extracellular terminal electron acceptors require that electrons generated by the oxidation of organic compounds are transferred to the cell´s exterior (Kumar Int J Energy Res 2015). Multiheme cytochromes located in the inner membrane, periplasm, and outer membrane have already been identified as involved in these pathways, but the redox partners were not identified. The inner membrane associated cytochrome CbcL from Geobacter sulfurreducens is essential for electron transfer to extracellular electron acceptors with a low redox potential, as iron oxides or electrodes poised at –100 mV (Zacharoff Bioelectrochemistry 2016). We recently characterized CbcL and its interaction with the periplasmic triheme cytochrome PpcA (Antunes Front Microbiol 2022). In the search for additional CbcL redox partners, in the present study, NMR spectroscopy and the distinct spectral features of the putative candidates were explored to probe the electron transfer reactions between CbcL and four triheme periplasmic cytochromes homologous to PpcA (PpcB/C/D/E). The results show that despite the different redox properties of the five periplasmic cytochromes, CbcL can transfer electrons to all of them. These results corroborate the promiscuity described for Geobacter's periplasm (Choi J Bacteriol 2022).

Poster Presentations
– Session 1 –

P-9
Nanoscale Confinement Enhances T Cell Activation and Transduction in 3D-Printed Nanoporous Scaffolds
Morteza Aramesh, Lotte Duijn, Cecilia Persson
[1] Uppsala University, Sweden

Immunotherapy is a promising approach to cancer treatment by manipulating the immune system to attack cancer cells. To enhance its effectiveness, researchers are exploring the use of engineered materials that interact with cells at the molecular and cellular level. Recent studies suggest that surface porosity of a material can affect the behavior of cells that come into contact with it. We have developed 3D nanoporous scaffolds using polymer-based materials that are biocompatible and biodegradable. The scaffolds have controlled micro-/nanoporous surface topography, achieved through controlled bubble formation by ultrasonic waves in the bioink prior to printing. The induced porosity in the structure contributes significantly in enhancing T cell activation and induction yields by increasing the surface area available for cell-material interactions as well as confinement of the cell's microvilli. T cells cultured on the nanoporous scaffolds exhibited increased gene expression of key T cell activation markers, improved cytokine production, and enhanced cellular uptake. The enhanced lentiviral uptake by the cells induced by the surface topography of the material leads to better efficiency in CAR-T cell production. This finding suggests that 3D printed nanoporous scaffolds have the potential to be used for developing personalized treatments in vitro, tailoring the structures to an individual's immune system. The use of nanoporous scaffolds in immunotherapy holds great promise for the future of cancer treatment by providing a 3D microenvironment to improve T cell activation and induction yields, allowing for more effective and personalized immunotherapy.

P-10
Mefloquine binding to the eukaryotic ribosome
Anastasia Atamas[1], Olga Kolosova[2], Yury Zgadzay[2], Artem Stetsenko[1], Andrei Rogachev[3], Lasse Jenner[2], Albert Guskov[1], Marat Yusupov[2]
[1] Groningen Biomolecular Sciences and Biotechnology Institute (GBB), University of Groningen, Groningen, 9747 AG, the Netherlands, [2] Department of Integrated Structural Biology, Institute of Genetics and Molecular and Cellular Biology, University of Strasbourg, Illkirch, 67400, France, [3] Moscow Institute of Physics and Technology, Dolgoprudny, Russia

Nonsense mutations (which introduce stop codons) cause a premature termination of translation, resulting in a synthesis of incomplete proteins. In turn these manifests in various diseases, including rare genetic disorders, cancer, metabolic disorders, and neurological disorders. Currently no effective treatment of such diseases is available, and one of the ways being extensively explored is application of aminoglycosides which were shown to induce premature termination codon (PTC) read-through. Unfortunately, it requires excessive drug concentrations, which lead to serious side effects. Recently, it has been demonstrated that certain small molecules, such as CDX5-1 or mefloquine (MFQ) can augment the PTC read-through effect of aminoglycosides. Importantly these compounds have a synergistic effect allowing aminoglycosides to be administered at considerably lower concentrations, which can overcome their harmful toxic effects. To better understand the read-through enhancement effect of MFQ, we aimed to get a structure of the eukaryotic ribosome in complex with aminoglycosides and MFQ. The obtained cryo-EM structure revealed the novel MFQ binding sites which help to understand the mechanism of PTC read-through. This work was supported by Fondation pour la recherche médicale grant #FDT202204014886 and Russian Science Foundation grants 20-65-47031 and 20-64-47041

P-11
Investigating the molecular dynamics of BCL-2 proteins in apoptosis
Jonas Aufdermauer[1], Ana Garcia-Saez
[1] University Cologne, Germany

Lymphoma treatment remains challenging despite the advent of novel targeted drugs, such as the B cell lymphoma 2 (BCL-2) inhibitor venetoclax (VEN). Mechanisms leading to VEN resistance are not well understood. In an effort to understand the underlying principles, we identified 18 de- novo gene alterations in BCL-2 associated with VEN resistance via mouse experiments. We are quantifying and examining alterations in protein function and morphological changes resulting from these mutations. Also, protein translocation plays a key role in the BCL-2 interaction network which we want to correlate to our BCL-2 mutations. We use super-resolution imaging to identify changes in mitochondrial morphology and localization of BCL-2 and perform dose response experiments with VEN to quantify resistance of the individual mutations. Additionally, we do flourescence correlation spectroscopy (FCS) and scanning fluorescence cross-correlation spectroscopy (sFCCS) to measure the binding of the different BCL-2 mutants to tBid and BAX. With all this, we hope to yield a better understanding of VEN resistance, protein homeostasis and apoptotic pathways.

P-12
Underwater adsorption, adhesion and cohesion of protein films on solid surfaces
Daniela Barragàn[1]
[1] University of Calabria (UNICAL), Italy

Bioadhesion is the stable attachment of an organism to a surface due to natural macromolecules. This mechanism has attracted much attention recently due to the ongoing progress in the study of marine bioadhesives. This research work is focused on an adhesive protein from a flatworm of the Macrostomum genus, distributed in the intertidal zone. Its reversible adhesion to solid substrates in wet conditions involves two proteins Mlig-1 and Mlig-2. The aim is to quantify the adsorbed mass, adhesion, and cohesion energy of repeating domains in these proteins, which are expected to enable Macrostomum bioadhesion. This study is conducted using a surface forces apparatus (SFA) and an atomic force microscope (AFM). In parallel, collagen, the most abundant protein in mammalians, was analyzed as a molecular structural model to understand adhesion mechanisms in the aqueous phase. Employing the AFM, the mechanical characteristics of individual tropocollagen type I and type III nanofibrils adsorbed on a mica substrate were characterized in terms of persistence length and intrinsic curvature. This information will provide essential for the design of collagen-based biocompatible and biodegradable adhesives.

Poster Presentations
– Session 1 –

P-13
Proton-coupled electron transfer in cytochrome c oxidase: Observing protonation reactions in real time
Federico Baserga[1], Pit Langner[1], Luiz Schubert[1], Julian Storm[1], Ramona Schlesinger[1], Joachim Heberle[1]
[1] Freie Universität Berlin, Germany

Complex IV of the mitochondrial respiratory chain (cytochrome c oxidase) contributes largely to the proton motive force necessary for ATP synthesis. It has been shown to decrease the formation of reactive oxygen species in mitochondria and it probably plays a role in protecting us from Alzheimer's, Parkinson's, and cardiovascular diseases. Even though the study of cytochrome c oxidase is key to physiology and drug development, the exact molecular mechanisms leading to its proton pumping function are still elusive after more than 50 years of research. The catalytic cycle of the enzyme is often analyzed by splitting up its reductive and oxidative phases. The redox state of its metallic cofactors is intimately connected to structural changes and proton pumping via proton-coupled electron transfer: Some of these changes can be investigated by photolyzing the 2- electron reduced, CO-bound state (R2CO). We monitored the reaction of the nanodisc-reconstituted aa3 oxidase from Rhodobacter sphaeroides upon photolysis of its R2CO state using transient mid-IR spectroscopy. We identify an intermediate in which the cofactors have the same redox state as in the first catalytic intermediate E. We report on structural changes and observe the deprotonation of the glutamic acid moiety of E286 at 13 µs, after formation of the E state. E286 reprotonates when the electronic reaction ends. We propose that the transient reduction of heme a induces the deprotonation of E286, controlling PCET in the transition between E and R2 in the reductive half of the physiological cycle.

P-14
Shikonin depolymerizes microtubules and inhibits the proliferation of oral squamous cell carcinoma: Evidence of shikonin sharing its binding site with taxol in tubulin
Pooja J. Batra[1], Anuradha Kumari[1], Shweta S. Prassanawar[1], Dulal Panda[1,2]
[1] Department of Biosciences and Bioengineering, Indian Institute of Technology Bombay, Mumbai, 400076, India, [2] National Institute of Pharmaceutical Education and Research, SAS Nagar, Punjab, 160062, India

We found that a natural product, shikonin, exerts potent antiproliferative effects on oral squamous cell carcinoma (OSCC). When monitoring the kinetics of shikonin action in OSCC cells, we observed that depolymerization of microtubules preceded ROS generation, DNA damage, and reduction in mitochondrial membrane potential, which leads to cell death. Shikonin inhibited tubulin assembly in the presence of DMSO but not in the presence of taxol. Further, shikonin produced synergistic cytotoxic effects in OSCC cells when combined with vinblastine or combretastatin-A4 (a colchicine site binder). Interestingly, shikonin showed an antagonistic cytotoxic effect in combination with taxol in OSCC cells. Competitive binding experiments with colchicine and vinblastine revealed that shikonin does not bind to either vinblastine or colchicine binding sites in tubulin. Taxol inhibited the binding of shikonin to microtubules. Further, a docking analysis supported the finding of the biochemical experiments that the shikonin binding site in tubulin overlaps with taxol. This study provides evidence that shikonin exerts its antiproliferative effects at least partly by targeting microtubules and that shikonin may have cancer chemotherapeutic potential in combination with microtubule depolymerizing agents.

P-15
Identifying the intracellular response in surface acoustic wave-stimulated wound healing
Kathrin Baumgartner[1,2,3,4], Paul Täufer [2], Manuel Schleicher[1,2], Hanna Engelke[5], Manuel Brugger[2], Christoph Westerhausen[1,2,3,6]
[1] Institute for Theoretical Medicine, Physiology, University of Augsburg, 86159 Augsburg, Germany, [2] Institute of Physics, Experimental Physics I, University of Augsburg, 86159 Augsburg, Germany , [3] Center for NanoScience (CeNS), Ludwig-Maximilians-Universität Munich, 80799 Munich, Germany, [4] Hanns-Seidel-Stiftung e.V., 80636 Munich, Germany, [5] Institute for Pharmaceutical Sciences, Pharmaceutical Cell Biology, University of Graz, 8010 Graz, Austria, [6] Augsburg Center for Innovative Technologies (ACIT), 86159 Augsburg, Germany

Surface Acoustic Waves (SAW) are a powerful tool for biophysical applications like manipulation, sensing and stimulation of cellular properties or functions. We here stimulate epithelial wound healing assays on LiNbO3 substrates with an interdigital transducer (IDT), generating travelling Rayleigh waves with a frequency of f = 160 MHz. By variation of the SAW intensities and pulse parameters, we reveal an optimal SAW stimulation intensity range between P = 4 and 8 mW, accelerating wound healing up to 235 %. The stimulation efficiency increases with increasing pulse width. Following the hypothesis that the cytoskeleton is responsible for faster cell migration in SAW-stimulated wound healing, we show that the orientation of actin fibres is significantly enhanced parallel to the SAW propagation direction. Ultimately, we aim to identify the contribution of the protein YAP and SPPL3 membrane proteases to the stimulation process. Thereby, we hope to unravel the underlying biophysical and biochemical mechanisms in SAW-stimulated wound healing and gain a better understanding of the impact of SAW on cellular processes.

P-16
Synergy between anillin and myosin II promotes the contraction of actin networks
Alexandre Beber[1], Zdenek Lansky, Pavlina Slikova, Roman Podhajecky
[1] BIOCEV, Czech Republic

Actin-based contractility underlies the dynamics of essential actin networks, such as the cytokinetic ring. Myosin is the canonical molecular motor propelling this contractility. Anillin is an actin filament crosslinker essential for cytokinesis in many organisms, which can interact with myosin and which can autonomously generate contractile forces. How anillin affects the functions of myosin is however unknown. Here, we show that anillin promotes the contractile properties of myosin. Using single molecule reconstitution experiments and mathematical modeling we show that anillin and myosin synergistically control the contraction of actin filament bundles, two-dimensional actin networks and actin rings. Based on our simulations and experimental findings we explain this process by the observed direct interaction between anillin and myosin and the diffusible nature of anillin-actin interaction. Our results suggest anillin as a direct regulator of myosin-dependent contractile rearrangements within actin networks, such as in the cell cortex or in the cytokinetic ring.

Poster Presentations
– Session 1 –

P-17
A novel chalcone derivative, IPHBO-36 inhibits FtsZ assembly, promotes GTPase activity of FtsZ and inhibits bacterial proliferation.
Prajakta Bhondwe[1], Neha Sengar[2], Hardik Bodiwala[2], Inder pal Singh[2], Dulal Panda[1,2]
[1] IIT Bombay, India [2] NIPER mohali

Chalcones are known for their antimicrobial activity. We identified (E)-N-(4-(3-(3,4-Dihydroxyphenyl)acryloyl)phenyl)-1-adamantylamide (IPHBO -36), a chalcone derivative as a new antimicrobial agent. We found that, IPHBO-36 induced filamentation in Mycobacterium smegmatis and Bacillus subtilis cells indicating that the compound inhibits cytokinesis. Our molecular docking results showed that this compound binds to the Mycobacterium smegmatis and Mycobacterium tuberculosis FtsZ at the interdomain cleft. We confirmed the binding of IPHBO-36 to FtsZ by monitoring tryptophan fluorescence spectra of Mycobacterium smegmatis FtsZ (MsFtsZ). Using sedimentation and light scattering assays, we found that IPHBO-36 inhibited purified FtsZ polymerization. Further in vitro study of this compound on MsFtsZ showed that it changes the secondary structure of FtsZ thereby affecting its GTPase activity. With the help of the transmission electron microscopy, we could visualize the reduction in the mean length and thickness of the FtsZ polymer upon IPHBO-36 treatment. Interestingly, IPHBO-36 disturbed FtsZ localization in the Bacillus subtilis at the septum without affecting the DNA segregation. Our results suggest that IPHBO-36 compound exerts its antibacterial effect by acting as an anti-FtsZ agent. We propose this compound to be a novel FtsZ inhibitor for the development of antimicrobials.

P-18
Interaction between Human Serum Albumin and miRNA as revealed by fluorescence, FRET, Atomic Force Spectroscopy and computational modelling
Anna Rita Bizzarri[1], Valentina Botti
[1] University of Tuscia, Italy

Human serum albumin (HSA), one of the most abundant globular proteins in plasma, plays a variety of physiological functions and it is characterized by a high capability to non-covalently bind different ligands. The interaction of HSA with the microRNA, (miR-4749 and miR-155), which are short, single-stranded, noncoding oligonucleotides, was investigated by Atomic Force Spectroscopy (AFS), static and time-resolved fluorescence spectroscopy combined with computational methods. A Stern-Volmer analysis of steady-state fluorescence quenching of the lone Trp residue (Trp214) emission revealed that HSA forms a complex with miRNA with an affinity of about 104 M-1. FRET measurements of fluorescence lifetime of the HSA/miRNA complex, carried out in the absence and in the presence of an acceptor chromophore linked to miRNA, led us to determine the distance between the lone Trp of HSA and a dye bound to miRNA. Such a structural parameter, combined with computational docking and binding free energy calculations, allowed us to identify a positively charged HSA pocket able to accommodate the negatively charged miRNA molecule. These results suggest that HSA is a suitable miRNA carrier under physiological conditions for delivering to appropriate targets.

P-19
Structure and dynamics of circular permutated mutants of the sweet protein MNEI
Andrea Bologna[1], Rosanna Lucignano[2], Lars-Oliver Essen[3], Delia Picone[2], Roberta Spadaccini[1]
[1] Department of Science and Technology, University of Sannio, Benevento, Italy, [2] Department of Chemical Sciences, University of Naples Federico II, Naples, Italy, [3] Unit for Structural Biochemistry, Department of Chemistry, Philipps University Marburg, Marburg, Germany

The process of protein aggregation is a fundamental biological phenomenon, often associated with neurodegenerative pathologies, and is characterised by the formation of amorphous aggregates, oligomers, and amyloid fibrils from monomeric proteins that are longitudinally associated with each other and have mutated their conformation from α-helix to β-sheet. Among the many proteins capable of giving rise to aggregation is MNEI, a sweet protein of plant nature that can arise a sweet sensation in humans when eaten and that under extreme conditions of temperature, pH and ionic strength can form amyloid fibrillar aggregates. Using the circular permutation technique, three new mutant proteins were obtained. Combining Circular Dichroism, fluorescence assays with ThT, TEM and molecular dynamics simulations we have mapped proteins stability and dynamics at chemical physical conditions (pH, temperature, ionic strength) close to those used in aggregation experiments on MNEI. Permutated mutants are comparable to MNEI from the point of view of thermal stability and capable of forming amyloid fibrillar aggregates at lower temperatures than the parent protein MNEI, which can be used as nanomaterials for various technological, biological and pharmaceutical applications.

P-20
Detection of succinylation in charge rich proteins using a label free approach: ProCharTS
Simangka Borsaikia[1], Rajaram Swaminathan[1]
[1] Indian Institute of Technology Guwahati, India

Succinylation is type of protein post-Translational modification that regulates various protein function. Dysregulation of succinylation is attributed with different diseases such as cardiovascular disease and cancer. Present techniques that detect Lysine succinylation includes mass spectrometry and chemically labelled probes2. However, detecting succinylation using a label free intrinsic probe is limited. Recently our group discovered a new intrinsic non-aromatic chromophore in a monomeric charged rich protein. The charged residues (Lysine, Arginine, Glutamate and Aspartate) participate in photoinduced electron transfer with the peptide backbone or among themselves. This gives rise to broad UV- Vis electronic absorption ranging from 250-800 nm called as Protein Charge Transfer Spectra (ProCharTS). Herein we established ProCharTS as a comprehensive detection and analysis tool to study succinylated protein. We use α3W and Human Serum albumin (charge rich proteins) and titrate with succinic anhydride to obtain different degrees of succinylation. We further perform CD spectroscopy and Tryptophan fluorescence to analyse the change in the structure of protein post succinylation. Our studies show that change in the charge of amino group of Lysine after succinylation perturbs the ProCharTS profile of both the proteins by altering the pool of charge in the protein.

Poster Presentations
– Session 1 –

P-21
Sustainable hydrogen production through optimizing [FeFe] hydrogenases in microalgae strains
Simone Botticelli, Giovanni La Penna, Velia Minicozzi
[1] University of Rome Tor Vergata, Italy

[FeFe]-hydrogenase is an iron-sulfur protein that catalyzes the chemical reduction of protons into the H2 molecule. HydA1 enzyme of the unicellular alga Chlamydomonas Rheinhardtii is very efficient in reducing protons in water to molecular hydrogen, but it is very sensitive to dioxygen (O2), which irreversibly degrades the enzyme. Molecular engineering of hydrogenase has been proposed as a possible workaround to the problem of O2 sensitivity. With this work, we aim at understanding how the [FeFe] hydrogenases (Hyd) variants expressed by these strains can better sustain hydrogen production in the presence of O2. The project focus on two microalgae strains: CrHydA1 and CvuHyd211/11P. We extensively use AlphaFold to generate 3D structures and GROMACS to perform simulations.Generally, [FeFe]-hydrogenase is made by two domains: H-domain, within H-cluster principal active site, and F-domain, within a variable number of auxiliary active sites. The most characterized [FeFe] hydrogenase is the bacteria Clostridium Pasteurianum [FeFe]-hydrogenase, made of H-cluster and four auxiliary F-clusters. In some microalgae organisms, the evolutionary process guided [FeFe]-hydrogenase protein to lose F-domain and replace it with a smaller disordered domain of variable sequence. We are focusing on the possible effect of the disordered N-terminal segment of Hyd on the accessibility of dioxygen to the H-cluster.

P-22
Effect of O-Glycans on Structure and Friction of the Intrinsically Disordered Synovial Joint Protein Lubricin
Saber Boushehri[1,2,3], Camilo Aponte-Santamaría[2,3], Frauke Gräter[2,3]
[1] Institut für Physikalische Chemie (IPC), Karlsruhe Insitute of Technology (KIT), Germany, [2] Heidelberg Institute for Theoretical Studies (HITS), Germany , [3] Heidelberg University, Germany

Lubricin plays a crucial role in facilitating efficient lubrication and reducing friction within synovial joints. This protein is characterized by extensive glycosylation, an intrinsically disordered region, and two folded domains. The role of glycans on the Lubricin conformation and, more generally, the molecular mechanisms by which Lubricin acts as a lubricant are yet to be fully understood. In this study, we employed atomistic molecular dynamics simulations to investigate the molecular mechanism underlying Lubricin's low friction properties, both under equilibrium and shearing conditions. To accomplish this, we generated a series of intrinsically disordered fragments from the Lubricin sequence, which are representative of its primary physicochemical features such as glycosylation and proline content. Our molecular dynamics simulations have demonstrated that an increase in glycosylation sites results in a rise in negative charge density, leading to elongation of Lubricin's intrinsically disordered fragments. Accordingly, the size of the fragments appears to follow the same charge-dependency as other intrinsically disordered proteins. By utilizing non-equilibrium molecular dynamics simulations, we have also investigated the effect of Lubricin, specifically its glycans, on the shear viscosity of the surrounding medium. Our results show glycans increase viscosity at equimolar concentrations but decrease shear viscosity when present at the same mass density compared to non-glycosylated peptides. These results highlight the local impact of glycans on Lubricin's conformation, providing valuable insight into the molecular factors that contribute to the remarkable low friction properties conferred by this intrinsically disordered protein.

P-23
Spatio-temporal analysis of the impact of antibiotics on Staphylococcus aureus' cell wall biosynthesis and cell division machinery
Dominik Brajtenbach[1], Jan-Samuel Puls[2], Tanja Schneider[2], Fabian Grein[2,3], Ulrich Kubitscheck[1]
[1] Clausius Institute of Physical and Theoretical Chemistry, University of Bonn, Wegelerstr. 12, 53115 Bonn, Germany., [2] Institute for Pharmaceutical Microbiology, University Hospital Bonn, University of Bonn, Meckenheimer Allee 168, 53115 Bonn, Germany., [3] German Center for Infection Research (DZIF), Partner site Bonn-Cologne, 53115 Bonn, Germany.

The study of pathogenic bacteria such as Staphylococcus aureus is of great importance because of their massive clinical impact. We focused on the
S. aureus cell wall synthesis machinery, which provides a target for numerous antibiotics. We investigated the influence of the β-lactam antibiotic oxacillin and glycopeptide antibiotics vancomycin and telavancin on the division cycle of S. aureus, in particular on the location of the fluorescently labelled FtsZ and penicillin binding protein 2 (PBP2) using time-lapse fluorescence microscopy. Using the glycopeptide antibiotics, we were able to demonstrate that cell wall synthesis is essential during cell division and that cell division immediately stops in the presence of these antibiotics, regardless of the exact division phase. Oxacillin inhibits cell division by preventing the recruitment of PBP2 to the site of cell division. The altered dynamics of PBP2 together with the unchanged behavior of other key proteins involved in cell division (FtsW, RodA, MurJ, PBP4) highlight the special role of the major peptidoglycan synthase PBP2 during cell division. Further investigations using β-lactams targeting PBP1, 3 and 4 will be presented. A new semi-automated analysis routine significantly accelerated the speed of the required complex image analysis.

P-24
The electrochemical behavior of methionine residues oxidation
Mihaela-Cristina Bunea[1], Teodor Adrian Enache[1]
[1] National Institute of Materials Physics, 405A Atomistilor, 077125 Magurele, Romania

The oxidation of methionine (Met), acetyl-methionine (Ac-Met) and a series of synthetic peptides, Ac-Gly-Met-Gly, Ac- (Gly)2-Met-(Gly)2 and Ac- (Gly)3-Met-(Gly)3 was investigated by differential pulse voltammetry, in a wide pH range, at glassy carbon electrode and an oxidation mechanism was proposed. The obtained results revealed that the first oxidation step consists in one-electron oxidation of thioether component leading to the formation of a cation radical. Following this, according to the experimental conditions and the side chain position of methionine, the formed radical can be stabilized by the nucleophilic attack of water molecules or by catalytic support of the neighboring carbonyl and amide groups in an intermediate structure, finally converted in methionine sulfoxide which can be further oxidized, at more positive potential, into methionine sulfone. For methionine, at acid media, and for Ac-(Gly)3-Met-(Gly)3, at high alkaline media, the amino and amide groups are active involved in the oxidation process and the electrode reaction take place with proton transfer. Forwards, in order to evaluate the activity of methionine sulfoxide reductase, a biosensor based on Ac-(Gly)3-Met(O)-(Gly)3 and screen printed electrodes modified with zinc oxide nanowires was developed. The regeneration capacity of this biosensors will be evaluated through voltammetric techniques.

Poster Presentations
– Session 1 –

P-25
Small molecules targeting the intrinsically disordered oncoprotein MYC

Alina Castell[1], Wesam Bazzar[1], Lars-Gunnar Larsson[1]
[1] Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Stockholm, Sweden.

Deregulated expression of MYC family oncogenes occurs frequently in human cancer and is often associated with aggressive disease and poor prognosis. MYC is a highly desired target but has been considered "undruggable" as it does not have any druggable pockets or enzymatic activity, and no specific anti-MYC drugs are available in the clinic. In a screening campaign to find potential molecule probes for targeting the interaction between MYC and its essential partner MAX, we identified small molecules named MYCMIs that directly target MYC. Two molecules, MYCMI-6 and MYCMI-7, efficiently inhibit MYC:MAX and MYCN:MAX interactions in cells, bind directly to recombinant MYC, and reduce MYC-driven transcription at single-digit micromolar concentrations, as validated by split Gaussia luciferase-, in situ proximity ligation-, microscale thermophoresis- and surface plasmon resonance (SPR)-assays. The MYCMI molecules inhibit tumor cell growth in a MYC-dependent manner with IC50 concentrations as low as 0.5 μM, while sparing normal cells. The response to the MYCMIs correlates with MYC expression based on data from 60 human tumor cell lines and is abrogated by MYC depletion. Further, they inhibit MYC:MAX interaction, reduce tumor growth and induce massive apoptosis in tumor tissue in MYC-driven xenograft mouse tumor models without causing severe side effects. The MYCMIs are unique molecular tools to specifically target MYC:MAX in vitro and in vivo, and they have good potential for clinical drug development.

P-26
Hydrogen Bond Fingerprints: Revealing the Complexities of Protein Function

Ebru Çetin[1], Melike Berksoz[1], Asli Yenenler[2], Canan Atilgan[1], Ali Rana Atilgan[1]
[1] Sabanci University, [2] İstinye University, Turkey

Mirsky and Pauling were pioneers in the study of protein structures, and their work included the first comprehensive description of the native state of a protein. Since then, researchers have extensively studied the role of hydrogen bonding in protein structure, folding, and function. Initially recognized as critical determinants of the native state, hydrogen bonds have been found to play additional roles in protein function. In this study, we employed a hydrogen bond tracking algorithm utilizing the VMD program Timeline plugin. By setting the distance criteria at 3.0 Å and the donor-acceptor-hydrogen angle at 20°, we tracked instantaneous occurrences of hydrogen bonds. Our algorithm merges multiple hydrogen bonds that occur between any pair of atoms of two residues at a time point into a single occurrence, i.e., if there are multiple hydrogen bonds between a given pair of residues, they only contribute to the occupancy once. We then calculated the differences in hydrogen bond occupancies and conducted a frequency distribution analysis to understand the distribution of changes. Using an appropriate threshold, we determined the fingerprints of the changes in hydrogen bond occupancies for three different systems: trimethoprim-resistant dihydrofolate reductase to explain evolutionary fitness6, a biosensor consisting of GFP-bound ferric binding protein to understand its functioning, and differences in mutations of MeCP2 protein related to Rett syndrome. Our study provides a better understanding of the complex roles that hydrogen bonds play in protein function and how they can be tracked and analyzed to gain insights into protein dynamics and function.

P-27
SITE-SPECIFIC RAMAN SPECTROSCOPY AND THIOL CONJUGATION WITH A NOVEL GENETICALLY ENCODED NON- CANONICAL AMINO ACID

ADITI CHATTERJEE[1,2], Gustavo Vives Fuertes[1], Bohdan Schneider[1], Aditya S. Chaudhari[1,2], Spyridon Kaziannis[3], Petra Čubáková[3], Miroslav Kloz[3], Atripan Mukherjee[3], Jan Štursa[1], Lukas Werner[1]
[1] Institute of Biotechnology, Cech Academy of Science, Czech Republic, [2] Charles University, Czech Republic, [3] Eli Beamlines, The Extreme Light Infrastructure ERIC, Czech Republic

Recent advances in genetic code expansion technology have enabled the co-translational incorporation of non-canonical amino acids (ncAAs) into proteins for many applications e.g. as spectroscopic probes. Time-resolved vibrational spectroscopy can reveal the temporal evolution of protein conformational changes upon light absorption. In this field of spectroscopy, ncAA can be utilized as “transparent window” reporters to alleviate the congestion typically found in infrared and Raman spectra of proteins. However, suitable Raman-active tags are scarce. We hereby show the genetic encoding of a new ncAA, diacetylene-phenylalanine (DAF), in E.coli. DAF features a conjugated diyne moiety (C□C-C□C) that acts as a strong and solvatochromic Raman probe. We incorporated DAF in several residue positions of the bacterial blue-light photoreceptor EL222 to follow signal propagation and fold-switching upon light activation by steady-state and time-resolved femtosecond-stimulated Raman spectroscopy. On the other hand, we found that the diyne readily reacts with sulfhydryls to yield stable conjugates. We used such a property to conjugate SH-containing dyes to EL222-methionine151DAF and calculate the protein’s diffusion coefficient by fluorescence correlation spectroscopy. Taken together, we suggest that DAF is a dual-purpose ncAA, suitable as both a Raman reporter of protein microenvironments and a chemical handle for the attachment of thiols.

P-28
The human small heat shock protein HSPB8 inhibits protein aggregation without affecting the native folding process

Dhawal Choudhary[1,2,3], Laura Mediani[4], Mario Avellaneda[1], Simon Alberti[5], Edgar Boczek[5], Alessandro Mossa[2,6], Serena Carra[4], Sander Tans[1], Ciro Cecconi[3]
[1] AMOLF, Netherlands [2] Center S3, CNR Institute Nanoscience, [3] Department of Physics, Informatics and Mathematics, University of Modena and Reggio Emilia, [4] Department of Biomedical, Metabolic and Neural Sciences, and Centre for Neuroscience and Neurotechnology, University of Modena and Reggio Emilia, [5] Max Planck Institute of Molecular Cell Biology and Genetics, [6] INFN Firenze

Poster Presentations
– Session 1 –

P-29
Identification of small molecules as potential correctors for defective apolipoprotein A-I structure and function
Christina Gkolfinopoulou[1], Angeliki Bourtsala[1], Daphne Georgiadou[1], Anastasia-Georgia Dedemadi[1], Angeliki Chroni[1]
[1] National Center for Scientific Research Demokritos, Greece

Apolipoprotein A-I (apoA-I), is the major protein component of high-density lipoprotein (HDL) and responsible for many of HDL atheroprotective properties. The L178P natural-occurring mutation was shown to induce major defects in structural integrity and functions of apoA-I that may underlie the increased cardiovascular risk observed in carriers of the mutation. Here, a library of marketed drugs (~1000 compounds) was screened against apoA-I[L178P] to identify molecules that can prevent mutant apoA-I from adopting its pathological conformation. Screening was performed by the thermal stability shift assay in the presence of fluorescent dye SYPRO Orange. As an orthogonal assay the monitoring of the change of fluorescence intensity of ANS upon its binding on hydrophobic sites on apoA-I was used. Screening analyses identified four potential structure correctors. Subsequent biophysical analyses (measurement of α-helical content and thermal unfolding by CD spectroscopy) narrowed the potential structure correctors to two. Functional analyses showed that these two compounds can restore the defective capacity of apoA-I[L178P] to promote cholesterol removal from macrophages. Overall, our findings indicate that small molecules can correct defective apoA-I structure and function and may lead to novel therapeutic approaches for apoA-I-related dyslipidemias and increased cardiovascular risk.

P-30
Mechanical stimulation of organotypic cell systems using 3D microstructures printed by a two-photon laser
Federico Colombo[1], Federico Colombo[1], Mohammadreza Taale[1], Fereydoon Taheri[1], Maria Villiou[1], Teresa Debatin[1], Gent Dulatahu[1], Philipp Kollenz[1], Malin Schmidt[1], Christine Selhuber-Unkel[1]
[1] Institute for Molecular Systems Engineering and Advanced Materials (IMSEAM), Ruprecht- Karls-Universität Heidelberg, Im Neuenheimer Feld 225, 69120 Heidelberg, Germany.

P-31
Acoustic wave induced stroboscopic optical elastography of adherent cells
Thomas Combriat[1,2,3], Petter Angel Olsen[1,4], Silja Borring Låstad[3], Anders Malthe-Sørenssen[2,3], Stefan Krauss[1,4], Dag Kristian Dysthe[3]
[1] University of Oslo, Hybrid Technology Hub, Institute of Basic Medical Sciences P.O. Box 1110 Blindern, 0317 OSLO, Norway, [2] University of Oslo, Center for Computing in Science Education, P.O. Box 1048 Blindern, 0316 Oslo, Norway, [3] University of Oslo, Department of Physics, Njord Centre, P.O. Box 1048 Blindern, 0316 Oslo, Norway, [4] Oslo University Hospital, Department of Immunology and Transfusion Medicine, P.O. Box 4950, Nydalen, 0424 Oslo, Norway

The stiffness of adherent cells relates to many biological functions like motility and metastasis in carcinoma cells. Many high throughput (HT) techniques for mechanotyping suspended cells exist, but there is no firm correlation with adherent state stiffness. Thus, there is a need for HT measurement of adherent, not suspended cell stiffness. We present a novel, HT technique using an acoustic transducer, stroboscopic fast imaging and homodyne detection to recover the mechanical properties of living adherent cells in a petri dish on an ordinary inverted microscope. The technique can be combined with standard fluorescence imaging to correlate biological and mechanical information. The technique is tested by mechanotyping co-cultures of different cell types. The entire field of view, which comprises on the order of 70-80 cells, is probed simultaneously at sub-cellular resolution yielding high statistical significance from a single experiment of 1s duration.

P-32
MEMBRANE-BASED ISOTROPIC CELL STRETCHER FOR MECHANOBIOLOGICAL STUDIES
Martina Conti[1], Giorgia Demontis[2], Simone Dal Zilio[1], Laura Andolfi[1]
[1] Istituto Officina dei Materiali-Consiglio Nazionale delle Ricerche (IOM-CNR), Basovizza, 34149, Italy , [2] Department of Physics, University of Trieste, 34100 Trieste, Italy

Cells sense various mechanical signals from the microenvironment and respond to them via specific mechanotransduction cascades. Mechanical and physical forces have been shown to play an important role in regulating physiological and pathological cell behavior. To study the mechanoresponse of single cells or cell monolayers, we developed a cell stretcher system that combines elastic polydimethylsiloxane (PDMS) membranes, whose stiffness and topography can be modulated, with a microfluidic circuit connected to a syringe pump that inflates the membrane and generates a local isotropic stress at its center. The cell stretcher can be integrated to an optical microscope to monitor its z-displacement and perform a variety of optical assays, such as live calcium imaging. As a preliminary result, by using our system, we observed both morphological and calcium handling changes happening within MDA-MB-231 cells when subjected to such mechanical stress. Therefore, this device represents a useful tool to study how cells respond to isotropic mechanical stress and may be useful to understand the involvement of mechanosensitive channels in the response of cells to this type of mechanical stimulus.

Poster Presentations
– Session 1 –

P-33
Studying the incorporation of polyA cofactor into Tau fibrils using total internal reflection tip-Enhanced Raman spectroscopy

Gary Sean Cooney[1], David Talaga[1], Vicky Ury-Thiery[2], Yann Fichou[2], Yuhan Huang[1], Sophie Lecomte[2], Sébastien Bonhommeau[1]
[1] Institute of Molecular Science (ISM), 33400 Talence, France, [2] Institute of Chemistry & Biology of Membranes & Nano-objects (CBMN), 33600 Pessac, France

The microtubule associated protein Tau is an intrinsically disordered protein associated with several tauopathies such as Alzheimer's disease. The formation of pathogenic tau fibrils is still poorly understood with little known about the exact nature of incorporation of negatively charged cofactors such as heparin, RNA, or lipids. Tip-enhanced Raman spectroscopy (TERS) combines atomic force microscopy (AFM) with surface- enhanced Raman scattering (SERS) which allows for the nanoscale chemical imaging of non-resonant biomolecules. Fibrils formed from the disease-associated mutation P301L of the full-length Tau with poly-Adenine cofactor were studied using AFM-TERS in total-internal reflection geometry. TERS mapping was performed under 532 nm laser excitation using Ag-coated AFM silicon tips. Poly-Adenine incorporation into the fibril structure was observed by correlation of the protein Amide I bands with the characteristic ring breathing mode of Adenine.

P-34
Functionally important C-terminus of small GTPase Ran: exploring its nucleotide-specific conformational surface

Janka Czigleczki[1], Pedro Tulio de Resende Lara[2], Balint Dudas[1,3,4], Hyunbum Jang[5], David Perahia[4], Ruth Nussinov[5,6], Erika Balog[1]
[1] Department of Biophysics and Radiation Biology, Semmelweis University, Budapest, Hungary, [2] Department of Medical Genetics and Genomic Medicine, School of Medical Sciences, University of Campinas—UNICAMP, Campinas, Brazil , [3] Inserm U1268 MCTR, CiTCoM UMR 8038 CNRS— Université Paris Cité, Paris, France, [4] Laboratoire et Biologie et Pharmacologie Appliquée, Ecole Normale Supérieure Paris-Saclay, Gif-sur-Yvette, France, [5] Computational Structural Biology Section, Frederick National Laboratory for Cancer Research in the Cancer Innovation Laboratory, National Cancer Institute, Frederick, MD, United States, [6] Department of Human Molecular Genetics and Biochemistry, Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel

As a member of the Ras superfamily of small GTPases, Ran (Ras-related Nuclear protein) is the main regulator of the nucleo-cytoplasmic transport through the nuclear core complex. It functions as a molecular switch cycling between the GDP-bound inactive or "off" and GTP-bound active or "on" state. Since deregulation of Ran is linked to numerous cancers from the stage of cancer initiation to metastasis, understanding the complexity of its interaction, especially the regulatory mechanism, is critical for drug discovery. Ran consists of a globular (G) domain and a C-terminal region, which is bound to the G-domain in the inactive, GDP-bound states. The crystal structures of the GTP-bound active form complexed with Ran binding proteins (RanBP) show that the C-terminus undergoes a large conformational change, embracing Ran binding domains (RanBD), whereas in the crystal structures of macromolecular complexes not containing RanBDs the structure of the C-terminal segment remains unresolved, indicating its large conformational flexibility. This movement could not have been followed either by experimental or simulation methods. Here, by using molecular dynamics (MD) and MDeNM (Molecular Dynamics with excited Normal Modes) simulation methods, we present how rigid the C- terminal region is in the inactive RanGDP form and for the first time in the literature, we were able to follow its conformational flexibility in the GTP-bound form. This conformational mapping allows us to envisage how the C-terminus can embrace RanBDs during the function of Ran.

P-35
Spatiotemporal regulation of p53 condensates and their functional implications

Debalina Datta[1], Ambuja Navalkar[1], Ajoy Paul[1], Arunima Arunima Sakunthala[1], Komal Patel[1], Shalaka Masurkar[1], Laxmikant Gadhe[1], Manisha Poudyal[1], Shinjinee Dasgupta[1], Jyoti Devi[1], Satyaprakash Pandey[1], Kundan Sengupta[2], Samir K Maji[1]
[1] Indian Institute of Technology Bombay, India [2] Indian Institute of Science Education and Research, Pune

Transcription factor p53, regarded as the 'guardian of the genome,' is a tumor suppressor protein associated with several interacting macromolecules inside the cell. The protein comprises five major functional domains, consisting of multiple intrinsically disordered regions and a low-complexity region. Here, we show that p53 phase separates to form dynamic liquid-like condensates in vitro and in mammalian cells. Our data show a clear spatiotemporal regulation of these condensates inside cells and altered physical states with time. The liquid-to-arrested state transition of these condensates occurs in a nucleo-cytoplasmic localization-dependent manner. Nuclear condensates show a more liquid-like nature than cytoplasmic condensates. The state of these condensates is altered due to multiple genetic as well as environmental factors. Hotspot mutants of p53 (which are prevalent in cancer), the addition of stress, and the stabilization of p53 independently modulate the liquid-to-solid transition dynamics of these condensates. The formation of these condensates is also highly regulated by DNA and RNA in the microenvironment. This study provides us with mechanistic insights into the modus operandi of p53 as a transcription factor and how it is involved in regulating cell fate.

P-36
APLF and lncRNA NIHCOLE rejoin DNA-ends in the presence of the Ku heterodimer

Sara De Bragança[1], Clara Aicart-Ramos[1], Raquel Arribas-Bosacoma[2], Angel Rivera-Calzada[3], Juan Pablo Unfried[4,5], Laura Prats-Mari[5], Puri Fortes[5,6,7], Oscar Llorca[3], Fernando Moreno-Herrero[1]
[1] Spanish National Center for Biotechnology (CNB), Spanish National Research Council (CSIC), Spain, [2] University of Sussex, UK, [3] Spanish National Cancer Research Center (CNIO), Spain, [4], Weizmann Institute of Science, Rehovot, Israel, [5] University of Navarra (UNAV), Spain, [6] Navarra Institute for Health Research (IdiSNA), Spain, [7] Liver and Digestive Diseases Networking Biomedical Research Centre (CIBERehd), Spain

Most DNA double-strand breaks (DSBs) are repaired via the non-homologous end joining (NHEJ) pathway, which is inherently complex and involves many factors. The rejoining of the DNA broken ends is critical for the DSB correct repair; however, this step is difficult to assess with bulk assays. Using single-molecule technique Magnetic Tweezers we mimic individual DSBs and study the contributions of the many different core and accessory factors to the DNA-ends bridging. In a recent work, we report the first evidence that the Ku heterodimer (Ku70-Ku80) and the intrinsically disordered protein APLF are sufficient to bridge DNA ends. Moreover, the synaptic complex mediated by Ku70-Ku80 and APLF can resist pulling forces of 2 pN for several minutes. APLF has been described to increase DSB ligation efficiency by acting as a scaffolding protein recruiting XRCC4 and XLF to the DSB. In this new context of high DNA-end mobility and resistance to force, we propose an additional scouting role for APLF, probing for interactions within a short range, and securing the synaptic complex while reducing the DNA-end mobility. The intriguing role of lncRNAs as regulatory factors in NHEJ was also addressed. The lncRNA NIHCOLE, induced in hepatocellular carcinoma, enhances the ligation efficiency of DSB by the NHEJ pathway. Our results show that NIHCOLE, specifically a small domain named SM3, interacts with Ku70-Ku80 and fortifies the synaptic protein complex. We propose a model where Ku70-Ku80 can simultaneously bind DNA and structured RNAs to promote the stable joining of DNA ends.

Poster Presentations
– Session 1 –

P-37
Design Of class-G Monoclonal Antibodies for the treatment of cutaneous T-cell lymphoma
Alfredo De Lauro[1], Raffaele Passannanti[1], Fabio Polticelli[1]
[1] University of Roma Tre, Italy

One of the most common cutaneous T-cell lymphomas is Sézary syndrome, characterised by a median overall survival of only 2.4 years, which results from the clonal expansion of CD4+ T helper cells responding to chronic antigen stimulation. The aim of this work was to design therapeutic antibodies directed against the T-cell receptor (TCR) of Sézary syndrome patients by means of computational methods. Since lymphomas arise from neoplastic transformation of a single lymphocyte, the TCR uniquely distinguishes tumor cells from healthy ones. Starting from this point, the design of antibodies capable of binding the variable region of neoplastic TCRs is a potential therapeutic solution. To this aim, a computational pipeline for the design of therapeutic antibodies was developed. It is divided into 3 steps: in-silico modelling of the target protein; modelling of the antibody-antigen complex by a docking procedure; in-silico affinity maturation of the designed antibody to optimize the binding energy of the complex. Alphafold2.2 software was used for the modelling phase and two protocols from the RosettaSuite were used for the following steps: SnugDock for docking and RosettaAntibodyDesign for antibody affinity maturation. Experimental validation of the protocol is currently ongoing.

P-38
Surface driven nucleation regulates spontaneous Insulin amyloid self-assembly within sub-microliter compartments.
Giuseppe De Luca[1], Giuseppe Sancataldo[2], Valeria Vetri[2]
[1] University of Palermo, Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, Italy [2] University of Palermo, Department of Physics and Chemistry - Emilio Segrè

Here we present an experimental study on the supramolecular association of Human Insulin (HI) within sub-microliter aqueous compartments casted on a glass coverslip, eventually leading to the formation of spherical aggregates at the water glass interface. By means of quantitative fluorescence microscopy techniques, it is possible to image the formation of these aggregates in real-time, allowing for the acquisition of aggregation kinetics at pixel-size spatial resolution. Moreover, FLIM (Fluorescence Lifetime Imaging Microscopy) and FRAP (Fluorescence Recovery After Photobleaching) measurements provide an overall overview of the aggregates' molecular organization, specifically of the molecular structure at pixel-size resolution, and of the mobility of species with under-resolution size. The results show that the aggregates present a solid-like core, and fluid-like edges. The latter are characterized by higher permeability to external HI molecules, and an amyloid-like structure, identified by exploiting the spectroscopic properties of Thioflavin T (ThT), a gold standard fluorescence dye that recognizes details amyloid intermolecular β- structures. The analysis of the aggregation kinetics reveals that the velocity of the process and the aggregates size are affected by compartments size. This leads to the hypothesis that the aggregation is driven by the substrate surface and by the probability of a single molecule to hit it. Moreover, a distinct homogeneity in the formation, shape, and size of the aggregates within the compartment is observed, denoting a different behavior with respect to the well-known polymorphism and heterogeneous nucleation mechanism highlighted for Insulin in literature.

P-39
Hierarchical Binding of Adaptor Proteins to Clathrin Heavy Chain in Saccharomyces cerevisiae: Structural Insights and Functional Implications
Lucas Defelipe[1,2], Osvaldo Burastero[1,2], Katharina Veith[1,2], Knut Korbel[3], Isabel Bento[1], Michal Skruzny[4], Charlotte Uetrecht[2,3], Maria M. Garcia Alai[1,2]
[1] EMBL Hamburg, Germany [2] Centre for Structural Systems Biology, [3] Leibniz-Institut für Experimentelle Virologie, [4] EMBL Heidelberg

Clathrin-mediated endocytosis (CME) is a highly conserved process in eukaryotes, essential for nutrient uptake and membrane receptor recycling. The clathrin heavy chain (CHC) assembles into triskelions, forming the clathrin cage along with light chains (CLC). Adaptor proteins, responsible for membrane binding and invagination, interact with CHC through intrinsically disordered regions (IDRs) containing short linear interaction motifs (SLiMs). The structural determinants of CHC-adaptor protein interactions in Saccharomyces cerevisiae (Sc) remain uncharacterized. In this study, we identified CHC binding motifs in the yeast proteome using bioinformatics and characterized selected motifs through crystallography and biophysical techniques. We report the Sc CHC N-terminal domain structure in complex with adaptor protein peptides (Ent-1, Ent-5, APL2, SWA2, and Sla1) from the canonical Clathrin, Arrestin, and W-boxes. These structures show a conserved binding mode shared across the different binding sites. Binding affinities (Kd) of these peptides were determined using nanoDSF and native mass spectrometry, revealing distinct yet similar Kd values for each box. These findings provide insights into the hierarchical organization of adaptor protein binding to CHC in budding yeast and contribute to our understanding of the molecular mechanisms regulating CME.

P-40
Exploring mechanobiology in SKOV3 and OVCAR3 ovarian cancer cells
Giorgia Del Favero[1], Martina Karasova[1], Denise Framke[1], Maximilian Jobst[1,2], Christopher Gerner[3]
[1] University of Vienna-Faculty of Chemistry, Department of Food Chemistry and Toxicology, Vienna, Austria, [2] University of Vienna, Vienna Doctoral School in Chemistry (DoSChem), Vienna, Austria, [3] University of Vienna-Faculty of Chemistry, Department of Analytical Chemistry, Vienna, Austria

In the abdomen, ovarian cancer (OC) cells are exposed to constant mechanical stimuli: the latter originate for instance from the movement of the intestine and during the accumulation of ascitic fluids. It was postulated that physical cues could sustain crucial cellular functions as those required for tumor progression. To deepen the correlation between disease stage and biomechanical compliance, two cell lines were selected, namely the non-serous carcinoma cells SKOV3 (high motile), and the high-grade serous OVCAR3 (low motile). In addition to substantial differences in the migratory potential, the models diverged in the intracellular calcium ([Ca2+]i) management capacity, measured as response to the stimulation with YODA-1 (5μM), a chemical agonist of PIEZO-1 ion channels. Parallel to higher [Ca2+]i increase, OVCAR3 displayed a more efficient nuclear translocation of mechanosensitive transcription factor YAP-1 in response to shear stress. SKOV3, in turn, were characterized by higher expression levels of YAP-1 in static conditions but returned limited response to the stimulation protocols. Taken together, our data suggest biomechanical heterogeneity for OC cells related to tumor position and origin.

Poster Presentations
– Session 1 –

P-41
Assessing the Contribution of Lysine to the UV-visible Spectra of Charged Amino Acid Rich Proteins by Acetylation
Himanshi Devi[1], Simangka Borsaikia[1], Rajaram Swaminathan[1]
[1] Indian Institute of Technology, Guwahati, India

Recent studies have revealed that monomeric proteins rich in charged amino acids exhibit charge transfer transitions, forming a broad UV-visible absorption band spanning 250-800 nm. The new band termed Protein Charge-Transfer Spectra (ProCharTS) opens up a new label-free window to probe proteins and their interactions. Here we examine the possibility of using acetylation of Lys amino groups to assess their contributions to the ProCharTS profile of charged amino acid-rich proteins. We present the UV-visible absorption of monomeric Human Serum Albumin (HSA) and Alpha3C whose sequence is rich (> 30%) in charged amino acids and show a prominent ProCharTS profile exhibiting a distinctive tail that extends up to 800 nm. Titration of HSA and Alpha3C with acetic anhydride (chemical acetylation) is shown to perturb the ProCharTS profile of these proteins. We present computational studies to understand the impact of Lys acetylation on the ProCharTS profiles of HSA. Our studies indicate that the modulation of the ProCharTS profile by selective acetylation of Lys is sensitive to the salt-bridge forming propensities and charge alteration of these amino acids.

P-42
Modeling nuclear receptor:coregulator interaction with coarse-grained simulations
Marieli Dias[1,2], Cristina Gil Herrero[1], Ana Carolina Migliorini Figueira[2], Sebastian Thallmair[1]
[1] Frankfurt Institute for Advanced Studies (FIAS), Frankfurt am Main - Germany, [2] Brazilian Center for Research in Energy and Materials (CNPEM), Campinas – Brazil

PPARγ is a nuclear receptor involved in adipogenesis and glucose metabolism, and a potential therapeutic target for metabolic diseases. However, the optimization of its therapeutic function requires a deeper understanding of its regulatory mechanisms beyond the canonical pathway. Previous studies suggest that the coregulator PGC-1α may form a non-canonical contact with PPARγ following obesity-linked post-translational modification. In this study, we aimed to investigate the interaction interface between PPARγ and PGC-1α using a combination of experimental and computational approaches. Our results indicate that PPARγ interaction with PGC-1α is potentially affected by PPARγ phosphorylation, and PPARγ can interact with PGC-1α even with the canonical interaction domain blocked, suggesting a possible non-canonical interaction. To elucidate the interaction interface between PPARγ and PGC-1α, we employed molecular docking and coarse-grained modeling approaches, which enabled the exploration of the various conformations of intrinsically disordered proteins, such as PGC-1α. Our findings shed light on the regulatory mechanisms of PPARγ and provide insights for the development of novel therapeutic strategies for metabolic diseases. The combination of experimental and computational approaches can facilitate the study of complex protein-protein interactions and provide relevant information about the mechanism of action of PPARγ in the context of obesity.

P-43
The role of the HelD protein in bacterial transcription and antibiotic resistance
Jan Dohnálek[1], Tomáš Kouba[2], Tomáš Kovaľ[1], Petra Sudzinová[3], Nabajyoti Borah[3], Jiří Pospíšil[3], Jarmila Hnilicová[3], Hana Šanderová[3], Tereza Skálová[1], Mária Trundová[1], Libor Krásný[3]
[1] Institute of Biotechnology of the Czech Academy of Sciences, Prumyslova 595, 25250 Vestec, Czech Republic, [2] Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Flemingovo nám. 2, 16000 Prague, Czech Republic, [3] Institute of Microbiology of the Czech Academy of Sciences, Videnska 1083, 14200 Prague, Czech Republic

RNA polymerase (RNAP) is assisted in its function by a number of accessory factors, some of which play roles in recovery from stalled states, adaptation to environmental changes, or antibiotic resistance. HelD, a multidomain helicase-like protein, and RNAP form a tight complex. Here we present the current picture of the HelD structure-function relationship using small angle X-ray scattering, crystallography, and Cryo-EM, together with biophysical measurements and transcription assays. Our cryo-EM structure of a complex between the Mycobacterium smegmatis RNAP and HelD shows HelD simultaneously penetrating deep into two RNAP channels, thereby removing nucleic acids or other binding partners from these channels. The structures provide insights into the function of HelD in releasing stalled RNAP from DNA and its protective effect against antibiotics in mycobacteria and possibly also in Bacillus subtilis. The structural and functional data together with comparative analysis show how HelD helps maintain bacterial transcription functional. This work was supported by MEYS (LM2018127, LM2023042) and CSF (23-06295S).

P-44
Interaction of the respiratory syncytial virus non-structural protein 1 with the MED25 Mediator subunit
Jiawei DONG[1,2], Vincent Basse [1,3], Marie Galloux [1,3], Jean-Francois Eléouët [1,3], Monika Bajorek [1,3], Christina Sizun [1,2]
[1] University Paris-Saclay, France [2] Institute of Chemistry of Natural Substances, CNRS UPR2301, [3] Molecular Virology and Immunology, INRAE UR892

Human Respiratory Syncytial Virus is a major cause of severe respiratory disease. RSV evolved a unique strategy to evade the host innate immune response by two non-structural proteins acting as interferon antagonists. Recent findings suggest that NS1 plays a role in host gene transcription modulation via interactions with the Mediator complex. We analyzed the interaction between NS1 and the ACID domain of the MED25 Mediator subunit. By ITC we found nanomolar affinity. As the C-terminal α3 helix of NS1 reminded of a transactivation domain, we analyzed NS1α3 peptide binding to MED25ACID. Chemical shift perturbations delineated a primary binding site on MED25ACID, corresponding to the target of Herpes simplex virus transcription regulator VP16. Surprisingly NS1α3 displayed micromolar affinity, pointing to additional interactions with full-length NS1. Since NS1α3 is critical for MED25ACID binding and involved in inter-protomer contacts in the dimer, we wondered about the structural plasticity of NS1. DLS and SEC suggested that NS1 is in a monomer-dimer equilibrium. Broad NMR signals for NS1 pointed to exchange phenomena. The NS1Δα3 deletion mutant displayed sharp NMR signals. Backbone assignment was performed and transposed to full-length NS1, showing that exchanged broadening is observed in the dimer interface. These dynamics will be further investigated.

Poster Presentations
– Session 1 –

P-45
Targeting the copper binding sites of CopI a periplasmic protein involved in bacterial copper resistance

Pierre Dorlet[1,2,3,4], Melanie Rossotti[1,2,3,4], Diletta Arceri[1,2,3,4], Anne Durand[1,5,6], Pascal Mansuelle[1,2,3], Olivier Bornet[1,2,3], Guillaume Gerbaud[1,2,3,4], Hélène Launay[1,2,3,4], Soufian Ouchane[1,5,6]

[1] CNRS, France [2] Aix-Marseille Université (AMU), [3] Institut de Microbiologie de la Méditerranée (IMM), [4] Laboratory of Bioenergetics and Engineering of Proteins (BIP), [5] Université Paris-Saclay, [6] Institute of Integrative Biology of the Cell (I2BC)

CopI is a periplasmic protein of 15kDa which is induced by high copper concentration and is directly involved in the copper resistance of the purple photosynthetic bacterium Rubrivivax gelatinosus. Homolog exists in other environmental bacteria but also pathogens such as Vibrio cholerae or Pseudomonas aeruginosa. The 3D structure of CopI has not been determined yet and its mechanism of action remains unknown. In order to get insights into the different Cu binding sites and possible mechanism of action of the protein, we have performed spectroscopic studies on the wild- type protein as well as specific mutants targeting the Cu binding modules from its sequence. Electron and nuclear magnetic resonance experiments show that CopI possesses a scarce green-type cupredoxin site and is the first single domain cupredoxin with at least three Cu sites. The cupredoxin center and a highly conserved His/Met-rich region, which binds preferentially Cu(I), are required for Cu resistance. The non-conserved His-rich N- terminal region is not required for Cu resistance and is a binding site primarily for Cu(II). Moreover, sequential additions of Cu(II) and Cu(I) to the protein indicates its ability to oxidize Cu(I) into Cu(II) via the cupredoxin center. We therefore propose that CopI may have the dual function to detoxify Cu by oxidizing it to its less toxic cupric form that it can store in its N-terminal site.

P-46
Quantitative 3D live-imaging of self-organisation in embryonic organoids

Valentin Dunsing-Eichenauer[1], Sham Tlili[1], Claire Chardès[1], Remi Galland[2], Jean-Baptiste Sibarita[2], Virgile Viasnoff[3], Léo Guignard[4], Pierre- François Lenne[1]

[1] Developmental Biology Institute of Marseille & Turing Center for Living Systems, Aix-Marseille University/ CNRS, Marseille, France, [2] Interdisciplinary Institute for Neuroscience, University of Bordeaux/ CNRS, Bordeaux, France, [3] Mechanobiology Institute, National University of Singapore, Singapore, Singapore, [4] Computer and Systems Laboratory & Turing Center for Living Systems, Aix-Marseille University/ CNRS, Marseille, France

The emergence of asymmetries within a mass of equivalent cells is the starting event in the development of embryos, resulting in the formation of the main body axes. Despite its fundamental role, the mechanisms that induce symmetry breaking remain largely unknown because they are difficult to probe in vivo, particularly in mammalian embryos. A promising in vitro model to study such mechanisms are embryonic organoids, which undergo gastrulation-like movements similar to those observed in embryos. We aim to use live imaging to disentangle the interplay between signaling, cell differentiation and mechanics underlying self-organized symmetry breaking. Currently available imaging platforms are limited to low-throughput 3D or high-throughput 2D imaging. To overcome this limitation, we establish multi-view single-objective lightsheet microscopy, allowing us to image tens of organoids over hours to days with cellular resolution and sufficient temporal sampling to track cells in 3D. We present ongoing efforts using deep learning based segmentation and quantitative image analysis to correlate cellular dynamics and rearrangements with the expression of key differentiation markers during polarization of aggregates. We thereby analyze how spatially localized expression domains and collective cell movements establish symmetry breaking. Finally, we analyze the variability of spatiotemporal patterns across multiple specimen.

P-47
Characterization of actin-binding tail structure and dynamics in metavinculin mutants associated with cardiomyopathy

Zeynep Aslihan Durer[1,2,3], Tobias Gräwert[4], Hande Ipek Yetke[5], Aybike Sehriban Bulut[6], Emel Timucin[7], Zehra Sayers[8], Beki Kan[1,3]

[1] Acibadem Mehmet Ali Aydinlar University, Turkey , [2] Acibadem Mehmet Ali Aydinlar University, Turkey, [3] Acibadem Mehmet Ali Aydinlar University, Health Sciences Institute, Biophysics, Turkey, [4] EMBL, Biological Small Angle Scattering Group, Hamburg, Germany, [5] Istanbul University, Health Sciences Institute, Biophysics, Turkey, [6] Acibadem Mehmet Ali Aydinlar University, Health Sciences Institute, Genome Studies, Turkey, [7] Acibadem Mehmet Ali Aydinlar University, School of Medicine, Department of Biostatistics and Medical Informatics, Turkey, [8] Sabanci University, Faculty of Engineering and Natural Sciences, Department of Molecular Biology, Genetics and Bioengineering, Turkey

Vinculin is a cellular adhesion site adaptor protein that connects the actin cytoskeleton to integrin receptors. Metavinculin, a muscle-specific higher molecular weight splice isoform, contains a 68-residue insert within the vinculin actin-binding tail. Mutations in the metavinculin-specific insert (R975W and DelLeu954) have been associated to dilated and hypertrophic cardiomyopathies.Therefore, the goal of this study is to identify the structural differences between the actin-binding tails of wild-type metavinculin and cardiomyopathy mutants. Limited proteolysis, dynamic light scattering, SAXS, and microsecond timescale molecular dynamics (MD) simulations were used to assess WT and mutant metavinculin actin binding tails (MVt). Our analysis showed that all three proteins had similar hydrodynamic diameters, and SAXS showed minor differences in estimated molecular weights and radius of gyration between WT and mutant MVTs. The metavinculin loop insert changed conformations from WT to mutant in MD simulations, although all structures had a highly stiff four-helix bundle. We found that R975 potentiates a more compact metavinculin, improving tail domain structure and stability. Further analysis will reveal structural variations between wild-type and mutant MVTs and allow modeling of their interactions with actin and focal adhesion proteins. Our work was supported by TUBITAK (3501 program project number 118Z149) and EMBL BAG Proposal SAXS-994.

P-48
Epigenetic editing of beta cells using nanostraws

Frida Ekstrand[1], Sabrina Ruhrmann[2], Mokhtar Mapar[1], Diogo Volpati[1], Karl Bacos[2], Charlotte Ling[2], Christelle Prinz[1]

[1] NanoLund and Solid State Physics, Lund University, Lund, [2] Epigenetics and Diabetes Unit, Department of Clinical Sciences, Lund University, Malmö, Sweden

Transfection of cells is a powerful method in molecular biology research. The most commonly used techniques today are lipid- and viral-based, which have inherent problems such as low efficiency and cytotoxicity, but the transfection efficiency can also depend on cargo size and cell type. By using nanostraw electroporation (NS-EP) for transfecting cells, both transfection efficiency and cell viability are high. Nanostraws (NS) are vertical hollow nanostructures protruding from a polycarbonate (PC) membrane, which allow direct intracellular access when cells are seeded on top of them. The devices, with NS and cells, are exposed to a pulsed electric field which opens up pores in the cell membrane and has an electrophoretic effect on the charged biomolecules in the cargo solution at the backside of the PC membrane. In this work, NS-EP has been used to transfect beta cells with dCAS9 and gRNA, to epigenetically alter insulin gene expression. First, method optimization was carried out to ensure efficient transfection, where different parameters, such as cell density, voltage, and straw length, were investigated. After injection of the gene-altering plasmids, gene expression was analyzed using quantitative real-time PCR (qPCR). The results show that after optimization, this method successfully altered the expression of the target insulin gene.

Poster Presentations
– Session 1 –

P-49
Integration of cell cultures in electrochemical (bio)sensing platforms
Adrian Enache[1], Caroline Sanz[1], Daniela Oprea[1], Mihaela Beregoi[1], Madalina Barsan[1]
[1] National Institute of Materials Physics, Magurele, Romania

The most amazing biological structure is the living cell, a dynamic machine that integrates a wide variety of biochemical structures, continuously adapting and responding to the local environment. The cellular systems produce and transform signaling biomarker molecules to inter- communicate, and their integration in biosensing devices became an important tool to understand the underlying mechanisms of different diseases, as well as to perfect timely disease diagnosis and personalized therapeutic approaches. As in any scientific field, the optimum experimental conditions in terms of sensitivity and minimization of undesirable effects requires the appropriate model. Thus, the electrochemical sensing of cells biomarkers requires the cultivation of the cells at/near the (bio)sensor surface, maintaining an appropriate electroactive available surface, and avoiding the passivation. This can be achieved using electrospun nanofibers polymer scaffolds, which allow the cell cultivation in desired condition. These scaffolds have a dual-role, serving as polymeric support for cell culture and as an electrochemical transducer, the later requiring their metallization and/or functionalization with specific components. Based on this, the screening of cellular stress has been achieved integrating the cells and the biosensor components on the transducer whereas the melanin exocytosis was successfully quantified using a commercial electrode. Either directly on the surface of the (bio)sensor or spatially detached from it, the integration of cell cultures in (bio)sensing platforms based on electrospun polymeric nanofibers represents a powerful bioanalytical tool able to provide real-time information about the biomarkers release, enzyme activity or enzyme inhibition effects on cells, as well as monitoring of different cellular events.

P-50
Structural insights on stand-alone disaggregase ClpG regulation
Lisa Engelhardt [1], Stavros Azinas
[1] Department of Biochemistry and Biophysics, Science for Life Laboratory, Stockholm University, Stockholm, Sweden.

P-51
NMR spectroscopy analysis of c-Myc transient structure and proline conformation upon phosphorylation in key positions
Arvid Eskilson[1], Vivian Morad[1], Alexandra Ahlner[1], Maria Sunnerhagen[1]
[1] Department of Physics, Chemistry and Biology (IFM), Sweden

Myc is a family of oncogenic, intrinsically disordered transcription factors that have been estimated to bind up to 15% of all genes, regulating cellular activities such as cell cycle regulation, proliferation, differentiation, and cell death. Myc proteins are highly implicated in various cancer types, and some form of Myc deregulation has been estimated in up to 70% of human malignancies. The lifecycle of Myc proteins in cells is tightly regulated through the phosphorylation of Threonine 58 and Serine 62. Phosphorylation of Serine 62 activates Myc, which leads to sequential phosphorylation of Threonine 58, inducing ubiquitination and degradation through the proteasome. Inhibition of Threonine 58 phosphorylation through mutation has been shown to be a common cause of cancers such as Burkitt's lymphoma and AIDS-related lymphomas. Proline residues are often highly enriched in intrinsically disordered proteins and have higher rates of isomerization than other amino acids. The conformation of proline residues has been implicated to impact the function of Myc. We have used nuclear magnetic resonance (NMR) spectroscopy to identify the cis-trans conformation of prolines and elucidate the interconnection between phosphorylation of T58 and S62, prolyl isomerase Pin1, proline conformation and Myc transient structures and dynamics.

P-52
Physical stimulation regenerates hair muscle by inducing differentiation of resident stem cells, improving skin elasticity
Tomonobu Ezure[1], Kyoichi Matsuzaki[2], Nobuhiko Ohno[3,4]
[1] Shiseido Co., LTD., Japan [2] International University of Health and Welfare, [3] Jichi Medical University, [4] National Institute for Physiological Sciences

Skin physically protects internal organs and maintains body morphology, and deterioration of its condition causes loss of elasticity, which can lead to pressure ulcer, delay of wound healing and wrinkle formation. Skin elasticity depends upon a high density of aligned arrector pili muscles (hair muscles), but hair muscles decrease with aging, and there are few solutions to regenerate them. The aim of this study was to identify a method of hair muscle regeneration, and to clarify the mechanism involved. Human skin was organ-cultured for 7 days, and physically stimulated in various ways. Immunohistochemical study revealed the presence of cells double-positive for stem-cell markers CD49f (smooth muscle stem-cell marker) and CD34 in hair muscles. Further, stretching the organ-cultured skin increased these stem-like cells and in turn increased muscle cells (αSMA- positive cells). In addition, stretching the skin of volunteers along the hair muscle direction for 4 weeks significantly increased skin elasticity. Overall, these results suggest that hair muscles contain CD34/CD49f-positive stem-like cells that respond to physical stimulation (stretching) by proliferating and differentiating into hair muscle cells. Thus, stretching can be effective for regenerating hair muscles in skin to improve clinical and quality of life (QOL) issues due to the skin's physical deterioration.

Poster Presentations
– Session 1 –

P-53
Understanding the pro-survival role of AIF: structural and functional study of AIF-CHCHD4 complex
Elisa Fagnani[1], Eloise Mastrangelo[2], Mario Milani[2], Alessandro Aliverti[1]
[1] Department of Biosciences, Università degli Studi di Milano, Via Celoria 26, I-20133, Milan, Italy, [2] Biophysics Institute, CNR-IBF, Via Celoria 26, I-20133, Milan, Italy

Apoptosis Inducing Factor (AIF) is a mitochondrial flavoprotein generally known for its pro-apoptotic function. However, recent studies demonstrate that AIF also regulates cell energy homeostasis by promoting the biogenesis and the function of multi-subunit respiratory complexes. Although the underlying molecular mechanisms have not been yet elucidated, this role is played thanks to the interaction of AIF with CHCHD4, a soluble inner membrane space (IMS) protein which promotes the entrance in the IMS and the oxidative folding of substrates belonging to the respiratory complexes' subunits. To understand AIF vital role in mitochondria, we decided to investigate the AIF-CHCHD4 interaction from both the functional and the structural point of view. We studied the possible impact of the 27-residues N-terminal portion of CHCHD4, which effectively mimics its binding site for AIF, on the catalytic activity and NAD+-binding ability of AIF. The peptide turned out to stimulate the DCIP-NADH reductase activity of AIF, with an apparent Kd for AIF in the sub-micromolar range. Moreover, it has been found that AIF binds NAD+ only in the presence of the peptide and that the complexation is strongly stimulated by lowering the temperature. For the determination of relevant structural features of the complex, we used AlphaFold software to build up a model, which was then experimentally confirmed by mutagenesis experiments on AIF and mass-spectrometry analyses on the crosslinked AIF-CHCHD4 complex. Since peptide and NAD+ bindings display strong positive cooperativity, we are using this information to set up the optimal conditions for X-ray diffraction studies.

P-54
Mechanical properties of Jurkat cells measured by deformability cytometry
Lija Fajdiga[1], Nina Bernat[1], Lara Betocchi[1], Špela Zemljič Jokhadar[1], Jure Derganc[1]
[1] Faculty of Medicine, University of Ljubljana, Slovenia

CAR-T cell therapy has achieved remarkable success in treating certain blood cancers and is currently being investigated for the treatment of other cancers. Since this technology is based on the anticancer function of modified T lymphocytes, detailed knowledge of their characteristics is essential for any further optimization. The mechanical properties of T lymphocytes are important both for the process of ex-vivo cell transfection and for the migration of the cells to the target cancer when they are reinfused into the patient's body. The goal of our work is to better understand the mechanical properties of lymphocytes in different environments and thus contribute to the advancement of CAR -T therapy. The focus of the present work is on the mechanical properties of Jurkat cells, a standard lymphocyte model system. We use deformability cytometry with a custom-made open-source stroboscopic imaging system. We analyze how the mechanics of Jurkat cells is governed by the cytoskeleton and how it is affected by fatty acids. Fatty acids play an important role in lymphocyte metabolism and they are also part of intravenously administered nutrition for cancer patients. Preliminary results suggest that destabilization of the actin cytoskeleton by cytochalasin D has a lesser effect on Jurkat cells than on adherent epithelial cells. We also found that Omegaven, a clinical fish oil lipid emulsion, affects Jurkat cells significantly more than a mixed lipid emulsion SMOFlipid.

P-55
Computational optimization of non-viral CRISPR/Cas9-Gold-based delivery vehicle design
Alexandra Farcas[1,2], Lorant Janosi[1], Simion Astilean[2]
[1] National Institute for Research and Development of Isotopic and Molecular Technologies, 65-103 Donat Street, 400293, Cluj-Napoca, Romania, [2]Babeş-Bolyai University, Faculty of Physics, 1 Kogalniceanu Street, 400084, Cluj-Napoca, Romania

CRISPR/Cas9 therapy, which offers a number of benefits over traditional gene therapies, can be used to produce effective targeted gene editing. The main challenge in using the CRISPR/Cas9-based approach is creating gene delivery vectors that can target specific mutations in the genome. In order to treat inherited disorders like muscular dystrophy, CRISPR/Cas9 has also been delivered in vivo using oligonucleotide-functionalized gold nanoparticles (GNPs). Because that current CRISPR/Cas9-Gold treatment trials are in the early stages of development, delivery vectors with minimal insertional mutagenesis risk should be created based on molecular knowledge of the implicated systems. We computationally optimized such a design in two steps: (i) we optimized the DNA loading on a range of GNP sizes in nanoparticle-oligonucleotide conjugates (ii) we performed molecular dynamics simulations of Cas9/sgRNA system. This study is essential in optimizing the design of CRISPR/Cas9-Gold-based delivery vehicles.

P-56
Modulation of stability and activity of lysozyme in imidazolium-based ionic liquids – a cation role
Diana Fedunova[1], Vladimir Vanik[1], Zuzana Gazova[1]
[1] Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Košice, Slovakia

Ionic liquids (ILs) are novel solvents with increasing applications in biochemistry, biophysics, and biotechnology, particularly as stabilizers of proteins and protein-based products. The vast number of possible anion and cation combinations in ILs composition facilitates the selection of solvents with desired properties. We have studied the effect of imidazolium-based ILs with allyl, propyl, and benzyl side chains in combinations with Cl- anion in wide concentration range on thermal stability, activity, and structure of lysozyme using docking calculations and spectroscopic and calorimetric methods. We have characterized the effect of cations with different hydrophobicity and geometry on lysozyme stability and activity to understand the mechanism of de/stabilization of protein with aim to identify useful solvents for biotechnological applications. We have found that at lower ILs concentrations up to 25 mM, all ILs increase the activity of lysozyme along with the maintaining the native structure and slightly enhanced thermal stability as follows: benzyl > propyl > allyl. At higher concentrations, the decrease in thermal stability and activity occurs. The results indicate the important role of hydrophobic and pi-interactions in lysozyme stabilization.

Poster Presentations
– Session 1 –

P-57
Exploring the Mechanics of Clumping Factor A
Alba Fernandez-Calvo[1,2], David de Sancho[2,3], Raul Perez-Jimenez[1,2]
[1] Center for Cooperative Research in Biosciences - CIC BioGUNE, Spain [2] Universidad del Pais Vasco/Euskal Herriko Unibertsitatea - UPV/EHU, [3] Donostia International Physics Center - DIPC

Bacterial adhesins are cell-surface proteins that anchor to the cell wall of the host organism, thus initiating infection. The study of this family of proteins is hence essential to develop new strategies to fight bacterial infections. In the case of Staphylococcus aureus, adhesins are also known as MSCRAMMs. Here we focus in one of them, the Clumping Factor A (ClfA), a protein with an immunoglobulin-like structure that binds to fibrinogen (Fg) to initiate colonization in the heart valves through the dock-lock-latch mechanism, originating endocarditis. Interestingly, Bernardi et al. have shown that an homologous protein of ClfA utilizes a catch-bond to withstand forces exceeding 2 nN, making this system as mechanically strong as a covalent bond. Using single-molecule force spectroscopy and atomistic molecular dynamics (MD) simulations, we show that ClfA is not particularly strong in the absence of its human targets. Integrating the results from both theory and experiment, we dissect contributions to the mechanical stability of this protein.

P-58
Effect of the negative lipid fraction on alpha-synuclein cooperativity and binding
Marco Fornasier[1], Stefan Wennmalm[2], Alexandra Andersson[1], Emma Sparr[1], Peter Jönsson[1]
[1] Division of Physical Chemistry, Department of Chemistry, Lund University, SE-22000, Lund, Sweden, [2] Department of Applied Physics, Biophysics Group, SciLifeLab, Royal Institute of Technology-KTH, 171 65 Solna, Sweden

Alpha-Synuclein (alpha-Syn) aggregates in vivo are a hallmark of Parkinson's disease. The protein interacts strongly with negatively charged membranes, but its monomeric binding and the healthy function are not fully understood. Recently, we showed that alpha-Syn binds cooperatively to this kind of membrane in acidic conditions, but the effect of the lipidic composition has not been investigated. For this reason, we evaluated the binding of alpha-Syn on supported lipid bilayers (SLBs) composed of different fraction of the negative lipid DOPS, between 0 and 30 %, and the uncharged lipid DOPC at pH 5.5. The fluorescence microscopy measurements showed that at least 5% of negatively charged lipids are needed for the binding to occur, and that the surface is saturated around 20 mol % of DOPS. The conformational change to alpha-helix from random coil on vesicles were studied via Circular Dichroism, highlighting that the saturation of the vesicles surface occurs at lower lipid-to-protein ratio, as the DOPS fraction increases. After preliminary measurements on the lipid-protein interactions, we evaluated how the fraction of the DOPS influences alpha-Syn cooperativity, using Fluorescence Cross Correlation Spectroscopy. These findings can shed more light on the healthy function of the protein when the monomeric protein interacts with the cell membrane and the molecular mechanisms behind alpha-Syn cooperativity, which could be related to the pathogenesis of Parkinson's disease.

P-59
Development of a sensitive blood-based amyloid amplification assay for diagnosis of Parkinson's disease
Laxmikant Gadhe[1], Rakesh Kumar[1], Arunima Sakunthala[2], Sangeeta Rawat[3], Samir K. Maji[1,2]
[1] IIT Bombay, Powai, Mumbai 400076, India, [2] Sunita Sanghi Centre for Ageing and Neurodegeneration Diseases, IIT Bombay, Mumbai 400076, India , [3] Seth GS Medical College and KEM Hospital, Mumbai-400012, India

Parkinson's disease (PD) is one of the common neurodegenerative diseases affecting several million humans worldwide. However, the major challenge in PD treatment is the lack of sensitive diagnosis at an early stage of the disease. It is known that a minute amount of α-synuclein oligomers/fibrils have appeared in biological fluids (blood and CSF) before the appearance of clinical manifestation in PD patients. Detecting misfolded and aggregated protein in biological fluids (blood) based on Protein misfolding cyclic amplification (PMCA) has shown promise in many neurodegenerative diseases including Prion. The method, however, will produce false positive results where the original amyloidogenic protein is intrinsically disordered such as α-Synuclein. Based on amyloid structure-based mutant design, we found novel artificial mutants of α-Synuclein, that do not aggregate spontaneously (unlike WT α-Synuclein) but only aggregate in the presence of α-Synuclein fibril seeds. This modified substrate for PMCA assay of α-Synuclein can discriminate blood samples from patients diagnosed with PD and healthy control subjects with high sensitivity and specificity. Our findings suggest that the detection of misfolded α-Synuclein by α-Synuclein PMCA using novel substrate in the blood of PD- affected patients may offer a good non-invasive, sensitive, and specific biochemical assay for the diagnosis of the disease.

P-60
Engineered artificial fluorescent proteins for biological light-emitting diodes
Liher Garcia Gonzalez[1], Horst Lechner[2], Gustav Oberdorfer[2], Sara Ferrara[3], Rubén D. Costa[3], Giacomo Renno[4], Maksym Bokan[4], Claudia Barolo[4], Pedro B. Coto[5], Mantas Liutkus[1], Aitziber L. Cortajarena[1,6]
[1] CIC biomaGUNE, Basque Research and Technology Alliance (BRTA), [2] Graz University of Technology, Institute of Biochemistry, [3] Technical University of Munich, Chair of Biogenic Functional Materials, [4] Department of Chemistry and NIS Interdepartment Centre, University of Turin, [5] Spanish National Research Council (CSIC) and Donostia International Physics Center (DIPC), [6] Ikerbasque, Basque Foundation for Science, Spain

Artificial fluorescent proteins (AFPs) composed of synthetic fluorophores stabilised within designed protein scaffolds have been developed to address the ever-growing need for efficient and environmentally sustainable lighting. Current white light-emitting diodes (WLEDs) use toxic and difficult-to-source materials, leading to high production costs and significant ecological impact. Protein-based light down-converters for integration into WLEDs provide an attractive eco-friendly alternative to the currently chemical-based components. Taking inspiration from natural external fluorophore-stabilising fluorescent proteins, like UnaG-bilirubin complex, protein scaffolds with large internal cavities/pockets were screened for their capability to accommodate selected large polyaromatic dye molecules with desired photophysical properties. All the designed dyes showed promising stokes shifts for white diode construction. β-barrel-based protein scaffolds and custom-designed α-helix bundle proteins, were reengineered for cavity complementarity with the fluorophore chemical frameworks to maximise host-guest interactions and dye stabilisation by the protein scaffolds. The most successful constructs exhibited no loss of photoluminescence quantum-yield (PLQYs) and showed good dispersion. Based on this promising results achieved through host-guest complexation, covalent chemical bioconjugation including cysteine-maleimide condensation, click chemistry (azide-alkyne cycloaddition), and thiol-yne coupling, are being developed to further stabilise the novel AFPs.

Poster Presentations
– Session 1 –

P-61
What properties of biomolecular condensates influence oxygen gradients?
Ankush Garg[1], Klaus Koren[1], Magnus Kjaergaard[1]
[1] Aarhus university, Denmark

Condensates can create a special internal milieu to regulate biochemical reactions through partitioning of biomolecules. Here, we explore how oxygen partitioning is affected by condensates. We propose two hypotheses to what might determine oxygen partitioning. Firstly, molecular oxygen is hydrophobic and is expected to get concentrated into the more hydrophobic interior of condensates. Secondly, the high density of polymers in the condensate excludes oxygen from part of the volume of the condensate and thus decreases oxygen concentrations inside. Here, we investigate which of these effects dominate. We investigate oxygen partitioning into condensates formed by synthetic repeat of intrinsically disordered proteins. Oxygen concentrations are measured using phosphorescence lifetime imaging microscopy (PLIM) using metal-organic sensors and electrochemical probes. We observe less oxygen concentration in the droplet compared to that of bulk which signifies the importance of excluded volume concept. We hope to understand the basis behind oxygen partitioning which will help us create anaerobic reaction crucibles that might be useful in production of complex natural compounds in microbial cell factories.

P-62
Crystal structure of a selective α□Hydroxy-β-amino Acid Bestatin Derivative inhibitor of ER aminopeptidase 2
Galateia Georgaki[1,2], Emmanuel Saridakis[1], Alexandros Athanasoulis[1], Anastasia Mpakali[1,2], Athanasios Papakyriakou[1], Efstratios Stratikos[1,2]
[1] National and Kapodistrian University of Athens, Greece
[2]Department of Chemistry, National and Kapodistrian University of Athens, Greece

Endoplasmic Reticulum aminopeptidase 2 (ERAP2), is an ER-resident aminopeptidase that trims antigenic peptides and their precursors before they can bind onto Major Histocompatibility Class I molecules (MHC-I) and presented on the cell surface to activate the adaptive cellular immunity. The activity of ERAP2 has been associated with resistance to pathogens such as HIV and Yersinia pestis (the cause of the Black Death pandemic) but is also a risk factor for the development of HLA-associated inflammatory autoimmune diseases such as Ankylosing Spondylitis, Psoriasis, Crohn's Disease, and Birdshot Chorioretinopathy. Thus, ERAP2 is an emerging pharmacological target for the treatment of autoimmunity. We have previously demonstrated that the α□Hydroxy-β-amino acid scaffold of the drug Bestatin can be exploited to make potent inhibitors for aminopeptidases. Here, we report the crystal structure of ERAP2 with a bound Bestatin derivative that is an nM inhibitor of the enzyme. The compound binds in the canonical orientation, engaging the active site Zinc (II) atom as well as the S1, S1' and S2' subsites. The compound utilizes a competitive mechanism of action and exhibits excellent selectivity versus the homologous enzyme ERAP1, primarily due to optimized interactions in the S1 specificity pocket but has limited selectivity against other M1 aminopeptidase family members. This crystal structure will be a valuable template for optimizing potency and selectivity towards pharmacologically relevant inhibitors of ERAP2 for applications in the treatment of autoimmune disease.

P-63
Modulation of enzymatic activity in biomolecular condensates
Marcos Gil-Garcia[1], Ana I. Benítez-Mateos[2], Florence Stoffel[1], Chiara Morelli[1], Lenka Faltova[1], Francesca Paradisi[2], Paolo Arosio[1]
[1] Department of Chemistry and Applied Biosciences, Institute for Chemical and Bioengineering, ETH Zurich, Zurich, Switzerland, [2] Department of Chemistry, Biochemistry and Pharmaceutical Sciences, University of Bern, Bern, Switzerland

A wide variety of biochemical reactions in vivo occurs within biomolecular condensates. However, the molecular mechanisms underlying the modulation of these reactions remain elusive. The development of cellular-mimic condensates in vitro can provide insights into how condensates modulate biochemical reactions in cells. Here we deploy intrinsically disordered, low complexity domains (LCDs) as building blocks to generate programmable enzymatic condensates of NADH-oxidase (NOX) with different sizes ranging from nanometers to microns. Using a variety of techniques as confocal microscopy, size-exclusion chromatography or dynamic light scattering, we demonstrate that LCDs derived from three different RNA-binding proteins induce condensates with similar enzyme concentration and different local environments. Only the droplets with the highest recruitment of substrate and cofactor increase the enzymatic activity, indicating that this effect is not due to a crowding mechanism but to the specific local microenvironment of the condensates. Importantly, we show that the increase in activity in nanocondensates and micron-size droplets is independent of the size of the condensates and is related to an improvement in the catalytic efficiency of the enzyme. These findings provide insights into the regulation of enzymatic reactions by condensation at different length scales and the design of condensates as new protein- based materials for heterogeneous biocatalysis.

P-64
Chaperone modulation of tau liquid liquid phase separation
Cláudio M. Gomes[1,2], Guilherme G. Moreira[1,2]
[1] Faculty of Sciences University of Lisbon, Lisbon, Portugal, [2] BioISI – Biosystems and Integrative Sciences Institute, Lisbon, Portugal

Tau is an intrinsically disordered protein implicated in several neurodegenerative tauopathies including Alzheimer's disease. While several studies evidenced tau liquid–liquid phase separation (LLPS) as an early event in tau pathogenesis with the potential to enhance aggregation, little is known on the regulation of this process by molecular chaperones. We recently established that S100B, an astrocytic brain protein implicated in neurodegeneration, has a novel chaperone activity that results in inhibition of the aggregation, toxicity and proteopathic seeding of Tau and Aβ42. Here we report that S100B modulates Tau LLPS in a Ca-dependent manner [4]. While apo S100B has no effect on PEG-induced tau LLPS, Ca- bound S100B prevents demixing, resulting in a shift of the phase diagram boundary to higher concentrations. Also, addition of Ca-bound S100B to tau droplets results in a sharp decrease in turbidity, indicating that interactions with the S100B chaperone promote transition of tau to the diluted phase. Using Alexa488-labeled tau we observed that indeed S100B reduces tau fluorescent droplets, without compromising its liquid-like behavior nor droplet fusion events. Indeed, both FLIM-FRET studies and electrophoretic analysis confirmed that S100B incorporates into tau liquid droplets. Altogether this work uncovers S100B as an important proteostasis regulator acting on Tau condensation and aggregation phenomena of relevance across the neurodegeneration continuum.

Poster Presentations
– Session 1 –

P-65
In vitro reconstituted artificial biosynthesis for one-pot production of pseudo-natural prenylated peptides
Yuki Goto[1], Yuchen Zhang[1], Dinh Thanh Nguyen[1], Sumika Inoue[1], Hiroaki Suga[1]
[1] The University of Tokyo, Japan

Prenylation is a post-translational modification widely found in primary and secondary metabolism. This modification can enhance the lipophilicity of molecules, enabling them to interact with lipid membranes more effectively. We recently discovered the first histidine-prenylating enzyme LimF from Limnothrix sp. CACIAM 69d. This peptide prenyltransferase selectively geranylates the electron-deficient C2 atom of His imidazole. Furthermore, we have integrated LimF with an in-vitro engineered translation apparatus, so-called flexible in vitro translation (FIT) system, to devise the FIT-LimF system. In this system, artificial thioether-closed macrocyclic peptides (teMPs) are expressed via genetic code reprogramming and subsequently modified by LimF in a one-pot manner, yielding prenylated teMPs. The FIT-LimF system validated the versatility of LimF and demonstrated the synthesis of a large array of teMPs with highly diversified sequence compositions. It should be noted that the FIT-LimF system also enabled the geranylation of nonproteinogenic His analogs including D-His and α-methyl-His. These results highlight the potential utility of the in vitro reconstituted system in analyzing the unique peptide-modification enzyme, producing designer prenylated peptides, and even constructing prenylated macrocycle libraries.

P-66
Microtubule-associated ATP facilitates protective functions of tau
Lenka Grycova[1], Tereza Humhalova[2], Darina Hlubuckova[1], Daria Khuntsariya[1], Arya Krishnan[3], Tomas Zdobinsky[2], Stefan Diez[4], Carsten Janke[3], Ivan Barvik[5], Lenka Libusova[2], Marcus Braun[1], Zdenek Lansky[1]
[1] Institute of Biotechnology, Czech Academy of Sciences, BIOCEV, Vestec, Prague, [2] Dept. Cell Biology, Faculty of Science, Charles University, Prague, [3] Institut Curie, Paris, [4] B CUBE – Center for Molecular Bioengineering, TU Dresden, [5] Faculty of Mathematics and Physics, Institute of Physics, Charles University, Prague, Czech Republic

ATP decrease is related to ageing and neurodegeneration. Neurodegeneration-related microtubule associated protein tau can cooperatively assemble into a cohesive layer termed microtubule envelope. In many neurodegenerative diseases tau dissociates from microtubules and the microtubules disintegrate. Mechanisms leading to tau dissociation from microtubules are, however, unclear. Here we show that ATP depletion causes disassembly of protective tau envelopes from microtubules. Using reconstitution experiments, live-cell imaging, and molecular modeling, we found that microtubule-associated ATP stabilizes tau molecules on the microtubule surface, enhancing thus the envelope self-assembly. We show that decrease in ATP level can lead to destabilization of tau envelope and microtubule disintegration through microtubule severing enzymes. Our results thus introduce microtubule-associated ATP as a modulator of tau function.

P-67
Deciphering catalytic mechanisms in Mo and W-enzymes through EPR spectroscopy, isotopic enrichment and DFT calculations
Bruno GUIGLIARELLI[1], Frédéric BIASO[1], Guillaume GERBAUD[1], Bénédicte BURLAT[1], Stéphane GRIMALDI[1]
[1] BIP-UMR7281- AMU-CNRS, France

Molybdenum (Mo) and tungsten (W) enzymes are found in virtually all living organisms where they catalyse a wide diversity of redox reactions involved in major biogeochemical cycles. In prokaryotes, most of these enzymes harbour a large Mo/W-bis pyranopterin guanosine dinucleotide cofactor. During catalysis, the metal ion cycles between the +IV and +VI redox states, the intermediate Mo(V) and W(V) states being EPR-active (S=1/2). In the various Mo/W enzymes investigated so far, several Mo(V) and W(V) species have been identified, but despite of numerous crystallographic and spectroscopic studies their structure and catalytic relevance are still largely debated. As EPR is very sensitive to spin-spin interactions between close paramagnetic species (magnetic nuclei or other metal cofactors), such magnetic coupling can be analyzed by multifrequency EPR or by the most advanced pulsed EPR techniques (HYSCORE, ENDOR, ELDOR-DEER) to give high resolution structural data. Combined with selective isotopic enrichment, hyperfine interaction analysis and DFT calculations, this approach is shown to be a powerful tool to decipher catalytical mechanisms in enzymes of nitrogen cycle and CO_2 reduction.

P-68
High-throughput biophysical mapping for diagnostic
Cenk Gurdap[1], Luca Andronico[1], Erdinc Sezgin[1]
[1] Karolinska Institutet, Sweden

Physical remodeling of our cells as response to environmental changes is essential for their survival and function. The ability of immune cells to pass through tight epithelial cell layers from circulating blood during infection, the ability of tumor cells to travel throughout the body during metastasis, migration potential of the cells after epithelial-to-mesenchymal transition could be examples where cells undergo extensive remodeling. Although numerous studies aimed at finding protein markers during these key steps, there is a major gap in our understanding of how collective biophysical properties of the cells (such as stiffness, fluidity, and viscosity) alter during these crucial biological processes. Similarly, our understanding of how biophysical properties of cells change in diseases is extremely limited. Moreover, biophysical properties can vary without notable changes in protein or RNA levels. Therefore, these physical properties can be exploited as complementary to current protein or nucleic acid markers to diagnose and treat diseases. However, current biophysical technologies suffer from low sampling, which is a major obstacle to apply them to medical problems that require measuring thousands of cells. Here, we describe a high-throughput platform based on spectral flow- cytometry and environmental sensitive probes, tackling this bottleneck and enabling fast measure of the biophysical properties. Combining with the machine learning, this method enables automated prediction of health state.

Poster Presentations
– Session 1 –

P-69
Investigating the Effect of Substrate Elasticity on Talin-1 Distribution in Fibroblasts Employing Semi-Automatic Image Analysis Software

Joanna Hajduk[1,2], Zbigniew Baster[3], Patrycja Twardawa[1,2], Zenon Rajfur[2,4]
[1] Doctoral School of Exact and Natural Sciences, Jagiellonian University, Cracow, Poland, [2] Faculty of Physics, Astronomy and Applied Computer Science, Jagiellonian University, Cracow, Poland, [3] Cell and Developmental Biology Center, National Heart Lung and Blood Institute, National Institutes of Health, Bethesda, USA, [4] Jagiellonian Center of Biomedical Imaging, Jagiellonian University, Cracow, Poland

Adhesion structures connect cells to the extracellular matrix (ECM) through integrins. They play a vital role in cell migration, proliferation, and wound healing. Adhesion structures are also involved in mechanotransduction, allowing cells to sense and respond to mechanical signals from the environment. Talin-1 is a key focal adhesion protein that binds integrins and the actin cytoskeleton. In this study, we investigated how microenvironment elasticity affects the morphology of adhesion structures in fibroblasts, particularly Talin-1 distribution. We studied adhesion structures in MEF 3T3 fibroblasts seeded on polyacrylamide substrates of varying elasticity with confocal microscopy. To classify, segment, and analyze the chosen parameters of focal adhesions, such as their number in the cell, size, shape, and relative fluorescence intensity, we developed a semi-automatic image analysis software. Our initial findings show that microenvironment elasticity significantly influences Talin-1 distribution. On the softer substrates, talin-1 is arranged in more elongated structures running through the center of the cell. Understanding how the microenvironment affects adhesion structures architecture can provide valuable insights into the mechanobiology of cell behavior.

P-70
Insights into the Binding of ScCBM2 to Polymer Substrates using NMR Spectroscopy and Spectrophotometry

Susanne Hansen Troøyen[1], Gaston Courtade[1]
[1] Norwegian University of Science and Technology (NTNU), Norway

We present a study on the binding mechanism of a carbohydrate-binding module (ScCBM2) from Streptomyces coelicolor to cellulose and polyethylene terephthalate (PET) substrates. Carbohydrate-binding modules (CBMs) are non-catalytic binding modules that are tethered to carbohydrate-active enzymes via a linker peptide. By localizing the enzyme towards the surface of an insoluble substrate, these binding modules are important for regulating enzymatic activity. Despite their importance, the actual mechanism of how CBMs bind to their substrates is still not fully understood, and it is interesting to explore how their binding properties are influenced by various factors. ScCBM2 has a flat binding surface with three aromatic amino acids that interact with crystalline regions of cellulose through π-stacking interactions and hydrogen bonding. Although cellulose is the natural substrate for ScCBM2, it has also been observed to bind to PET. Our investigation aims to understand how ScCBM2 binds to both cellulose and PET, and how the structure of the substrates affects this interaction. Additionally, we explore how site-directed mutagenesis of specific amino acids in the binding site can tune the binding affinity. We use a combination of NMR spectroscopy and spectrophotometry to study the binding event in detail. Our findings shed light on the complex binding mechanism of CBMs, which is important for developing new strategies for enzymatic depolymerization of both cellulose and polyester substrates.

P-71
Cross-linking and Hydrogen-Deuterium Exchange Mass Spectrometry as a National Infrastructure Service

Lotta Happonen[1,2,3], Simon Ekström[2,3]
[1] Division of Infection Medicine, Department of Clinical Sciences, Lund University, Lund, Sweden, [2] Swedish National Infrastructure for Biological Mass Spectrometry (BioMS), Lund University, Lund, Sweden, [3] Structural Proteomics, SciLifeLab Integrated Structural Biology platform (ISB), Lund, Sweden

Cross-linking (XL-MS) and hydrogen-deuterium exchange mass spectrometry (HDX-MS) are powerful techniques in structural proteomics. XL-MS provides evidence of proteins interacting with each other as well as structural information of individual proteins or protein complexes by providing distance constraints between two cross-linked peptides. The distance constraints arise from the defined length of the cross-linker used. HDX-MS analysis can be used to obtain information on structure, protein-protein interaction sites, allosteric effects, intrinsic disorder, and conformational changes. HDX-MS takes advantage of the labile nature of the exchangeable protons present on the protein backbone amides. When dissolved in solution, proteins exchange these protons with hydrogen groups present in a deuterated buffer. The rate of hydrogen to deuterium exchange can be measured by MS and provides a measure of solvent accessibility and is used to infer structural information. We demonstrate examples from published user projects, including antibody epitope mapping and studies using integrative methods in structural biology.

P-72
Unravelling the unique role of glutamine dynamics in disease-related aggregation

Karin Hauser[1], Ho-Wah Siu[1], Benjamin Heck[1]
[1] University of Konstanz, Germany

Polyglutamine (polyQ) diseases, like e.g. Huntington disease, are caused by expanded polygQ repeats in the affected protein and are accompanied by the formation of polyQ fibrils. The unique role of glutamines in the aggregation onset is undoubtedly accepted. Although the morphology of the fibrils has been studied in detail, the side-chain specific structural dynamics inducing oligomerization are not well understood yet. We designed various template-assisted glutamine-rich beta-hairpin monomers mimicking the structural motif of a polyQ fibril. In a top-down strategy, a soluble hairpin was used as template and polyQ-rich sequences were inserted into each strand. CD, IR, and NMR spectroscopic studies reveal a decreasing hairpin stability with increased glutamine content and demonstrate the enormous impact of only a few glutamines to destabilize structure. Furthermore, we could access sub-ms conformational dynamics of oligomeric precursor states by laser-excited temperature-jump IR spectroscopy. Both, the increased number of interacting glutamines and higher concentrations are key parameters to nucleate a fibril. Early oligomeric states were characterized by a highly dynamic nature in contrast to the slow dynamics observed for fibril expansion. Site-specific isotope labelling provided insights into the side-chain dynamics of individual glutamines. We propose that a high structural flexibility of oligomers is required to nucleate a fibril, but not anymore after a fibrillar structure has consolidated. Our studies reveal that structural dynamics changes at different stages in the aggregation process, from fibril initiation to fibril growth.

Poster Presentations
– Session 1 –

P-73
Towards understanding transcription factor oligomerization regulation in live cells

Katherina Hemmen[1], Anay Fernanda Lazaro Alfaro[2], Thomas-Otavio Peulen[1], Exequiel Antonio Medina[3], Katrin G. Heinze[1], Hugo Sanabria[2]

[1] Julius-Maximilians University Wuerzburg, Rudolf Virchow Center for Translational and Integrative Bioimaging, Wuerzburg, Germany, [2] Clemson University, Department of Physics and Astronomy, Clemson, South Caroline, U.S. A., [3] University of Chile, Department of Biology, Santiago, Chile

Transcription Factors (TF) are often multidomain proteins that gate access to specific DNA sequences crucial for gene activation or repression. The P-family of the Forkhead-box TFs (FoxP) contains a highly conserved Forkhead-box DNA binding (FKH) and a leucine Zipper (ZIP) domain connected by an unstructured region. The FKH and ZIP domains are known to dimerize, but the exact dynamics and mechanisms under physiological conditions are still unclear. We study the assembly of FoxP1 oligomers by multiparameter fluorescence imaging spectroscopy. Experiments on full-length and truncated FoxP1 domain variants (ZIP-FKH, FKH) tagged by eGFP or mCherry in live cells delineate the oligomerization propensity of individual domains. An automated analysis pipeline processes fluorescence-polarization resolved confocal PIE- FRET-FLIM data to determine mean fluorescence lifetime and fluorescence anisotropies and integrates machine learning based segmentation, and nuclei classification to spatial resolved compositions of molecular complexes. Our preliminary results hint to a cell-cycle dependent redistribution of FoxP1, in which FoxP1 seems to gather at hot spots in the nucleus.

P-74
Detection of amyloid polymorphism using super resolution fluorescence microscopy

Elnaz Hosseini[1], Qiqi Yang[2], Sapun Parekh[2,3], Xiaomin Liu[2]

[1] University of Tehran, Iran, Institute of Biophysics and Biochemistry, [2] Max Planck Institute for Polymer Research, [3] Department of Biomedical Engineering, University of Texas at Austin

Amyloids are fibrillar protein states that have been correlated with neurodegenerative disorders including Alzheimer's and Parkinson's diseases. Investigations have shown that each amyloid protein assembles into specific morphologies that can have distinct toxicity. Recent studies show that amyloid fibrils have polymorphic structures at the molecular level. Polymorphism is a classical feature of amyloid fibrils, and there is no exact mechanism to explain these structural differences for a particular polypeptide chain. The ability to investigate various structural mechanisms of fibrils with high resolution techniques is thus essential. Super-resolution imaging fluorescence microscopy techniques allow us to image structures with nanoscale resolution under in situ conditions using (relatively) straightforward preparation techniques. In this study, we use single-molecule localization microscopy (SMLM) to image fibril structures using the standard amyloid probe Thioflavin T (ThT) with stable binding. ThT binds to β- sheet structure of amyloid and becomes emissive upon this interaction. To probe structural polymorphism, we also used a covalently bound dye (Cy3) and compared images of two different dyes. We report SMLM from different fibril morphologies and polymorphisms formed by different protein sequences (Amyloid-β, α-synuclein and insulin). Also, we find that the affinity of ThT to bind to the different morphology of fibrils changes with specific peptide sequences. In summary, SMLM microscopy is a simple and rapid imaging technique to complement other microscopic techniques such as atomic force microscopy (AFM) that can also detect heterogenous structure of amyloids with nanometer resolution.

P-75
Fabrication of Gold-Coated AFM-based TERS Tips by Electrochemical Methods and Its Application on L34T Fibrils

Yuhan Huang[1], Patrick Garrigue[1], David Talaga[1], Gerardo Salinas[1], Gary Cooney[1], Laurent Bouffier[1], Sébastien Bonhommeau[1]

[1] The Institute of Molecular Sciences, France

Nanoscale analysis is important for areas such as molecular electronics, materials, biophysics, and biology. Tip-enhanced Raman spectroscopy (TERS) has gained considerable interest due to its nanoscale spatial resolution, high sensitivity, and ability of simultaneous topographic and spectroscopic imaging. By using a sharp metallic nanotip to confine and enhance the light field near the tip apex, a single hot-spot is excited and nanoscale chemical imaging is achieved. Different biological samples such as amyloid fibrils, nucleic acids and membranes have been investigated with TERS to get detailed characterization including chemical and structural information. In this work, we fabricated gold-coated AFM-based TERS tips through electrochemical methods and performed TERS measurements on L34T fibrils in air with side-illumination TERS system. This result demonstrates the feasibility of electrochemical deposition as an easy and powerful tool to fabricate metal coated AFM-based TERS tips and the applicability of such modified tips for the analysis of biological samples. This method is promising in order to electrodeposit different metals on the surface of AFM tips and use these modified probes to analyze biological samples in both air and liquid medium.

P-76
Molecular interactions between human diamine oxidase and heparins

Jenny Isaksson[1], Liisa Pösö[1,2], Elisabeth Gludovacz[3,4], Tomi Airenne[1], Bernt Jilma[3], Thomas Boehm[3], Tiina Salminen[1]

[1] Åbo Akademi University, Turku, Finland, [2] University of Turku, Finland, [3] Medical University of Vienna, Austria, [4] University of Natural Resources and Life Sciences, Vienna, Austria

Human diamine oxidase (DAO) is the enzyme responsible for extracellular deamination of histamine. Using DAO as a therapeutic drug in conditions with elevated histamine levels, e.g., mastocytosis and anaphylaxis, is desirable but still not possible due to the rapid clearance of DAO from circulation. Recent studies show the importance of heparins for the binding and cellular internalization of DAO. High, but not low, molecular weight heparins reduce the internalization of DAO leading to increased plasma concentrations of DAO. Binding of heparins to DAO is supported by our computational analysis showing that a heparin-derived hexasaccharide can be successfully docked into the heparin-binding motif located on the DAO surface. The aim of the ongoing study is to elucidate the atomic details of the complexes between human DAO and different heparins. The results contribute to a better understanding of the molecular interactions between DAO and heparins, which is essential for the development of DAO as a treatment to histamine-related disorders.

Poster Presentations
– Session 1 –

P-77
Catalytic center of as isolated and high-energy metastable forms of oxidized cytochrome c oxidase: Protonation and ligand binding study

Daniel Jancura[1], Adriana Tomkova[1], Tereza Sztachova[1], Vladimir Berka[2], Maian Fabian[3]
[1] Department of Biophysics, Faculty of Science, University of P.J. Safarik in Kosice, Slovakia, [2] Department of Internal Medicine, University of Texas Health Science Center, Houston, USA, [3] Center for Interdisciplinary Biosciences, Technology and Inovation Park, University of P.J. Safarik in Kosice, Slovakia

Cytochrome c oxidase (CcO) catalyzes the electron transfer from ferrocytochrome c to O2 and contributes to the formation of electrochemical proton gradient on the inner mitochondrial membrane. One of the current models of the proton pumping relies on the existence of a high-energy metastable OH state, which is formed immediately after oxidation of the fully reduced CcO with oxygen. In the absence of external electron donor, the high-energy OH form should relax to the resting oxidized form. In this work. the reaction of as "isolated fast form" (O) and OH state of the oxidized CcO with hydrogen peroxide have been investigated by stopped-flow spectroscopy. The rate constants for the formation of ferryl forms of CcO after its interaction with hydrogen peroxide are almost identical for both forms of the fully oxidized CcO. Using phenol red as a pH indicator we have found that a proposed relaxation of OH to O state is not accompanied neither with the proton uptake or proton release. These results together with our previous findings suggest that there is no difference in the ligation and protonation states of the catalytic site of O and OH forms of the oxidized CcO.

P-78
Constant-pH Molecular Dynamics Simulations of a Proton-Gated Ion Channel

Anton Jansen[1,2], Paul Bauer[1,2], Rebecca Howard[1,3], Berk Hess[1,2], Erik Lindahl[1,2,3]
[1] SciLifeLab, [2] KTH, [3] Stockholm University, Sweden

Conformational cycling in many macromolecules is modulated by pH. Molecular dynamics (MD) simulations are used routinely to study a variety of structural transitions, for example between functional states of an ion channel. However, in classical MD simulations titratable residues such as aspartic and glutamic acid are assigned fixed protonation states. In reality, such charge states can change dynamically, and may play an important role in functionally relevant mechanisms. Although various MD algorithms have been previously proposed to capture dynamic protonation, their implementations can be computationally intensive or restricted in features, rendering them of limited applicability to large complex systems. Here, we applied a recent constant-pH implementation in the GROMACS molecular dynamics package to model protonation-dependent conformational states of the ion channel GLIC. Simulations of structures determined in apparent resting or activated states, under both neutral and acidic conditions, enabled the identification of key titratable amino-acid residues involved in initiating or propagating transitions on the channel gating pathway. This work not only provides insight into the gating of a ligand-gated ion channel, but also into the practical utility of constant-pH simulations for modeling dynamic protonation effects in a multimeric, multi-domain, pH-sensitive membrane protein.

P-79
Tissue tension of cysts – the role of viscoelasticity and superelasticity

Andreas Janshoff[1], Amaury Tirado[1]
[1] University of Goettingen, Germany

MDCK cells form multicellular cysts (acini or spheroids) comprising a closed monolayer of polarized cells that encloses liquid. The tissue tension of these cysts is typically assessed through Laplace's law by measuring the internal pressure. Here, we present force-relaxation experiments performed on MDCK II cysts and describe the response to external deformation by a theoretical framework accounting for possible superviscoelasticity of the spheroids. It was found that the cells provide excess tissue area by thinning of the cell monolayer to reduce tension upon deformation. This mechanism that also protects single cells from lysis is used on larger length scales as a universal mechanism to withstand external stress.

P-80
Dynamics of γD-crystallin undergoing Liquid-Liquid phase separation observed through EPR, Fluorescence spectroscopy and MD simulations.

Abhishek Kalarikkal[1], Dominik Gendreizig[2], Laura Galazzo[1], Saumyak Mukherjee[1], Arnulf Rosspeintner[2], Alexandre Fuerstenberg[2], Lars Schaefer[1], Christian Herrmann[1], Enrica Bordignon[2]
[1] Ruhr University Bochum, Germany [2] University of Geneva

γD-crystallin belongs to a family of highly homologous mammalian eye lens crystallin proteins. This globular protein undergoes liquid-liquid phase separation (LLPS) at high concentrations and low temperature, marked by a cloudy appearance of the protein solution which is similar to what is observed in cataract formation. The onset of LLPS in γD-crystallin can be modulated by cosolutes and temperature. To probe the site-specific sidechain dynamics of γD-crystallin during phase transition, we labelled the protein with a nitroxide label. Using continuous-wave EPR, we investigated the spectral fingerprint of the protein at varying temperature and cosolutes and identified the onset temperature of LLPS. To discern the sidechain dynamics from the overall rotational tumbling of the protein, we additionally performed bulk anisotropy measurements, fluorescence lifetime and time resolved fluorescence anisotropy on fluorescein-labelled γD-crystallin in the dilute and condensed phase. The challenges in data analysis in each type of measurement are discussed. The rotational correlation times of the protein from fluorescence anisotropy are compared to those calculated based on MD simulations done on the diluted and condensed phase. This study aims to reveal the timescale of the rotational motion of a globular protein during phase separation through an integrated biophysical approach.

Poster Presentations
– Session 1 –

P-81
Probes for Single-Molecule Microscopy Analysis of ErbB4 Biophysical Properties
Barbora Kalouskova[1], Tayde Gabriela Serrano Cano[2], Peter Nagy[2], Mario Brameshuber[1]
[1] Institute of Applied Physics, Biophysics Group, TU Wien, Austria , [2] Department of Biophysics and Cell Biology, University of Debrecen, Hungary

Receptor tyrosine kinase ErbB4 is an outstanding epidermal growth factor receptor (EGFR or ErbB) family member. It cooperates with other family members (EGFR, ErbB2, and ErbB3) in promoting various cellular physiological responses. It affects proliferation, migration, and cell survival, thus having an impact not only on healthy tissue but also on tumor suppression or progression. Upon binding of a growth factor (e.g. heregulin-β1), ErbB4 changes its conformation, enabling homodimerization or heterodimerization with other ErbB family members. However, the higher organization of ErbB4 into oligomers in the context of the cell membrane environment has not yet been well described because of the lack of stoichiometric probes and quantitative live cell approaches for the characterization of these single molecule oligomerization events. We will apply the quantitative single-molecule microscopy method TOCCSL (thinning out clusters while conserving the stoichiometry of labeling) as well as single-molecule tracking to exploit ErbB4 global organization on the cell surface, focusing on the differences between activated and non-activated receptor status. We generated and tested fluorescently labeled high-affinity monovalent DARPins (designed ankyrin repeat proteins) with a close to 1:1 labeling stoichiometry. This probe, together with the quantitative imaging, will enable to elucidate ErbB4 plasma membrane organization on the single-molecule level.

P-82
Structural analysis and engineering of β-trefoil lectins
Kenichi Kamata[1,2], Yuki Fujii[3], Yasuhiro Ozeki[2], Jeremy Tame[2], Arnout Voet[1]
[1] KU Leuven, Belgium [2] Yokohama City University, [3] Nagasaki International University

Protein design is a rapidly evolving field with great potential for applications in biocatalysis, drug discovery, and materials science. In this study, we aimed to functionalize lectins using protein design. Lectins are glycan-binding proteins with a wide range of biological functions, including cell adhesion, signalling, and immune response. We focused on the β-trefoil lectin, aiming to create a symmetrical structure and analyze it using X-ray crystallography, Isothermal Titration Calorimetry (ITC), and NMR spectroscopy. By introducing symmetry, the thermal stability and resistance to denaturants of the designed lectin was increased. These results demonstrate the potential of protein design to create lectins with improved properties for glycan profiling, diagnostics, and therapies. Further research in this field will expand the scope of lectin-based biotechnology, leading to new and exciting applications.

P-83
Conflicting Interfacial Electrostatic Interactions as a Design Principle to Modulate Long-Range Inter-Domain Communication
Adithi Kannan[1], Dhruv Kumar Chaurasiya[1], Athi N. Naganathan[1]
[1] Department of Biotechnology, Indian Institute of Technology Madras, India

The precise molecular features underlying inter-domain allosteric communication and hence, function in multidomain proteins is an open question. One possible, simple evolutionary strategy could involve the selection of conflicting or favorable long-range electrostatic interactions at the interface of two closely spaced domains to modulate the inter-domain connectivity. In this work, we study a two-lobed domain FF34, a part of the eukaryotic p190A RhoGAP protein, to explore a potential design principle that can be exploited to tune the local and global traits of a protein. We integrate a collection of spectroscopic techniques, calorimetry with statistical mechanical modeling, simulations and sequence-structure analysis to study and tune the behavior of FF34. We find that while the individual subdomains FF3 and FF4 are marginally coupled with low cooperativity, they display distinct intrinsic stabilities, manifesting as slow folding. Their interface harbors a network of highly conserved but frustrated charged residues — charge troika — that enables the protein to sample multiple metastable structures, stabilised by non-native interactions on a rugged landscape. Perturbing this network via a charge reversal mutation flips the protein into a highly stable, cooperative and coupled entity with dampened fluctuations, a smooth landscape and hence, a faster folding rate by at least an order of magnitude. Our work highlights how a non-optimal selection of interface electrostatics shapes a native ensemble of a bilobed protein, a feature that could be harnessed to design molecular systems with enhanced long-range coupling and cooperativity.

P-84
IgG oligomer mediated Fc Receptor clustering in the spotlight
Andreas Karner[1], Maryam Marefat[1], Martina Hofmann[1], Christine Siligan[2], Johannes Preiner[1]
[1] TIMed Center, FH Upper Austria, Linz, Austria, [2] Institute of Biophysics, Johannes Kepler University, Linz, Austria

Poster Presentations
– Session 1 –

P-85
Diverse structural landscape of the engineered potato virus Y coat protein assemblies
Luka Kavčič[1], Andreja Kežar[1], Neža Koritnik[1], Magda Tušek-Žnidarič[2], Franci Merzel[3], Elena Holden[4], Justin L.P. Benesch[4]
[1] Department of Molecular Biology and Nanobiotechnology, National Institute of Chemistry, Ljubljana, Slovenia, [2] Department of Biotechnology and Systems Biology, National Institute of Biology, Ljubljana, Slovenia, [3] Theory Department, National Institute of Chemistry, Ljubljana, Slovenia, [4] Department of Chemistry, Physical & Theoretical Chemistry Laboratory, University of Oxford, Oxford, United Kingdom

The capsids of plant RNA viruses represent a collection of evolutionarily optimized structural assemblies of different shapes and sizes. Potato virus Y (PVY) is a flexible filamentous single-stranded RNA virus and the most important viral pathogen of potato worldwide. Its coat protein (CP), which has a high degree of intrinsic disorder, is the only structural component of infectious virions. It plays many other roles in the viral life cycle, however, the structural landscape of PVY CP facilitating its multitasking has never been explored previously. Using combined structural and biophysical approaches, we elucidate the role of intrinsically disordered regions (IDRs) in the polymorphic CP self-assembly leading to the simultaneous formation of three distinct filament types. Based on their high-resolution cryo-EM models and additional mutagenesis, we can redirect the association of protomers towards the desired filament morphology. Furthermore, by partially truncating CP IDRs in combination with single amino acid substitutions, we unlock entirely novel modes of CP self-assembly, leading to single or double octameric rings, highly ordered cubes, spherical particles and more. By fusing it to other proteins, we can additionally control the self-assembly process in space and time. In summary, we demonstrate the enormous potential of PVY CP intrinsic plasticity to generate diverse structural assemblies with desired architectural and chemical properties not previously observed for CPs from filamentous viruses.

P-86
Cooperativity between skeletal myosin molecules evaluated by information theory
Motoshi Kaya, Arun Kasimchetty, Hideo Higuchi
[1] University of Tokyo, Japan

Previous single-molecule studies have shown that the function of a single protein molecule is extremely variable and deviates from the higher-order function of biomolecular tissue. Therefore, we have focused on skeletal and cardiac myosins, and measured force of their molecular ensembles, myofilaments, revealing the existence of cooperative force generation between molecules, which is essential for contractile functions in muscle and the heart. In this study, we apply information theory as a method to quantitatively evaluate how the fluctuations of the molecules can be utilized to respond more accurately to changes in the external environment during the process of molecular assembly. In particular, by evaluating the amount of information transfer, we will clarify the mechanism of how the fluctuation of skeletal myosin molecules can be utilized to enhance the amount of information transfer between molecules in the molecular assembly. In the experiment, forces in a myofilament were measured by optical tweezers, and the amount of mutual information was obtained from the probability distribution of the input-output relationships between force and velocity changes observed in myofilaments. We have found that the mutual information of myofilaments, that contain approximately 20 interacting molecules, indicates an information transfer rate of about 60-80% for 3-4 bits of input. In the future, we plan to evaluate the amount of information transfer in myofilaments with reduced interacting molecules and a single myosin molecule embedded in myofilament and understand the cooperative phenomena between myosin molecules from the viewpoint of information theory.

P-87
Coral acid-rich protein-mediated process of calcium carbonate spherulites formation
Barbara Klepka[1], Tomasz Wojciechowski [1], Anna Niedzwiecka[1]
[1] Institute of Physics, Polish Academy of Sciences, Poland

One of the most pressing issues these days is predicting the future consequences of global climate warming. The rising temperatures and acidification of the oceans can have a great impact on the marine life. To be able to determine how those factors can affect the marine animals one has to fully understand the molecular apparatus governing the processes related to their development and survival including skeleton formation. It is postulated that coral acid-rich protein (CARPs) that are secreted to the extracellular matrix are of great importance in terms of biomineralization. So far, only four CARPs have been cloned and partially characterized. It was shown that these proteins bind calcium ions stoichiometrically and alter the morphology of calcium carbonate crystals in vitro. However, it is still quite elusive what are the molecular basis of CARP-mediated (mezo) crystal formation, how are CARPs incorporated into the $CaCO_3$ phases and how CARPs can contribute to the emergence of spherulitic structures. We have cloned for the first time two novel CARPs – secreted acidic protein 1A and aspartic and glutamic acid-rich protein of Acropora millepora coral species. By means of i.a. combination of fluorescence-based methods and scanning electron microscopy we aim to shed some light on the molecular mechanism of CARP-driven calcium carbonate precipitation starting with the emergence of CARP-Ca^{2+} clusters and ending with the fully grown spherulitic $CaCO_3$ phase.

P-88
Structure and dynamics of low density lipoprotein targeted by cryo-EM and HS-AFM
Karin Kornmueller[1,2], Sarah Stainer[3], Aline Cisse[2,4], Ambroise Desfosses[5], Eaazhisai Kandiah[6], Gerd Leitinger[7], Gerd Hoerl[8], Peter Hinterdorfer[3], Judith Peters[2,4,9], Ruth Prassl[1]
[1] University of Graz, Austria, [2] Institut Laue-Langevin, France, [3] University Linz, Austria, [4] Université Grenoble Alpes, CNRS, LiPhy, France, [5] Institut de Biologie Structurale, Université Grenoble Alpes, CEA, CNRS, IBS, France, [6] European Synchrotron Radiation Facility, France, [7] Gottfried Schatz Research Center for Cell Signaling, Metabolism and Aging, Division of Cell Biology, Histology and Embryology, Medical University of Graz, Graz, Austria, [8] University of Graz, Austria, [9] Institut Universitaire de France

Low density lipoprotein (LDL) plays a crucial role in the human cholesterol transport system and is a key-player in the development of cardiovascular diseases. The development of novel treatment strategies requires a deep understanding of LDLs structure and dynamics on a molecular level. Structural analyses are challenging – LDL is a complex nano-assembly comprising different species of lipids and one large monomeric protein, apolipoprotein B-100 (apo B-100). Here we combined two powerful techniques to study LDL in its near-native state: cryo- electron microscopy (cryo-EM) and high-speed atomic force microscopy (HS-AFM). Single particle analysis of the cryo-EM micrographs yielded a 9 Å resolution map, in which we resolved substructures in the layered LDL core that led to a new model for the cholesterol-ester core organization.
We found that two protrusions emerged from the particle, which we attributed to specific domains of apo B-100. Variability analysis demonstrated that the protrusions are highly flexible. Using HS-AFM their dynamics and flexibility was directly visualized. We tracked large domain fluctuations of apo B-100 over several milliseconds, with nanometer spatial resolution. Apo B-100's flexibility is a crucial property of LDL, in accordance with its role in metabolism. An in-depth knowledge of structural and dynamical features of LDL will open new avenues for rational drug-design, combating cardiovascular diseases.

Poster Presentations
– Session 1 –

P-89
14-3-3 dimer vs monomer – (dis)similarities in Tau protein binding

Aneta Kozeleková[1,2], Lucia Ilkovičová[2], Radek Crha[1,3], Alena Hofrová[2], Jozef Hritz[1,4]
[1] Central European Institute of Technology, Masaryk University, Kamenice 5, Brno, 625 00, Czech Republic, [2] National Centre for Biomolecular Research, Masaryk University, Kamenice 5, Brno, 625 00, Czech Republic, [3] Institute for Molecular Modeling and Simulation, Department of Material Sciences and Process Engineering, University of Natural Resources and Life Sciences, Muthgasse 18, 1190, Vienna, Austria, [4] Department of Chemistry, Faculty of Science, Masaryk University, Kamenice 5, Brno, 625 00, Czech Republic

Neurofibrillary tangles (NFTs), composed of aggregated hyperphosphorylated Tau protein, are one of the hallmarks of Alzheimer's disease (AD). Up to now, the mechanism of Tau aggregation is not well understood. In the late 1990's, 14-3-3 proteins, highly abundant proteins in brain, were found in NFTs. 14-3-3s usually exist as dimers, but after Ser58 phosphorylation they monomerize. In our project, we aimed to characterize the interaction between dimeric and monomeric 14-3-3s and PKA-phosphorylated Tau (pTau). The binding affinity, stoichiometry and interacting residues were studied using native-PAGE, NMR spectroscopy, cross-linking and tandem MS. 14-3-3 dimer interacted with pTau with higher affinity and different stoichiometry compared to 14-3-3 monomer. Using NMR, interaction dissociation constants (KDs) of individual PKA-phospho-sites on pTau were determined. Tau bound to 14-3-3 predominantly by microtubule binding domain, which suggests competition between 14-3-3s and microtubules in Tau binding and relation to AD pathology. In conclusion, we provide an insight into numerous aspects of binding between monomeric and dimeric 14-3-3s and Tau protein.

P-90
Investigation of Sponge Phase Nanoparticles for Drug Delivery Purposes

Thea Kristersdotter[1], Pedro Aoki[1], Jennifer Gilbert[2], Marshall R. Machingauta[2], Tommy Nylander[2], Christelle N. Prinz[1]
[1] Division of Solid State Physics, Lund University, [2] Division of Physical Chemistry, Lund University, Sweden

Sponge phase nanoparticles are lipid-based nanoparticles composed of a combination of lipids and a stabiliser. Their size ranges from 100 to 250 nm with pore sizes of up to 13 nm. This "large" pore size possibly allows for delivery of larger cargos into cells than the commonly used ionizable lipid nanoparticles (used in e.g. novel cancer therapies and mRNA vaccines). However, the interactions between sponge phase nanoparticles and cells are not well understood. It is unclear whether these nanoparticles can escape endosomes, and avoid autophagy and degradation once in the cytosol. Here, we investigate the fate of sponge phase nanoparticles after delivery to human breast cancer cells via incubation and nanopore- injection. Incubation results in internalisation via endocytosis, enabling the evaluation of endosomal escape, whereas nanopore-injection results in direct delivery to the cytosol, allowing for the investigation of additional cellular degradation mechanisms such as autophagy and enzymatic degradation. The nanoparticle fate within the cells is examined using fluorescence and STED microscopy and the internalisation is quantified and compared between the delivery methods. Our aim is to gain a better understanding of the nanoparticle fate and their ability to deliver cargos into cells.

P-91
Native doublet microtubules from Tetrahymena thermophila reveal the importance of outer junction proteins

Shintaroh Kubo[1,2], Corbin S. Black[2], Ewa Joachimiak[3], Shun Kai Yang[2], Thibault Legal[2], Katya Peri[2], Ahmad Abdelzaher Zaki Khalifa[2], Avrin Ghanaeian[2], Caitlyn L. McCafferty[4], Melissa Valente-Paterno[2], Chelsea De Bellis[2], Phuong M. Huynh[2], Zhe Fan[2], Edward M. Marcotte[4], Dorota Wloga[3], Khanh Huy Bui[2]
[1] The University of Tokyo, Japan, [2] McGill University, Canada, [3] Nencki Institute of Experimental Biology of Polish Academy of Sciences, Poland, [4] University of Texas, Austin, USA

Cilia are eukaryotic organelles responsible for cellular motility and sensory functions. The ciliary axoneme is a microtubule-based cytoskeleton consisting of two central singlets and nine outer doublet microtubules. Cryo-EM has revealed a complex network inside the lumen of both tubules composed of microtubule-inner proteins (MIPs). However, the functions of most MIPs remain unclear. Here, we present the Tetrahymena thermophila native doublet microtubule and identify 42 MIPs by single-particle cryo-EM-based analyses and MD simulations. These data make clear on the conserved and diversified roles of MIPs. In addition, we identified MIPs potentially responsible for the assembly and stability of the doublet outer junction. Knockout of the evolutionarily conserved outer junction component CFAP77 moderately diminishes Tetrahymena swimming speed and beat frequency, indicating the important role of CFAP77 and outer junction stability in cilia beating generation and/or regulation.

P-92
Molecular mechanisms of amyloid-β self-assembly and chaperone-mediated inhibition can be translated to in vivo-derived fibril seeds

Rakesh Kumar[1], Luis Enrique Arroyo García[2], Shaffi Manchanda[1], Laurène Adam[1], Giusy Pizzirusso[2], Henrik Biverstål[1], Per Nilsson[3], Andrè Fisahn[3], Jan Johansson[1], Axel Abelein[1]
[1] Department of Biosciences and Nutrition, Karolinska Institutet, 141 52 Huddinge, Sweden, [2] Department of Women's and Children's Health, Karolinska Institutet, 171 64 Solna, Sweden, [3] Division of Neurogeriatrics; Center for Alzheimer Research; Department of Neurobiology, Care Sciences and Society, Karolinska Institutet, 171 64 Solna, Sweden

Alzheimer's disease (AD) is the most common neurodegenerative disease, and it is closely associated with amyloid-β peptide (Aβ) fibril formation. In vitro studies on Aβ self-assembly have resulted in detailed insights into Aβ self-assembly, however in vivo studies on Aβ self-assembly remains elusive. Here, we isolated in vivo-derived fibrils and investigated the ability of in vivo-derived Aβ extracts to seed Aβ42 aggregation in vitro using two different App knock-in AD mouse models. From our seeded kinetics data, we quantified the microscopic rate constants. Interestingly, the nucleation mechanism of in vivo-derived fibril seeded Aβ42 aggregation can be also described the same kinetic model as applied in vitro. Further, we investigated the effect of the anti-amyloid BRICHOS chaperone on in vivo-derived fibril seeded Aβ42 self-assembly and identified the inhibitory mechanism of BRICHOS. The results showed suppression of secondary nucleation and fibril elongation, which is strikingly similar as observed in vitro. These findings hence provide a molecular understanding of the Aβ42 nucleation process of in vivo-derived Aβ42 seeds and may provide a framework for the search of new AD therapeutics.

Poster Presentations
– Session 1 –

P-93
Structure determination and ab initio prediction provide new insights into the diversity of Magnaporthe oryzae MAX effectors

Mounia Lahfa[1], Karine De Guillen[1], Nathalie Declerck[1], Philippe Barthe[1], Jérôme Gracy[1], Florian Charriat[2], Pierre Gladieux[2], Stella Césari[2], Thomas Kroj[2], Christian Roumestand[1], André Padilla[1]
[1] Center for Structural Biology, University of Montpellier, CNRS, INSERM, Montpellier, France, [2] PHIM Plant Health Institute, University of Montpellier, INRAE, CIRAD, Agro Insitute, IRD, Montpellier, France

MAX effectors are a family of proteins widely distributed in the phytopathogen fungus Magnaporthe oryzae that share a conserved 3D structure despite low sequence identity. These effectors family of almost 100 members constitute the molecular arsenal for the fungus infection and contribute to the pathogenicity of the plant pathogen that causes blast disease of a wide variety of cereals. Determining the structure of these effectors is a challenging case study that was overcome by the development of a prediction pipeline combined with modeling using the Alphafold software. After sequence screening of M. oryzae secretomes and identification of MAX effectors the Alphafold models were confronted with experimentally validated NMR structures. The compilation of models and experimental structures highlighted the existence of variability within this structural family that was essentially reflected by the existence of structured N- or C-terminal extensions beyond the preserved MAX core. Folding pathways were studied by High Pressure NMR to further characterize folding intermediates that involved elements of the MAX core but also additional extensions. Taken together, this work constitutes the first step in mapping the functional network of these effectors through their structure by identifying possible interacting sites within the MAX effectors that are useful to prioritize plant biology studies of infected hosts.

P-94
New insights into the structural dynamics of intrinsically disordered proteins by field-dependent NMR relaxation measurements

Nils-Alexander Lakomek[1], Tobias Stief
[1] University of Dusseldorf, Germany

Intrinsically disordered proteins (IDPs) and proteins with large intrinsically disordered regions (IDRs) constitute about 30% of the human proteome. Their high internal dynamics complicate structural studies, e.g., by cryo-EM or X-ray crystallography. However, NMR spectroscopy is well suited to investigate IDP structural dynamics with atomic resolution. As a model system, we use the SNARE (soluble N-ethylmaleimide- sensitive-factor attachment receptor) proteins synaptobrevin-2 and SNAP25. They participate in forming the SNARE complex that drives synaptic vesicle membrane fusion, a prerequisite for neurotransmitter release. In their monomeric form (before fusion), the SNARE proteins' cytosolic part is intrinsically disordered. To assess the internal dynamics of synaptobrevin-2 and SNAP25, we recorded NMR relaxation experiments at different magnetic field strengths, between 14 and 28T, corresponding to Proton Larmor frequency between 600 and 1200 MHz. The field-dependent NMR measurements reveal novel insight into the structural dynamics of synaptobrevin-2 and SNAP25, both for the picosecond to nanosecond timescale and for the micro- to millisecond timescale. In particular, the 1200 MHz data allow for deeper insights and better discrimination of the picosecond to nanosecond motions. Our unpublished data will be presented at the conference.

P-95
Benchmarking the parametrization of protein S-nitrosylation in classical force fields

Matteo Lambrughi[1], Emmanuelle Bignon[1], Elena Papaleo[1,2]
[1] Cancer Structural Biology, Danish Cancer Society Research Center, 2100 Copenhagen, Denmark, [2] Cancer Systems Biology, Section for Bioinformatics, Department of Health and Technology, Technical University of Denmark, 2800 Lyngby, Denmark

The oxidative post-translational modification of cysteine residues by nitric oxide, protein S-nitrosylation, is an essential and ubiquitous regulatory mechanism on cellular function, mediating redox-based signaling. S-nitrosylation modulates protein stability, activity, localization, and interactions with partners, and its dysregulation is linked to various pathologies, including cancer. Despite this importance, we still have only sparse knowledge about the structural properties of S-nitrosylated cysteines and their associated molecular mechanisms in proteins. Molecular Dynamics (MD) simulations could contribute to investigating the structure-function-dynamics relationship of S-nitrosylation and the conformational changes they induce in proteins. An essential step is to benchmark against experimental data the reliability of the parameters of classical force fields developed for treating S-nitrosylated proteins. We used classical and enhanced sampling MD simulations of S-nitrosylated proteins to benchmark parameters for three force field families (CHARMM, AMBER, and GROMOS). We have been evaluating the robustness and accuracy of these parameters and their approximations using six target proteins. Our results provide a guide for studying S-nitrosylated proteins by MD simulations and indicate future directions for developing improved and more robust force-field parameters.

P-96
Data-driven multi-dataset refinement of macromolecular structures

Lena Lassinantti[1], Ying Luo[1], Nicholas M Pearce[1]
[1] Bioinformatics division, IFM, SciLifeLab, Linköping University, Sweden.

Structural studies of macromolecules in their apo forms or in complexes with ligands have long been used in biomedical applications. New experiments such as widespread fragment screening have led to a new era of high-volume data generation. However, the computational pre- and post-processing of structural data gathered from these methods remains underdeveloped and continues to utilize methods from a one-dataset one- structure paradigm. Modelling and refinement of the weakly bound ligands detected by such experiments remains difficult, and moreover contentious. We have developed a new multi-dataset refinement pipeline for preparing and refining structures for use in fragment screening experiments. We dynamically create restraints whose parameters are determined by the inherent variation within the data, and iteratively refine structures until these restraints have converged. These lead to highly consistent high-quality refinements that improve the performance of analytical programs such as PanDDA, particularly for low-resolution data. Additionally, we use this refinement approach to refine the output ensemble models from fragment experiments, leading to high quality ligand models, even for very low occupancy ligands. Data-driven structural refinement provides a new way to think about structural approaches in general and opens new possibilities for future method development.

Poster Presentations
– Session 1 –

P-97
Investigation of damage around aggregates of Ab1-42 in brain tissue by vibrational microscopies.
Sophie Lecomte[1], Youssef El Khoury[2], Ayikoe-Guy Mensah-Nyagan[3], Christine Matte-Mensah[3], Christian Klein[3], Petra Hellwig[2], Sandrine Villette[1]
[1] CBMN, UMR5248, University of Bordeaux, CNRS, 33600 Pessac, France, [2] LBS, UMR7040, University of Strasbourg-CNRS, 67081 Strasbourg, France, [3] BMNST, INSERM U1119, FMTS, University of Strasbourg, 67000 Strasbourg, France

Alzheimer's disease (AD) is the most common neurodegenerative disorder and cause of dementia. The disease is pathophysiologically characterized by aggregated amyloid protein, as Aβ (Aβ). The disease is identified pathologically by amyloid plaques composed of aggregated amyloid peptide, neurofibrillary tangles composed of aggregated, hyperphosphorylated tau protein and neuron loss. The objective of our work was to determine what damage is created within the tissue in the vicinity of the amyloid plaques of the Aβ1-42 peptide. To probe this damage, we chose to study brain sections of mice and humans with severe Alzheimer's disease by vibrational microscopy methods. These methods require no labeling and are non- destructive. Fourier transform infrared and Raman imaging on Alzheimer's diseased mice and human brain tissue were performed. Our finding suggests the accumulation of hemes in the senile plaques of both murine and human samples. We compared the Raman signature of the plaques to the ones of various hemeoproteins and to the hemin-Aβ-42 complex. The detected Raman signature of the plaques does not allow identifying the type of heme accumulating in the plaques. (ACS Chem. Neurosci. 2021, 12, 2940−2945) With the same approach, by FTIR and Raman imaging, we evidenced a reorganization of phospholipids in brain tissue from AD diseased tissues of mice with severe AD.

P-98
Exploring the alternative conformation of a known protein structure based on contact map prediction
Jiaxuan Li[1]
[1] Peking University, China

The rapid development of deep learning-based methods has considerably advanced the field of protein structure prediction. The accuracy of predicting the 3D structures of simple proteins is comparable to that of experimentally determined structures, providing broad possibilities for structure-based biological studies. Another critical question is whether and how multistate structures can be predicted from a given protein sequence. In this study, analysis of tens of two-state proteins demonstrated that deep learning-based contact map predictions contain structural information on both states, which suggests that it is probably appropriate to change the target of deep learning-based protein structure prediction from one specific structure to multiple likely structures. Furthermore, by combining deep learning and physics-based computational methods, we developed a protocol for exploring alternative conformations from a known structure of a given protein, by which we successfully approached the holo-state conformations of multiple representative proteins from their apo-state structures.

P-99
Dynamics and interactions in the 410 kDa RNA exosome complex studied by solution-state NMR
Jobst Liebau[1], Daniela Lazzaretti[1], Anna Bichler[1], Michael Pilsl[1], Remco Sprangers[1]
[1] University of Regensburg, Germany

Solution-state NMR is uniquely suited to study interactions and dynamics of biomolecules in solution and at near atomic resolution. However, conventional solution-state NMR is limited to proteins smaller than ~40 kDa. A range of methodological advances has made it possible to extend this size-limit substantially. Here, we combine selective 19F- and methyl-labeling schemes with sensitivity-optimized NMR pulse sequences to study the essential eukaryotic RNA exosome. This complex consists of 10 distinct subunits that form a 410 kDa molecular machine that degrades and processes RNA in 3' to 5' direction. We show that for the exosome solution-state NMR methods can be employed to validate and complement structural information that we obtained from cryo-EM, to study weak or transient substrate interactions and to investigate localizations and motions of dynamic regions and relate dynamics to function. We thus demonstrate that large, asymmetric complexes are amenable to a wealth of solution-state NMR techniques when recent methodological advances are combined.

P-100
A novel solubility assay for amyloid beta peptides
Max Lindberg[1], Emil Axell[1], Emma Sparr[2], Sara Linse[1]
[1] Department of Biochemistry and Structural Biology, Lund University, Lund, Sweden, [2] Department of Physical Chemistry, Lund University, Lund, Sweden

Proteins that can form amyloid fibrils have been extensively studied in the last decades, mainly due to their involvement in prevalent diseases such as Alzheimer's disease. However, the primary focus has been on the kinetic and structural aspects of these fibrils, while the solubility of the involved proteins remains less studied. Increased insight into the solubility of these proteins could be important both for therapeutic and materials science aspects. To facilitate the study of amyloid solubility, we've developed a quick, easy and inexpensive method to assay solubility, which is equal to the concentration of monomers at equilibrium. Thus by separating monomers from fibrils with 96-well filter plates after an apparent equilibrium is reached from various initial concentrations, the solubility can be determined from monomer concentration measurements using the amine-reactive fluorescent dye o-phthalaldehyde. Utilizing this new method we have determined the solubility of the Aβ40 peptide, with or without an extra N- terminal methionine from recombinant expression. Preliminary data show Aβ1-40 to be twice as soluble as Aβm1-40. Further applications of this method include several mutations and conditions relevant for pathology and physical chemistry, for further understanding of the thermodynamic driving forces of aggregation.

Poster Presentations
– Session 1 –

P-101
Computational Protein Structure Elucidation from Mass Spectrometry Data
Steffen Lindert[1]
[1] Ohio State University, United States

Mass spectrometry-based methods such as covalent labeling, surface induced dissociation (SID) or ion mobility (IM) are increasingly used to obtain information about protein structure. However, in contrast to other high-resolution structure determination methods, this information is not sufficient to deduce all atom coordinates and can only inform on certain elements of structure, such as solvent exposure of individual residues, properties of protein-protein interfaces or protein shape. Computational methods are needed to predict high-resolution protein structures from the mass spectrometry (MS) data. Our group develops algorithms within the Rosetta software package that use mass spectrometry data to guide protein structure prediction. These algorithms can incorporate several different types of mass spectrometry data, such as covalent labeling, surface induced dissociation, and ion mobility. We developed scoring functions that assess the agreement of residue exposure with covalent labeling data, the agreement of protein-protein interface energies with SID data and the agreement of protein model shapes with collision cross section (CCS) IM measurements. We subsequently rescored Rosetta models generated with de novo protein folding and protein-protein docking and we were able to accurately predict protein structure from MS labeling, SID and IM data. We are now extending this work to use MS data in deep learning-based predictions of protein structure.

P-102
Computational Insights into Xanthan Gum Glycosyltransferase Dynamics and Structure
Davide Luciano[1], Jochen Schmid[2], Gaston Courtade[1]
[1] Department of Biotechnology and Food Science, NTNU Norwegian University of Science and Technology, Trondheim, Norway, [2] Institute of Molecular Microbiology and Biotechnology, University of Münster, Münster, Germany

Exopolysaccharides are a diverse class of molecules with a wide range of industrial applications. Xanthan gum, a heteropolysaccharide synthesized by the Gram-negative bacterium Xanthomonas campestris, is a significant contributor to this class of polymers. Its versatile mechanical properties make it useful in various fields such as the food, material, and pharmaceutical industries. Modifications of the polysaccharide are being studied to modulate its properties for more specific applications. Here, we present a computational approach to identify key residues in the active site of three glycosyltransferases involved in Xanthan gum biosynthesis and their conformational properties. Our aim is to engineer these three enzymes allowing them to incorporate non-natural units into the polysaccharide and tailor its mechanical properties. We present preliminary in silico findings on the dynamic behavior of one of these enzymes and its potential binding site residues for a substrate, shedding light on the underlying molecular interactions that govern Xanthan gum biosynthesis.

P-103
Concentration dependent effect of bivalent cations on the stability of double-stranded DNA fragments
Maria Lugojanu[1], Stefania Iancu[1], Nicolae Leopold[1], Zoltán Bálint[1]
[1] Faculty of Physics, Babes-Bolyai University, Cluj-Napoca, Romania

The stability of cell-free DNA (cfDNA), a potential diagnostic tool in certain conditions, is primordial for a stable diagnosis. We aimed to determine the stability of ultrasonicated double-stranded DNA fragments at various concentrations of bivalent cations and pH range. We fragmented Calf Thymus dsDNA (D-1501, Merck) using a 1.8W/cm2 ultrasonic bath and visualized it using gel electrophoresis. The stability of dsDNA at 50ng/uL was tested by analyzing the fluorescence intensity of SybrGreen (S-7567, Thermo Scientific) bound to dsDNA under different pH and ionic strengths using Ca(NO3)2, NaCl, CuSO4, ZnSO4, MgSO4, FeSO4, and Pb(NO3)2. Our results showed that the labeled dsDNA fragments had maximum stability at pH 7, with a range of (4, 12). The addition of 1M of each ion resulted in a significant decrease or even disappearance of the signal. Ca2+ and Cl- increased the fluorescence signal, while Cu2+ inhibited it. Zn2+ and Mg2+ had no effect, and the signal was reduced for Fe2+ and Pb2+ at low concentrations. Studying the stability of dsDNA fragments at varying pH levels and ionic strengths is crucial for utilizing cfDNA as a diagnostic tool in cancer research where stability and accurate detection are critical.

P-104
Homo- versus Hetero-Oligomerization Drives the Thermo-Osmo Responsive Behavior of the Enterobacterial Sensory Protein H-NS
BINCY LUKOSE[1], Takahiro Maruno[2], Susumu Uchiyama[2], Athi N Naganathan[1]
[1] IIT MADRAS, India, [2] Osaka University,Japan

Environmentally regulated gene expression is critical for bacterial survival under a variety of conditions, including extremes in temperature, pH, osmolarity, and nutrient availability. Sensing the environment is orchestrated by macromolecules with diverse underlying mechanisms - e.g. changes in protein oligomerization status, unfolding, or DNA superhelicity - that eventually regulate gene expression in a finely tuned manner. Here, we dissect the thermo- and osmo-responsory behavior by H-NS, an archetypal sensory protein ubiquitous in enterobacteria. Employing an array of experimental spectroscopic, calorimetric, hydrodynamic methods and computational modeling, we show that H-NS exhibits a large degree of heterogeneity in its helical content and oligomeric nature with osmolarity- and concentration-dependent populations of monomer, dimer, tetramer, and octamer. The population of distinct oligomeric species is controlled by an intricate interplay between dimerization and oligomerization, which are dictated by two different sites on the protomer. This in turn, drives a competition between weak homo- versus hetero- oligomerization of protein-protein and protein-DNA complexes, which is also tuned in a concentration-dependent manner by the co-repressor Cnu. Additionally, H-NS forms liquid-like condensates at low concentrations of DNA and even in the absence of DNA but at high concentrations. Rationally engineered mutations and a phospho-mimetic variant dramatically suppress oligomerization and phase separation. Our work thus uncovers important organizational principles that act at different length scales in H-NS to ultimately determine its response to environmental cues in a finely tuned manner.

Poster Presentations
– Session 1 –

P-105
Large Scale Mapping of Motif-based Human Protein-protein Interactions

Priyanka Madhu[1], Caroline Benz[1], Johanna Kliche[1], Leandro Simonetti[1], Filip Mihalic[2], Per Jemth[2], Norman Davey[3], Ylva Ivarsson[1]

[1] Department of Chemistry BMC, Uppsala University, Box 576, Husargatan 3, 751 23 Uppsala, Sweden, [2] Department of Medical Biochemistry and Microbiology, BMC, Uppsala University, Box 582, Husargatan 3, 751 23, Uppsala, Sweden, [3] Division of Cancer Biology, The Institute of Cancer Research, London, U.K

A large proportion of protein-protein interactions (PPIs) that wire the cell involve short linear motifs (SLiMs; typically 3-10 amino acid stretches), which are present in the intrinsically disordered regions of the proteome. SLiM-based interactions are critical for cellular function, but they are underrepresented in the current maps of the interactome. We have developed proteomic-peptide phage display (ProP-PD) as a dedicated approach to capture the SLiM-based interactions. Here, we present our on-going large screening efforts for mapping SLiM-based interactions. We have created a phage based peptidome, named the human disorderome 2 (HD2), which displays one million of intrinsically disordered peptides on the surface of M13 phage. We have screened more than 300 globular domains from some of the most abundant domain families in the human proteome such as PSD-95/Dlg/ZO-1 (PDZ) domains, RNA Recognition Motifs (RRM) domains, SRC homology 2 (SH2) domains, and Phosphotyrosine-binding (PTB) domains against our HD2 phage library. We have analyzed the data using our in-house bioinformatic pipeline and identified thousands of SLiM-based interactions. A selected set of novel interactions have been validated by affinity measurements. Finally, we corroborated novel motifs using peptide SPOT arrays, and mapped binding sites on target proteins using a combination of AlphaFold2 and mutagenesis.

P-106
Rational design of a multimeric model hemoenzyme towards adaptable redox catalysis

Michelle Mahler[1,2], Patrycja Kielb[1,2]

[1] Transdisciplinary Research Area 'Building Blocks of Matter and Fundamental Interactions (TRA Matter)', University of Bonn, Germany, [2] Clausius Institute of Physical and Theoretical Chemistry, University of Bonn, Germany

Developing artificial metalloproteins is a promising research field that offers the possibility of creating novel catalysts for reactions that are either not catalyzed by natural enzymes or of superior properties tailored for technological or medical applications. This aim requires sophisticated tools for interpreting molecular structure-function relationships to unravel the impact of introduced structural modifications. In this work, we introduced rationally-designed structural modifications into the tyrosine-coordinated heme protein (HTHP) from Ruegeria pomeroyi and elucidated the structure of the cofactor using resonance Raman (RR) spectroscopy, while its redox functionality was assessed via cyclic voltammetry (CV). HTHP naturally shows catalase and peroxidase activity in vitro and forms a homohexameric structure, with each of the all-alpha-helical subunits harboring solvent-exposed heme b cofactors accessible even to bulk substrates. Several characteristics, such as high thermal and pH-range stability and resilience against some denaturing agents, combined with its multimeric yet minimalistic composition, make HTHP a promising target for protein engineering. By exchanging the natural coordinating amino acids with several classical axial ligand motifs found in other hemoproteins, we assess the applicability of HTHP as a model for studying the redox properties of hemoenzymes and as a tool for developing customizable redox reactions.

P-107
Conformational heterogeneity in the chromophore pocket of mEos4b impacts the observed photoswitching and photoconversion properties

Arijit Maity[1], Jip Wulffelé[1], Isabel Ayala[1], Adrien Favier[1], Virgile Adam[1], Dominique Bourgeois[1], Bernhard Brutscher[1]

[1] Institut de Biologie Structurale Grenoble, France

Photoconvertible fluorescent proteins (PCFPs) are an essential tool for PALM-type super-resolution microscopy. Upon application of UV light, the initially green fluorescence of PCFPs irreversibly turns red. This is the result of a light-induced extension of the chromophore conjugated electron system. One of the main factors limiting the maximal achievable resolution in PALM is the limited photoconversion efficiency (PCE) of the PCFPs, ie only a fraction of the molecules typically reach the red state. In order to improve PCE, detailed mechanistic knowledge of the photoconversion process is crucial, which remains actively debated till date. Using NMR spectroscopy coupled with in-situ light-illumination and fluorescence microscopy we studied the Green-to-Red photoconversion mechanism of mEos4b, a popular PCFP. As PCFPs also show pronounced reversible photoswitch between their green state and a metastable dark state, we also studied this photoswitching mechanism. We discovered that mEos4b (as well as other PCFPs) exhibits a well-defined two-state heterogeneous population in its green form. We found marked differences in these two states in terms of their structure and local chemistry around the chromophore pocket. We studied the conformational exchange between the two states, notably under UV light. I will describe how this hitherto undetected conformational partitioning impacts photoconversion and reversible photoswitching of mEos4b.

P-108
Structure-based rational design of Adenosine deaminase inhibitors against Mycobacterium TB (MtbADA)

Jansi Rani Malaiyappan[1], Ravindra Singh Rawat[1], Nagendra Singh[2], Sanjit Kumar[1]

[1] Vellore Institute of Technology (VIT), Vellore- 632014, Tamil Nadu, India, [2] School of Biotechnology, Gautam Buddha University, Greater Noida, U.P.-201312, India

The bacterium known as Mycobacterium tuberculosis is responsible for the chronic lung illness known as tuberculosis. In the world, tuberculosis is considered to be one of the leading causes of death. The World Health Organization estimates that 1.5 million people lost their lives to tuberculosis, of which around 14% were HIV positive. When it comes to the mortality caused by infectious organisms, tuberculosis ranks right behind only Covid -19 infections. In addition, the advent of Mycobacterium tuberculosis strains that are resistant to many drugs has made clinical care of the disease more difficult. Hence, it appears as though the task of discovering novel therapeutic targets and compounds to use against those targets will never cease. We were able to convincingly establish that taxifolin is a MtbADA inhibitor using a combination of in-silico and biochemical research. Taxifolin is a flavonol, a type of polyphenol generated by plants. It is possible to employ it as an adenosine deaminase inhibitor to suppress the growth of Mycobacterium tuberculosis, which may lead to the development of a therapeutic molecule. This is possible due to the fact that it occurs naturally and has a low cytotoxicity. Although several inhibitors of this enzyme have been found, the transition state inhibitors like deoxycoformycin are the most effective inhibitors of ADA. Other inhibitors of this enzyme have also been reported. Deoxycoformycin has potential as a medicinal chemical, however its cytotoxicity severely restricts its application. We have employed deoxycoformycin as a control molecule to ensure the reliability of our results.

Poster Presentations
– Session 1 –

P-109
Orienting intact proteins in the gas phase
Maxim Brodmerkel[1], Emiliano Di Santis[1], Carl Caleman[1,2], Charlotte Uetrecht[3,4,5]
[1] Uppsala University, Sweden[2] Center for Free-Electron Laser Science, [3] University of Siegen, [4] Leibniz Institute of Virology, [5] Centre for Structural Systems Biology

Structural biology in the gas phase can circumvent some of the limitations of condensed-phase techniques. Native mass spectrometry is an ideal component in such experiments, as it can single out specific proteoforms among multiple coexisting variants from crude mixtures, while maintaining noncovalent interactions. We have used molecular dynamics simulations to investigate if electric fields can be used to orient gas-phase proteins and shown that is advantageous for X-ray diffraction in the gas phase. We have also used our simulations to see if the proteins retain a "memory" of their conformations through their flight, by simulating two coexisting conformations of the Ms2 capsid protein dimer and discovered that no crossing of conformation happens in the gas phase or in subsequent rehydration simulations. Building on those investigations, we then simulated the rehydration of oriented proteins, to see if the exposure to the orienting electric field causes perturbations to the structures that cannot readily be reverted by in silico resolvation. We find that while the vacuum exposure causes some lasting effects on the structures, the orienting field does not, which augurs well for the use of strong electric fields in gas-phase structural biology.

P-110
Spectroscopic and micobiological insight into synergistic effect demonstrated by the selected 1,3,4-thiadiazoles and polyene-based antibiotics
Arkadiusz Matwijczuk[1,2], Lidia Ślusarczyk[1], Klaudia Rząd[1], Mikołaj Gurba[3], Dariusz Karcz[2,4], Kamila Rachwał[5], Agnieszka Dróżdż[6], Mariusz Gagoś[6,7], James Hooper[3]
[1]Department of Biophysics, University of Life Sciences, Lublin, Poland , [2]ECOTECH-COMPLEX—Analytical and Programme Centre for Advanced Environmentally-Friendly [3]Faculty of Chemistry of Jagiellonian University, Kraków, Poland , [4]Department of Chemical Technology and Environmental Analytics, Cracow University of Technology, Krakow, Poland , [5]Department of Biotechnology, Microbiology and Human Nutrition,University of Life Sciences, Lublin, Poland , [6]Department of Cell Biology, Maria Curie-Sklodowska University, Lublin, Poland , [7] Department of Biochemistry and Molecular Biology, Medical University of Lublin, Lublin, Poland

Multidrug-resistant microbial strains are often source of serious difficulties in the treatment of infections and hence the development of new antimicrobial agents is an extremely important challenge [1]. Herein we report on a more detailed studies of the synergistic effects observed, namely a representative examples of microbiological and spectroscopic studies on the synergistic systems mentioned. In addition, the experimental data are supported with a set of quantum-mechanical studies, namely the DFT calculations with use of BLYP-D3 and B3LYP-D3 methods [7]. Moreover a screening tests using the checkerboard microdilution technique. In parallel, electronic absorption, fluorescence emission, RLS, fluorescence anisotropy and fluorescence lifetimes measurements were carried out aimed at a determination of the mechanism of synergism observed [5]. Based on a screening tests the most promising 1,3,4- thiadiazole derivative was selected as compound which allowed for a significant decrease of the Amphotericin B dose while maintaining the synergistic antifungal effect against C. Parapsilosis strain. Based on the set of spectroscopic experiments a molecular mechanism responsible for the synergistic effect observed was proposed. The mode of synergistic action proposed was additionally supported by quantum mechanical studies, which in turn enabled a more detailed examination of the nature of interactions between the thiadiazole derivatives and Amphotericin B.

P-111
Trapping Proteins in Nanoscale Chambers
Jesper Medin[1], Justas Svirelis[1], Andreas B. Dahlin[1]
[1] Chalmers University of Technology, Sweden

This work presents a new platform for trapping of biomolecules to be observed by fluorescence microscopy. In comparison with existing methods, we can trap many more molecules (>100 proteins) in a volume as small as one attoliter without tethering them to a surface. Instead of confining the proteins by forces (field gradients, hydrodynamic etc.) they are trapped inside solid state containers (nanochambers) functionalized with macromolecular gates. The molecules are kept at physiological conditions for extended observation (hours). As an application example, we show how an enzymatic cascade reaction (multiple enzymes) can be run inside the nanochambers with continuous exchange of reactants and products with the surrounding environment. We propose that the technology can be useful for studies of protein oligomerization.

P-112
Probing the distinct conformational signatures of the flanking polyQ regions in huntingtin exon 1 at the membrane interface:
insights into Huntington's disease
Ana M. Melo[1,2], Tânia Sousa[1,2], Gonçalo Damas[1,2], Ana Coutinho[1,2,3], Nuno Bernardes[1,2], Ana Azevedo[1,2], Manuel Prieto[1,2]
[1] iBB–Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, Lisboa 1049-001, Portugal, [2] Associate Laboratory i4HB–Institute for Health and Bioeconomy at Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, Lisboa 1049-001, Portugal, [3] Dep. Química e Bioquímica, Faculdade de Ciências, Lisbon, Portugal

Huntington's disease (HD) is a fatal neurodegenerative disorder caused by an abnormal extension of the polyglutamine (polyQ) repeat within the first exon of huntingtin protein (httex1). Lipid membranes play a critical role in httex1 aggregation. Recent evidence supports that flanking polyQ regions [the N17 segment and the proline-rich domain (PRD)] strongly modulate the httex1-lipid interaction. However, its structural description is highly challenging due to the disordered features of httex1 and its aggregation propensity. Here, we employed acrylodan and Atto-A88 fluorescence to report on membrane binding and conformational dynamics, respectively. Globally, our acrylodan data revealed a favored partitioning of httex1 towards anionic membranes. Still, httex1-lipid interaction has an important hydrophobic component and does not show preference for an ordered phase. In addition, time--resolved anisotropy measurements of httex1 site-specific labeled with Atto A488 at each flanking polyQ domain indicate distinct conformational signatures. The N17 segment adopts a less flexible state, along with a deeper membrane insertion into anionic lipid vesicles. Notably, the PRD domain preserves its conformational dynamics and remains highly solvent exposed in the membrane-bound state. Our work provides unique insights into the roles of each adjacent polyQ region in regulating httex1-lipid interaction.

Poster Presentations
– Session 1 –

P-113
Dances with enzymes: Multiscale simulation of glycolytic enzyme assembly formation
Tom Miclot[1], Stepan Timr[1]
[1] J. Heyrovský Institute of Physical Chemistry, Czech Academy of Science,Czech Republic

In living cells, many enzymes are able to interact with each other to form dynamic enzyme assemblies. In particular, this is the case for phosphofructokinases, which have been identified as prominent members of glycolytic enzyme assemblies and shown to form filaments. However, the mechanism of the formation of these structures and their physiological roles remain poorly understood. Here we use a combination of all-atom and coarse-grained molecular dynamics simulations to describe the assembly formation by phosphofructokinase enzymes. By obtaining contact maps between proteins, comparing their interaction networks, and by performing free energy calculations, we characterize the role of specific amino acids and secondary structures in the mechanism of formation of these superstructures. The work provides a starting point for the elucidation of mechanisms governing the localized metabolism in cells.

P-114
Micro-Exon Gene (MEG) products: IDP and variability at the host-parasite interface
Adriana Erica Miele[1,2], Stepanka Nedvedova[2,3], Florence Guillère[2], Jan Dvorak[3], Olivier Walker[2], Maggy Hologne[2]
[1] Sapienza University of Rome - Italy, [2] Université Claude Bernard Lyon 1, [3] CZU University of Life Sciences Prague, Czech Republic

The parasitic worm Schistosoma mansoni has a complex genome structure, including 7 autosomes and a pair of sexual chromosomes. Among other genes, it expresses a peculiar superfamily of secreted proteins called micro-exon genes (MEG) products. MEGs are encoded by very short exons (6 -81 bp), interspersed by long introns (0.5-1.5 kbp) and flanked by transposon-like sequences. We have determined by multidimensional NMR, with normal isotopic abundance, the first 3D structure of 3 isoforms of the MEG 2.1 family, demonstrating their IDP nature and their stickiness. In parallel, we have also analysed all the 87 validated protein sequences and proposed a model for their filiation, duplication and spread over the genome. In the poster we shall also present the strategies we have applied after the failure in heterologous expression in bacteria, yeast, insect cells and cell-free, in order to perform the structural analysis by Circular Dichroism, Dynamic Light Scattering and NMR.

P-115
ESty: A new tool for evaluating energetics and stability of protein structures
Karolina Mikulska-Ruminska[1], James Krieger[2], Anupam Banerjee[3], Ivet Bahar[3]
[1] Institute of Physics, Faculty of Physics Astronomy and Informatics, Nicolaus Copernicus University in Torun, PL87100 Torun, Poland., [2] Centro Nacional de Biotecnología-CSIC, C/Darwin, 3, 28049 Cantoblanco, Madrid, Spain., [3] Laufer Center for Physical and Quantitative Biology, and Department of Biochemistry and Cell Biology, School of Medicine, Stony Brook University, Stony Brook, NY 11794, USA.

The propensity of proteins to adopt well-defined conformations and the impact of individual residues on the protein function and stability have intrigued scientists for many decades. With the development of bioinformatics tools and the improvements of experimental methods in obtaining high-resolution structures, this knowledge has become more accessible to relevant analyses. We developed a new module ESty of the ProDy API to evaluate the contribution of a single residue (or group of residues) towards the stability of individual proteins and protein-protein interactions (PPI). Along with distinguishing highly important protein regions, the module can predict crucial hotspots in protein-protein complexes based on intermolecular interactions predicted using a single PDB structure or ensemble of structures from experiments or simulations. ESty can also be used to predict the impact of mutations on individual protein stability and on the interaction affinity of PPIs.

P-116
Head or tail? A molecular dynamic approach to the complex structure of TNF-associated factor TRAF2
Velia Minicozzi[1], Fulvio Erba[2], Luisa Di Paola[3], Almerinda Di Venere[2], Eloise Mastrangelo[4], Federica Cossu[4], Giampiero Mei[2]
[1] Department of Physics and INFN, Tor Vergata University of Rome, Via Della Ricerca Scientifica 1, 00133 Rome, Italy, [2] Department of Experimental Medicine, Tor Vergata University of Rome, Via Montpellier 1, 00133 Rome, Italy, [3] Unit of Chemical-Physics Fundamentals in Chemical Engineering, Department of Engineering, University Campus Bio-Medico of Rome, Via Álvaro del Portillo 21, 00128 Rome, Italy, [4]National Research Council (IBF-CNR) Milan Unit, Institute of Biophysics, Via Celoria 26, 20133 Milan, Italy

TRAFs are trimeric proteins that play a fundamental role in signaling, acting as intermediaries between the TNF receptors and the proteins that transmit the downstream signal. The monomeric subunits of all the TRAFs family members share a common tridimensional structure: a C-terminal globular domain and a long coiled-coil tail that characterize the N-terminal section. In this study we have analyzed in silico the dependence of the TRAF2 dynamics on the length of its tail. In particular, we used the available crystallographic structure of a C-terminal fragment of TRAF2 (168 out of 501 a.a.), TRAF2-C, and that of a longer construct, addressed as TRAF2-plus, that we have re-constructed using the AlphaFold 2 code. The results indicate that the longer N-terminal tail of TRAF2-plus has a strong influence on the dynamics of the globular regions in the protein C- terminal head. In fact, the quaternary interactions among the TRAF2-C subunits change asymmetrically in time while the movements of TRAF2- plus monomers are rather limited and more ordered than those of the shorter construct. Such findings shed a new light on the dynamics of TRAFs subunits and on the protein mechanism in vivo, since TRAFs monomer-trimer equilibrium is crucial for several reasons (receptor recognition, membrane binding, hetero-oligomerization).

Poster Presentations
– Session 1 –

P-117
Exploring the potential of the Bri2 BRICHOS domain as a drug-carrier for lysosomal storage disorders
Willem Molenkamp[1], Nina Kronqvist[1], Janne Johansson[1], Axel Abelein[1]
[1] Karolinska Institutet, Sweden

The blood-brain barrier (BBB) is crucial for the protection of the central nervous system by preventing molecules such as toxins to enter. However, in the case of treatment of brain-related disorders, the BBB imposes a challenge as it also prevents the passage of almost all potential drugs. In this project, we aim to explore the potential of the Bri2 BRICHOS protein domain in transporting drug candidates over the BBB. This protein domain was initially found to act against protein aggregation involved in neurodegenerative disorders. However, recent in vitro and in vivo experiments suggests that BRICHOS is efficiently transported over the BBB and ends up in the lysosomes of neuronal cells. When fused to other proteins, BRICHOS is still able to pass and could therefore help to transfer other proteins over the BBB. In this project we will explore whether BRICHOS can transfer lysosomal enzymes over the BBB that can help against lysosomal storage disorders such as Parkinson's disease, Gaucher disease and neuronal ceroid lipofuscinosis. Different lysosomal enzymes will be fused to BRICHOS and produced in a mammalian cell system to maintain the enzymatic activity. Subsequently, by using a monolayer of brain microvascular endothelial cells as BBB model system, the passage of these enzymes can be tested to explore the potential of BRICHOS as a drug-carrier against lysosomal storage disorders.

P-118
The Effect of Chaperones on Tau Pathogenic Mutants
Yuval Mor[1], Rose Irwin[1], Ofrah Faust[1], Rina Rosenzweig[1]
[1] Weizmann institute of science, Israel

The abnormal aggregation of the microtubule-associated protein tau is related to over 20 neurodegenerative conditions, including Alzheimer's and Pick's disease. While the tau aggregation trigger is not well understood, it is known to be driven by local structural changes to the microtubule- binding repeats (MTBRs). Such changes trigger the conversion of tau from an intrinsically disordered protein to stable, disease-associated, cross-β amyloids. In the cell, proteins called chaperones engage with tau during these pathogenic events, counteracting its aggregation and targeting the misfolded species for degradation. Chaperones prevent tau aggregation usually by binding directly to the hydrophobic MTBRs, thus preventing their misfolding and self-association. Over 50 pathogenic mutations in tau were identified, usually associated with early onset of the disease. Most mutations are located in the MTBRs and are thought to induce conformational change in this region, leading to enhanced tau aggregation. Furthermore, it was hypothesized that these mutants interfere with the chaperone binding sites, allowing these tau variants to evade the cellular chaperone network. To test this, we used NMR spectroscopy and biochemical methods to characterize the effect of tau pathological mutations on its conformation and interaction with chaperones. Using three chaperone systems, each affecting different stages of aggregation, we show that indeed most chaperone interactions are reduced by the disease-associated mutations. Further mechanistic understanding of this chaperone system, could provide new avenues for treating tau related and aggregation induced diseases.

P-119
Aurora kinase A interact with the intrinsically disordered N-terminus (TAD) of c-Myc through MBx0
Vivian Morad[1], Johanna Hultman, Alexandra Ahlner, Maria Sunnerhagen
[1] Linköping University, Sweden

Myc is a master gene regulator associated with several normal cellular processes, but its overexpression in many human cancers is often associated with poor prognosis. The Aurora kinase A is essential for cell division and spindle assembly, however it is overexpressed in several human cancers. The expression of Aurora A is tightly regulated by Myc and Aurora A interacts with N-Myc and protects it from proteasomal degradation through the interaction with the region flanking MBI. Several studies have confirmed the link between Aurora A and c-Myc showing that both these two oncogenes are often amplified and co-expressed in cancer cells. But does Aurora A interact directly with c-Myc, even though this flanking MBI region identified in N-Myc is not conserved in c-Myc? Here we report that Aurora A binds directly to c-Myc with a dissociation constant Kd of 8 μM but this binding completely disappeared when MBx0 was removed from the construct. Titration experiments using nano-DSF show that c-Myc is thermo-stabilizing Aurora A in the presence of MBx0. This dynamic interaction between Aurora A and c-Myc is confirmed by SAXS data showing a stable complex sufficient to be eluted on a SEC-MALS-column. The result confirms the direct interaction of Aurora A and c-Myc primarily by MBx0 which may facilitate future therapeutic targeting of c-Myc in tumors overexpressing Aurora kinase A.

P-120
Molecular thermodynamics of protein condensate formation from all-atom MD simulations
Saumyak Mukherjee[1], Lars Schäfer[1]
[1] Ruhr University Bochum, Germany

Liquid liquid phase separation (LLPS) of proteins that gives rise to membraneless organelles in the form of protein condensates have been widely recognized as a major driver for a multitude of intracellular processes. Associated with the process of LLPS is a thermodynamic tug-of-war among the water molecules that are released from the protein hydration layers into the bulk and those which are retained inside the protein droplets. Additionally, the intra- and inter-protein interactions play a vital role in condensate formation. Using atomistic molecular dynamics simulations on an intrinsically disordered protein domain (low complexity domain of Fused in Sarcoma RNA binding protein) we dissect the multiple thermodynamic contributions to LLPS and assess their individual significance in the process. Our investigations reveal that water plays a part as important as that of the protein itself. The analysis provides a detailed view of the thermodynamic driving forces associated with the process of LLPS and quantifies the spontaneity of condensate formation under suitable conditions.

Poster Presentations
– Session 1 –

P-121
A Spectro electrochemical study, detection and monitoring of uranium in LB media
Atripan Mukherjee[1,2], Martin Precek[1], Amirmanoor Ashrafi[2], Lukas Richtera[2], Miroslav Kloz[1]
[1] Extreme Light Infrastructure, ELI Beamlines Facilities, Czech Republic, [2] Department of Chemistry and Biochemistry, Mendel University in Brno

It goes without saying that clean and fresh water systems are essential for the local ecosystem's survival. They are precious resources for supplying fresh drinking water worldwide, leading to the growth of ancient civilizations towards urban and developed cities. The chemistry of uranium ions in a mixture of different phases at a particular metal composition in a solvent system is still unknown. The adsorption of uranium metal at anodic conditions was conducted by using methyl-β-glucuronide sodium salt (MGu) and 8-hydroxy quinone glucuronide (8-HQG) in Luria broth media. The uranium adsorption was done by electrochemical techniques by adsorptive stripping differential pulse voltammetry at -0.3 V for 180 sec on a screen-printed carbon electrode (SPCE). Uranium was expected to be adsorbed as a chelating complex with 8-HQG. This transition of uranium (IV) to another state was observed by combining electrochemistry with spectroscopy. This spectroelectrochemical technique can be vital for environment monitoring with the application of green chemistry. Understanding the basic uranium specification in monitoring systems through spectroelectrochemistry can expedite the development of eco-friendly nuclear fuel monitoring and recycling process.

P-122
The anti-amyloid chaperone BRICHOS regulates Tau liquid-liquid phase separation and inhibits Tau fibrillation
Cecilia Mörman[1,2], Axel Leppert[2], Jan Johansson[2], Jinghui Luo[1], Axel Abelein[2]
[1] Paul Scherrer Institut, [2] Karolinska Institutet, Sweden

One pathological characteristic of Alzheimer's disease is the accumulation of neurofibrillary tangles consisting of aggregated Tau protein. How this aberrant behavior of Tau is initiated in the misfolding process is not yet fully understood. Liquid-liquid phase separation (LLPS) has, apart from being involved in membrane-less organelles, also emerged as an important step for protein aggregation in neurodegeneration. Here, we identify the anti-amyloid chaperone activity of the BRICHOS domain to inhibit Tau aggregation and determine the effect of BRICHOS on Tau liquid droplet formation applying a combination of spectroscopic methods. We found that native Tau-441 fibrillation is inhibited by BRICHOS in a concentration- dependent manner, as monitored by fluorescence aggregation kinetics and electron microscopy imaging. To test if the inhibitory effect originates from monomer-monomer interactions, solution NMR measurements were performed, which elucidated a weak interaction of BRICHOS to monomeric Tau. Interestingly, BRICHOS enhanced the LLPS behavior of Tau in the determined phase diagrams. Preliminary data suggests that BRICHOS is incorporated into Tau droplets, despite a low tendency of secondary structural changes. This study can facilitate how modulated LLPS formation is linked to the inhibitory effect of protein aggregation mediated by molecular chaperones.

P-123
Role of disordered regions beyond the binding motif of the Measles virus NTAIL
Gabor Nagy[1], Lillian Otteson[2], John Kunkel[2], Gerdenis Kodis[2], Wenwei Zhend[3], Christophe Bignon[4], Sonia Longhi[4], Andrea C Vaiana[1], Helmut Grubmuller[1], Sara M Vaiana[2]
[1] Theoretical and Computational Biophysics, Max Planck Institute for Multidisciplinary Sciences, Goettingen, Germany, [2] Department of Physics, Arizona State University, Tempe, AZ 85287, USA, [3] College of Integrative Sciences and Arts, Arizona State University, Mesa, AZ 85212, USA, [4] Aix Marseille Universite and NRS, AFMB, Marseille, France

The Measles virus nucleocapsid is made of thousands of nucleoprotein (N) repeats, which hold the viral RNA in a helical structure. The last 125 amino acids of each N repeat (NTAIL) are intrinsically disordered and protrude radially outward from the nucleocapsid. NTAIL promotes virus replication by binding to the X domain of the phosphoprotein P (PXD), which in turn recruits the viral polymerase to transcribe and replicate the viral RNA. The majority of NTAIL, on either side of this molecular recognition region (MoRE), remains disordered. While it has been shown that these disordered regions dampen the binding affinity, their function and interactions have not been identified. We apply photo-induced electron (PET) transfer relaxation experiments of full-length NTAIL to probe intra-molecular contact formation times between tryptophan (W) and cysteine (C) residues introduced to the protein under different salt and pH conditions. To better understand NTAIL dynamics, we combine PET measurements with analytical models, molecular dynamics simulations, and co-evolutionary analysis. Our integrated approach identifies transient non-local interactions between two regions out of MoRE. These interactions dominate the dynamics of the entire NTAIL in solution and affect the conformational preferences of the MoRE

P-124
Large scale computational screening of cancer and disease mutations integrating machine learning and simulations
Michael Nagy[1]
[1] Karolinska Institute, Sweden

In the era of proteomics, large amounts of patient-observed mutation data associated with disease are stored in databases, but their information content remains elusive. Even among those mutations located in known cancer driver genes, many mutations are simple "passenger" mutations, mutated only by chance and not direct drivers of disease. In addition to known disease-related proteins, there is the possibility that evolutionarily advantageous traits are acquired through mutations in housekeeper proteins that are not currently classified as disease-related but may play an important role in disease treatment and progression. While conventional methods aim to predict cancer-causing mutations at the protein level based on static structural representations of proteins, the essential dynamic part of their conformational movements is missing. From an evolutionary perspective, cancer enhancers are allosteric modifications selected to alter native conformations ("phenotypes"). To better understand and classify potential novel mutations, we will train a state-of-the-art machine learning-based classifier based on whole exon sequencing datasets containing more than 14000 proteins and 14 million mutations. Using novel implementations of multidimensional contact maps that merge structural, evolutionary, chemical, and biophysical information in latent space with integrated dynamic data from multi-scale simulations, we expect to significantly improve performance over current prediction methods. For later potential clinical application, we will strongly emphasize the use of explainable AI tools (SHAP, etc.) to rationalize any prediction made.

Poster Presentations
– Session 1 –

P-125
An exquisite order-disorder equilibrium sculpts the folding-binding landscape of an antibiotic sequestering protein

Lawanya Natarajan[1], Maria Laura De Sciscio[2], Alessandro Nicola Nardi[2], Alessandra Del Giudice[2], Marco D'Abramo[2], Ashok Sekhar[3], Athi N Naganathan[1]
[1] Department of Biotechnology, Indian Institute of Technology Madras, India, [2] Department of Chemistry, Sapienza University of Rome, Italy, [3]Molecular Biophysics Unit, Indian Institute of Science, Bangalore, India

P-126
Investigating the Molecular Basis of c-FLIP/Calmodulin Interaction for Modulating Apoptosis

Kalyani Natu[1,2], Kakoli Bose[1,2]
[1] TMC-ACTREC, India [2] HBNI

Cancer in its various forms is a major health hazard across the globe. Despite the presence of multiple treatment modalities, there is a significant burden of death. Therefore, there is a dire need to identify new cancer therapeutics using novel/alternate approaches, a promising one being modulation of the functions of the molecules in the apoptotic pathway. Apoptosis is a normal cellular process to eliminate damaged cells and is critical to maintain balance between cell survival and death thus maintaining homeostasis. Resistance to apoptotic pathways is a hallmark of cancer and can be achieved through overexpression of anti-apoptotic proteins. A decoy of procaspase 8 cFLIP antagonizes the functionality of DISC assembly in the extrinsic apoptotic pathway. This protein is overexpressed in many tumors providing them with a survival advantage even in presence of death ligands such as TRAIL and FAS. Targeting this protein is difficult due to its structural similarity with the DISC protease procaspase 8 that enzymatically propagates the death signal and therefore might interfere with the procaspase 8 functions. Recent reports indicate that the ubiquitous 17-kDa dumbbell-shaped protein calmodulin (CaM) acts as a scaffold to recruit cFLIP to the DISC. Therefore, this work aims at developing Calmodulin/cFLIP interaction-disruptive peptides/analogues for apoptosis restoration in cancer cells. This will not only be effective in scenarios where cFLIP is overexpressed (such as prostate, colorectal, and gastric etc.) but will be generally applicable over a wide range of cancers to restore apoptosis through cFLIP inhibition.

P-127
TTLL11 as a Tubulin Polyglutamylase

Jana Nedvědová[1,2], Ambroise Desfosses[3], Cyril Bařinka[2]
[1] Department of Biochemistry, Faculty of Science, Charles University, Czechia, [2] Institute of Biotechnology AS CR, Czechia, [3] Institute of Structural Biology, France

Tubulin polyglutamylation is a common posttranslational modification (PTM) performed by the tubulin tyrosine ligase-like (TTLL) family of glutamylases. This PTM endows microtubules with distinct functional and structural properties as it influences microtubule stability as well as their interactions with microtubule associated proteins and molecular motors. Understanding molecular mechanisms of TTLLs is thus essential for elucidating the tubulin code and its implications for microtubule functions. Our research is focused on biochemical, biophysical, and structural characterization of TTLL11, one of the least studied members of TTLL glutamylases. We cloned, expressed, and purified a number of TTLL11 variants and established in vitro biochemical assays to elucidate TTLL11 substrate preferences and functional motifs critical for tubulin polyglutamylation. Furthermore, we used cryo-electron microscopy to describe structural determinants governing tubulin polyglutamylation by TTLL11 showing the unique binding mechanism and activity regulation. Overall, our work contributes to better understanding of physiological functions and functional consequences of TTLL11 activity in vitro as well as in the cellular context.

P-128
Mechanistic Insights into the Activation of Lecithin–Cholesterol Acyltransferase in Therapeutic Nanodiscs Composed of Apolipoprotein A-I Mimetic Peptides and Phospholipids

Tuomas Aarni Akseli Niemelä[1], Laura Giorgi[1], Esa-Pekka Kumpula[1,2], Ossi Natri[1], Petteri Parkkila[1,3], Juha Huiskonen[1,2], Artturi Koivuniemi[1]
[1] University of Helsinki, Finland [2] Helsinki Institute of Life Science (HiLIFE), [3] Chalmers University of Technology

Lecithin-cholesterol acyltransferase (LCAT) maturates high density lipoprotein (HDL) particles enabling reverse cholesterol transport (RCT). Its primary cofactor on HDL is apolipoprotein A1 (apoA1). In order to boost RCT efficiency to combat atherosclerotic cardiovascular diseases, apoA1 mimetic peptides have been developed, such as 22 amino acid long 22A. Despite being ~10 % the size of apoA1, when 22A is formulated to a synthetic HDL particle, called a nanodisc, it is able to activate LCAT as well as apoA1 based HDL. However, the mechanism of how 22A, or apoA1, activates LCAT is not known. We set out to explain this mechanism with a combination of coarse-grained molecular dynamics simulations, LCAT activity assays and electron microscopy (EM). The computational data matched the experimental data in two ways. First, EM imaging showed that LCAT binds to the perimeter of 22A based nanodiscs, which was replicated by the simulations. The overall binding pose was also consistent. Second, by simulating and assaying a set of 22A mutants, we were able to see a correlation between the in silico binding kinetics of a peptide to a specific site in LCAT and how well the peptide activates LCAT in vitro. Taken together, we've shown that these simulated systems can be exploited for designing peptides with increased efficacy. A more detailed description is found in our similarly titled article in Mol. Pharmaceutics 19: 4135 -4148, 2022.

Poster Presentations
– Session 1 –

P-129
Investigating the effect of bootstrap aggregation in model selection of NMR order parameters
Daniel Nilsson[1], Sven Wernersson[1], Göran Carlström[1], Mikael Akke[1]
[1] Biophysical Chemistry, Lund University, Sweden

Conformational dynamics is crucial for the biological function of proteins and may play a role in the kinetics and thermodynamics of ligand binding. Our research group has used 15N NMR relaxation experiments to measure order parameters, describing the residue-specific fluctuation amplitudes of the protein backbone, as a means to estimate the conformational entropy and its response to ligand binding. Clearly, high accuracy in the parameter estimates is required when investigating minor differences in binding thermodynamics among similar ligand–protein complexes. A statistical tool bootstrap aggregation (bagging) was recently developed by Crawley & Palmer in order to improve the confidence of order parameters fitted to NMR relaxation data. Here, we apply this method to two similar ligand–Galectin-3C complexes, using 15N R1, R2 and NOE relaxation data measured at four different static-magnetic field strengths. We observed that bagging led to a larger influence by complex models containing more parameters, compared to the conventional model selection approach. In addition, implementing bagging resulted in a reduced difference in order parameter values between neighbouring residues. We conclude, in line with the original authors, that bagging has the potential to increase fit robustness and accuracy in model selection.

P-130
The hybrid approach to structural characterization of tau aggregation domains
Stefana Njemoga[1,2], Exequiel Barrera[3], Ondrej Cehlar[2]
[1] Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia, [2] Institute of Neuroimunology, SAS, Bratislava, Slovakia, [3] IHEM, Universidad Nacional de Cuyo, CONICET, Mendoza, Argentina

The aggregation of neuronal protein tau is one of the hallmarks of tauopathies. The origin of tau pathology remains unclear, however the isolated tau filaments from patients‘ brains with different tauopathies suggest that repetitive regions R3 and R4 are primarily involved in the tau filament formation. The aim of the project is to obtain metastable tau structures to open the possibilities for designing an inhibitor of tau aggregation. For the crystallization of complexes, tau321-391 comprising R3 and R4 domains was incubated with DC11Fab in a 1.5:1 molar ratio. Crystallization was performed at 295 K by the vapor-diffusion technique in sitting drops in an MRC 96-well crystallization plate. The synchrotron data were collected at the DESY, Hamburg, Germany. Diffraction data were processed using XDS. The atomistic molecular dynamics simulations of shorter tau fragment within the R4 were performed in explicit solvent starting from extended conformations of peptides build in Chimera, using DES-Amber force field to enhance conformational sampling. Also, coarse-grained simulations of docking tau epitopes into corresponding monoclonal antibodies were done. All simulations were performed using the Gromacs 2021.3 package. Crystals of DC11Fab alone gave diffraction of up to 1.4 Å, while crystals of DC11Fab complexed with tau321-391 gave diffraction of up to 1.74 Å. The structure of DC11 Fab is being solved by molecular replacement and will be used for docking of tau peptides.

P-131
Time-resolved fluorescence spectroscopy characterize small-molecular compounds for inhibition of Aβ peptide aggregation
Sho Oasa[1], Valentina Kouznetsova[2], Sergej Masich[3], Ann Tiiman[1], Vladana Vukojevic[1], Igor Tsigelny[2], Lars Terenius[1]
[1] Department of Clinical Neuroscience, Karolinska Institutet, Sweden, [2] San Diego Supercomputer Center and Department of Neurosciences, University of California San Diego, USA, [3] 3D-EM Facility, Department of Cellular and Molecular Biology, Karolinska Institutet, Sweden

Alzheimer’s disease (AD) is a progressive neurodegenerative disease with the loss of the ability to communicate and adapt to the environment, which is characterized by structured amyloid beta (Aβ) aggregates depositions in the brain. A handful of antibody-based drugs have been approved by the U.S. Food & Drug Administration (FDA). We applied pharmacophore simulation as a pre-screening method to identify small-molecular compounds targeting the Aβ dimer to inhibit further aggregation. We characterized experimentally the efficacy of identified small-molecular compounds in vitro using time-resolved fluorescence spectroscopy, Fluorescence Correlation Spectroscopy with Thioflavin T (ThT-FCS) and FCS integrated Förster Resonance Energy Transfer (FRET-FCS) for Aβ aggregation processes. Several compounds changed the morphology of Aβ fibril structures and suppressed Aβ-mediated cell toxicity. Characterization of the identified small-molecular compounds in animal studies is in the early phase.

P-132
Cryo-EM structure of ASK1 kinase suggests a role for inter-domain interactions in its regulation
Tomas Obsil[1,2], Karolina Honzejkova[1], Dalibor Kosek[2], Veronika Obsilova[2]
[1] Department of Physical and Macromolecular Chemistry, Faculty of Science, Charles University, Prague, Czech Republic, [2] Institute of Physiology of the Czech Academy of Sciences, Laboratory of Structural Biology of Signalling Proteins, Division BIOCEV, Vestec, Czech Republic

ASK1 (apoptosis signal-regulating kinase 1), a member of MAP3K protein family, directs cells towards inflammation or apoptosis through p38 or JNK signaling pathways. Dysregulation of ASK1 activity has been associated with inflammatory, cardiovascular, tumor and neurodegenerative diseases, among others. Therefore, ASK1 represents a prospective target for therapeutic intervention in these disorders. However, lack of structural data and insufficient understanding of the complex regulation of ASK1 hinder the search for effective drugs. Here, we aim at structurally characterizing ASK1 and its interaction with thioredoxin by H/D exchange coupled to MS and cryo-EM. Our results show that ASK1 dimerizes in solution and thioredoxin further promotes this tendency. C-terminally truncated ASK1 forms an asymmetric dimer via thioredoxin-binding (TBD) and kinase (KD) domains, with the TBD and KD dimers separated by tetratricopeptide repeat and pleckstrin homology domains that ensure communication between TBD and KD. These interactions allow allosteric modulation of KD upon thioredoxin binding to the TBD. Binding of thioredoxin to the TBD disrupts its dimerization, which in turn allosterically modulates the KD near the active center. Overall, our results provide the first structural insight into the N-terminal part of ASK1 and suggest a role for interdomain interactions in regulating its activity.

Poster Presentations
– Session 1 –

P-133
Molecular basis of Nedd4-2 regulation by calcium and 14-3-3 proteins
Veronika Obsilova[1], Masa Janosev[1], Rohit Joshi[1], Tomas Obsil[1,2]
[1] Institute of Physiology of the Czech Academy of Sciences, Laboratory of Structural Biology of Signaling Proteins, Division BIOCEV, Prumyslova 595, Vestec, 25250, Czech Republic , [2] Department of Physical and Macromolecular Chemistry, Faculty of Sciences, Charles University, Albertov 6, Prague, 12843, Czech Republic

Nedd4-2 is a key regulator of Na+ homeostasis as it ubiquitinates various channels and membrane transporters, including the epithelial sodium channel ENaC. Dysregulation of Nedd4-2 leads to a number of pathologies, including electrolytic imbalance, respiratory distress, hypertension, and kidney diseases. Nedd4-2 is regulated by phosphorylation and interaction with 14-3-3 proteins, which inhibit Nedd4-2 through modulation of its conformation and possibly other still unclear mechanism(s). Furthermore, calcium is thought to enhance Nedd4-2 activity by inducing Nedd4-2 binding to the membrane via the N-terminal C2 domain, which in turn stabilizes the active conformation of Nedd4-2. The goal of this study was to investigate how 14-3-3 proteins and calcium affect the conformation of Nedd4-2 using HDX-MS and liposome binding assays. Our results show that calcium binding to the C2 domain induces an open conformation of the enzyme and its binding to the membrane. However, the 14-3-3η protein directly interacts with the C2 domain of Nedd4-2, thereby blocking its binding to the membrane. Thus, it can be assumed that 14-3-3 binding suppresses not only the catalytic activity of Nedd4-2 but also its localization on the membrane.

P-134
Polyphenolic plant extracts as cell protector against oxidative stress induced by reactive oxygen species
Daniela Oprea[1], Daniel Crisan[1], Adrian Enache[1]
[1] National Institute of Material Physics – INCDFM, Romania

The main characteristic of an antioxidant molecules is given by its ability to neutralize free radicals through electrons transfer, a process that employs the oxidation of the antioxidant molecule and the reduction of the free radical. Using the ultrasound assisted acidified methanol method, the polyphenolics content of several spring greens was extracted and the radical scavenger activity as well as the total antioxidant capacity were evaluated by the means of UV-Vis spectroscopy and electrochemistry. The spectrophotometric evaluation of the antioxidant capacity was performed by DPPH assay, measuring the absorption at 520 nm, and the half maximum effective concentration (EC50) of each extract was calculated as the concentration value which induced a 50 % decrease of DPPH radical maximum absorbance, i.e. neutralization of half amount of radical. Also, the antioxidant capacity of the extracts was evaluated using amperometry at fix potential; 0.3 V (vs. Ag/AgCl) was chose for the evaluation of high antioxidant content and 0.7 V for the estimation of total antioxidant fraction. The amperometric results were in good agreement with the EC50 values; the lowest EC50 values were obtained for the highest current oxidation peaks. Based on this result, the plant extracts, at the concentration of EC50 value, were assessed in a fibroblast cell culture, evaluating their protective effect against oxidative stress induced by LPS and TNF alpha.

P-135
Quantitative measurement of cellular active states during ATP depletion using diffusion of intracellular particles
Hideaki Ota[1], Hideo Higuchi[1]
[1] Graduate School of Science, University of Tokyo, Japan

When ATP in cells is depleted for a certain period, cellular active states will change, and cells will die with a certain probability. Here, we focused on intracellular particle diffusion to quantify the difference in cellular active states in ATP depletion. ATP depletion was achieved by inhibiting two ATP production pathways and diffusion coefficients of vesicles were calculated from mean square displacement plot. As a result, diffusion coefficients of vesicles decreased according to ATP depletion time and became about 1/300 with depletion for 3 hours. In addition, a correlation between decrease rate of vesicle diffusion coefficients and death rate means that vesicle diffusion coefficients can quantify cellular active states.
From fluorescent imaging, we found two types of vesicles in ATP depletion. One almost stop moving near microtubules, the other move quickly between cytoskeletal networks. Vesicle movement may be suppressed via motor proteins that stick to microtubules.

P-136 Short talk
Mechanistic insights into DNA movement during chromatin remodeling under single-turnover conditions
Mikhail Panfilov[1], Anton Sabantsev[1], Sebastian Deindl[1]
[1] Department of Cell and Molecular Biology, Science for Life Laboratory, Uppsala University, Uppsala, Sweden

ATP-dependent chromatin remodeling enzymes (remodelers) regulate DNA accessibility in eukaryotic genomes. Many remodelers reposition (slide) nucleosomes, the universal units for genome packaging. However, how DNA is propagated around the histone octamer during this process is still incompletely understood. Given that the DNA substrate is highly constrained in the nucleosome, sliding must be coupled to the disruption of many histone-DNA contacts, which cannot be achieved with a single catalytic cycle of the remodeler. We have recently demonstrated that during sliding by Chd1 and SNF2h remodelers, DNA is shifted discontinuously, with movement of entry-side DNA preceding that of exit-side DNA. This temporal delay indirectly implied the transient absorption of DNA inside the nucleosome. However, the buildup and propagation of such unstable 'buffering' intermediates during nucleosome sliding has not been characterized as of yet. Here we combine a new method for controlling NTP- driven reactions in single-molecule experiments via the local generation of NTPs (LAGOON) with 3-color single molecule FRET measurements to monitor nucleosomes sliding under single-turnover conditions. Our new data provide unprecedented insights into the formation of nucleosome sliding intermediates over multiple sequential translocation steps and allow us to estimate the efficiency of ATP usage by the remodeler.

Poster Presentations
– Session 1 –

P-138

CATALYTIC ACTIVITY IMPROVEMENT OF BIOTECHNOLOGICAL ENZYME NITRILE HYDRATASE USING MOLECULAR MODELLING TECHNIQUES

Lukasz Peplowski[1], Julia Berdychowska[1]
[1] Nicolaus Copernicus University, Faculty of Physics, Astronomy and Informatics, Poland

Nitrile hydratase (NHase) is a non-standard metalloenzyme that converts nitriles to the corresponding amides. It has an active site with a CoIII metal ion coordinated by post-translationally oxidized cysteines. More than 600 kilotons of amides are produced each year using NHase. Enzymes used in biotechnology should exhibit high catalytic activity. Mutations introduced close to the active site may improve catalytic activity but are risky due to the high possibility of mutating important residues. In this report, we show two strategies that may be used to improve catalytic efficiency. The first approach is to mutate amino acids at the entrance to the channel leading to the active site. Such modifications proposed by molecular modeling tools allowed us to speed up the production of amides by one order of magnitude in the two NHase strains coming from Pseudonocardia thermophila JCM 3095 and Streptomyces thermoautotrophicus. Another approach to enhance catalytic activity is thermostability improvement. We showed mutations increasing thermostability, far away from the active site, proposed by the FireProt server as a side effect, gave us the better activity of Pseudonocardia thermophila JCM 3095 NHase. In all the above cases, molecular dynamics simulations were crucial in explaining, at the single atom level, the purposes of enhanced properties. Improvement of activity was proved by experiments.

P-139

Drp1 C-terminal region regulates its fission activity

Isabel Pérez-Jover[1,2], Kristy Rochon[3], Di Hu[4], Pooja Madan Mohan[4], Jason A. Mears[3,5], Rajesh Ramachandran[4,5], Anna V. Shnyrova[1,2]
[1] Department of Biochemistry and Molecular Biology, University of the Basque Country, 48940 Leioa, Spain, [2] Biofisika Institute (CSIC-UPV/EHU), 48940 Leioa, Spain, [3] Department of Pharmacology, Case Western Reserve University School of Medicine, Cleveland, OH 44106, USA, [4] Department of Physiology & Biophysics, Case Western Reserve University School of Medicine, Cleveland, OH 44106, USA, [5] Cleveland Center for Membrane and Structural Biology, Case Western Reserve University School of Medicine, Cleveland, OH 44106, USA

Mitochondria are highly dynamic organelles constantly merging and dividing in response to cellular requirements. The large GTPase dynamin- related protein 1 (Drp1) self-assembles on the mitochondrial outer membrane leading to constriction and further scission of the mitochondrial double membrane. However, its self-sufficiency as a mitochondrial fission catalyst is still under debate. Recently, a study noted the importance of a disordered short segment at its C-terminus (CT) for targeting Drp1 to the mitochondria from the cell periphery. In this work, we aimed to elucidate a possible regulatory role for that Drp1 CT region in mitochondrial fission. Combining in vitro functional reconstitution with in vivo assays, we show that the CT region of Drp1 is a critical structural determinant that controls Drp1 oligomerization, assembly-stimulated GTPase activity, as well as membrane constriction and fission. We further provide evidence of direct modulation of Drp1 fission activity by the interaction of its CT region with specific binding partners.

P-140

Structural and functional insights into interactions of two key membrane binding virulence factors of Listeria monocytogenes

Nejc Petrišič[1,2], Maksimiljan Adamek[1], Gregor Anderluh[1], Marjetka Podobnik[1]
[1] National Institute of Chemistry Slovenia, [2] University of Ljubljana

Listeriosis is one of the deadliest foodborne diseases caused by the intracellular bacterium Listeria monocytogenes (Lm). Broad-range phospholipase C (PC-PLC) is a zinc metalloenzyme and a major virulence factor of Lm. PC-PLC together with the cholesterol-dependent pore- forming toxin listeriolysin O (LLO) and other bacterial and host proteins, disintegrates the lipid membrane of the phagosome and releases Lm into its replicative niche, the cytosol. We determined the crystal structure of PC-PLC and complemented it with the functional analysis of this enzyme. This revealed that PC-PLC has evolved several structural features to regulate its activity, from the structurally plastic active site to the Zn2+-dependent activity and the tendency to form enzymatically inactive oligomers. Next, we investigated the interaction of PC-PLC and LLO on the model lipid membranes. We show that the phospholipase activity of PC-PLC facilitates the binding of LLO to lipid membranes and enhances its pore-forming activity. Using cryoEM, we observed enhanced binding and changes in the topology of LLO oligomers on the lipid membrane. In addition, we have demonstrated specific inhibition of the mature PC-PLC by its propeptide in trans, providing a new platform for the development of alternative solutions to combat listeriosis. Overall, our studies aim to discover the molecular mechanisms of listerial virulence factors acting on the membrane, which is crucial for understanding the interaction of the pathogen with the host.

P-141

A key GPCR phosphorylation motif discovered in arrestin2•CCR5 phosphopeptide complexes

Ivana Petrovic[1], Polina Isaikina[1], Roman P. Jakob[1], Parishmita Sarma[2], Ashutosh Ranjan[2], Minakshi Baruah[2], Vineet Panwalkar[1], Timm Maier[1], Arun K. Shukla[2], Stephan Grzesiek[1]
[1] Focal Area Structural Biology and Biophysics, Biozentrum, University of Basel, CH-4056 Basel, Switzerland, [2] Department of Biological Sciences and Bioengineering, Indian Institute of Technology, Kanpur, India

The two non-visual arrestin isoforms, arrestin2 and arrestin3 recognize and bind hundreds of G protein-coupled receptors (GPCRs) with different phosphorylation patterns leading to distinct functional outcomes. However, the molecular basis of phosphorylation-driven arrestin interactions is still poorly understood. We have characterized the interactions between the phosphorylated CC chemokine receptor 5 (CCR5) and arrestin2. Using mass spectrometry, several new CCR5 phosphorylation sites necessary for stable complex formation with arrestin2 were detected. Crystal structures of arrestin2 in apo form and in complexes with CCR5 C-terminal phosphopeptides together with complementary NMR experiments, biochemical and functional assays in the cells revealed three phosphoresidues in a pXpp motif that are essential for the arrestin2 interactions and activation. The same phosphoresidue cluster is present in other receptors, which form stable complexes with arrestin2. We propose that the identified pXpp motif is responsible for robust arrestin2 recruitment in many GPCRs. An analysis of available sequences, as well as structural and functional information on other GPCR•arrestin interactions suggest that a particular arrangement of phosphoresidues within the GPCR intracellular loop 3 and C-terminal tail determines arrestin2 and 3 isoform specificity. Taken together, our findings demonstrate how multi-site phosphorylation controls GPCR•arrestin interactions and provide a framework to probe the intricate details of arrestin activation and signaling.

Poster Presentations
– Session 1 –

P-142
Apoptotic changes in 3D spheroids caused by photodynamic therapy

Viktória Pevná[1], Veronika Huntošová[2], Pavol Miškovský[1,2,3,4]
[1] Department of Biophysics, Pavol Jozef Šafárik University in Košice, Slovakia, [2] Center of Interdisciplinary Biosciences, Technology and Innovation Park, Pavol Jozef Šafárik University in Košice, Slovakia, [3] Cassovia New Industry Cluster, Košice, Slovakia, [4] SAFTRA Photonics Ltd., Košice, Slovakia

Photodynamic therapy (PDT) represents a therapeutic approach, which uses light of an appropriate wavelength, photosensitizer, oxygen, and exhibits a low degree of invasiveness. To understand the mechanism at a tumor stage, a 3D spheroid model was prepared using hanging drop method. Hypericin was used as a photosensitive molecule which was administered to spheroids before spheroid irradiation. The irradiation was carried out by a LED system at 590 nm (2-10 J/cm2) and the effect of therapy was monitored 24 hours after irradiation. Results showed increase in caspase-3 protein level which has a key role in apoptotic pathways. Heterogeneity of caspase-3 distribution in 3D spheroid cell was detected by flow cytometry and vital confocal microscopy. Caspase-3 levels through NucView 488 were preferentially localized in spheroidal periphery cell layers after irradiation. Finally, the differences in phototoxicity due usage increasing dose of irradiation were revealed by lactate dehydrogenase assay.

P-143
Solving the mystery around viral 2A Hbox/NC proteins from Picornaviruses

Marion Pichon[1], Anna Wehlin[1], Eleonore von Castelmur[1,2]
[1] Linköping University, Sweden [2] Wallenberg Centre for Molecular Medicine

The Picornaviruses are a large family of small, non-enveloped RNA viruses, responsible for human and animal diseases including the common cold, poliomyelitis, or foot-and-mouth disease in cattle. During infection, the virus is internalized and releases its ss(+)RNA viral genome into the cytoplasm, to act as mRNA. Due to the nature of these viruses and their high mutation rate, it is crucial to identify host factors early in the infection process to develop efficient drug targets. Recently, the non-essential human phospholipase and acetyltransferase PLAAT3 was identified as a host factor for some picornaviruses. However, studies have demonstrated that several 2A proteins of picornaviruses, including members of the Parechovirus and Kobuvirus genera share conserved sequences with PLAAT3, namely the Hbox/NC motif, which constitutes the lipase active site required for its host factor function. This suggests that these viruses may have acquired this motif through evolution to pursue their infection independently of the host factor. However, its role in the viral life cycle remains unclear. In this study, we use a combination of biophysical, structural, and cell-based techniques to study 2A proteins from several branches of the phylogenetic tree to investigate and understand their role in the viral life cycle. Despite sharing the same motif, the different X-ray structures we obtained revealed that while Aichivirus 2A has a similar structure as PLAAT3, the Parechovirus 2A proteins have undergone a structural rearrangement, destroying the active site. This makes us wonder how this 2A participates in the viral life cycle.

P-144
Interrogating electrochemical approaches for the determination of electron transfer rates of immobilized redox proteins

Frank Hendrik Pilz[1,2], Patrycja Kielb[1,2]
[1] Transdisciplinary Research Area 'Building Blocks of Matter and Fundamental Interactions (TRA Matter)', University of Bonn, Germany, [2] Clausius Institute of Physical and Theoretical Chemistry, University of Bonn, Germany

Interfacing redox proteins on electrodes revolutionized the field of bioelectrochemistry, providing a way to study electrocatalytic activity and offering direct thermodynamic control of the redox state of the protein. As heterogeneous electron transfer (HET) between a protein and electrode is intrinsically non-native, it requires careful choice of immobilization technique and method of analysis. The sheer number of analytical techniques often makes choosing the most suitable one difficult. Here, we compare the HET rate of cytochrome c immobilized on an Ag electrode using cyclic voltammetry (CV), square wave voltammetry (SWV) and electrochemical impedance spectroscopy (EIS). We found both CV and SWV to be suitable for the system of interest. As reliable analysis of CVs via the popular Laviron method proved experimentally inaccessible, we have developed a novel simulation-based approach. This work provides a comprehensive list to choose the most applicable technique for the protein of interest.

P-145
Structural elucidation of a new member of chitin oligosaccharide deacetylases from the marine bacterium Vibrio harveyi

Sirikan Pongnan[1], Robert C. Robinson[1], Wipa Suginta[1]
[1] School of Biomolecular Science and Engineering (BSE), Vidyasirimedhi Institute of Science and Technology (VISTEC), Rayong, Thailand

Poster Presentations
– Session 1 –

P-146
Intermolecular interactions underlie protein/peptide phase separation irrespective of sequence and structure at the crowded milieu

Manisha Poudyal[1], Komal Patel[1], Laxmikant Gadhe[1], Ajay Singh Sawner[1], Pradeep Kadu[1], Debalina Datta[1], Semanti Mukherjee[1], Soumik Ray[1], Ambuja Navalkar[1], Siddhartha Maiti[1], Debdeep Chatterjee[1], Jyoti Devi[1], Riya Bera[1], Nitisha Gahlot[1], Jennifer Joseph[1], Ranjith Padinhateeri[1], Samir K. Maji[1,2]

[1] Indian Institute of Technology Bombay, Powai, Mumbai-400076, India, [2] Sunita Sanghi Centre for Aging and Neurodegenerative Diseases, IIT Bombay, Powai, Mumbai, India

Liquid-liquid phase separation has emerged as a proteome-wide phenomenon underlying the formation of biomolecular condensates, which are involved in widespread cellular functions. Failure in the regulation of the condensate state of proteins might lead to its phase transition to the solid- like state, which is mostly disease-related. Multiple studies have elucidated that intrinsically disordered regions and low-complexity domains promoting intermolecular interactions are the key organizing principle for phase separation. We hypothesize that liquid-liquid phase separation is an inherent property of proteins and polypeptides under specific solution conditions. To investigate this, we evaluated the tendency of 23 proteins/polypeptides with varying sequence and structure to undergo homotypic phase separation in the presence as well as the absence of molecular crowder, polyethylene glycol-8000. We showed that disordered, folded proteins and designed polypeptides (polar, hydrophobic and charged) can form dynamic liquid-like condensate but with different phase regimes and kinetics. We also demonstrated that electrostatic, hydrophobic and H-bonding interactions, alone or in combination play an important role in governing phase separation of proteins/polypeptides, however, the nature of interaction differs from protein to protein.

P-147
Gold-enhanced visible photocatalysis for antibacterial photodynamic therapy

Giorgia Puleo[1,2], Vittorio Ferrara[3], Giuseppe Sancataldo[3], Mariano Licciardi[1], Vito Foderà[2], Valeria Vetri[3]

[1] Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo, Viale delle Scienze Bld.18, Palermo, Italy, [2] Department of Pharmacy, University of Copenhagen, Denmark, [3] Department of Physics and Chemistry, University of Palermo, Viale delle Scienze Bld, Italy

Photodynamic and photothermal therapies are gaining attention as antimicrobial strategies. With the aim of developing new hybrid systems with effective activity against localised infections, we here present an experimental study on the photocatalytic properties of nitrogen-doped titanium dioxide (N-TiO2), a photodynamic agent that, when illuminated, produces reactive oxygen species (ROS), in combination with gold nanoparticles (AuNPs) with well characterised photothermal properties. N-TiO2 was synthesized as a visible-light photocatalyst and the effect of N-doping on the TiO2 band-gap was tested using blue light to trigger ROS production. The photocatalytic activity of these materials was assessed by spectroscopy on standard dye, then tested on target biomolecules revealing that DNA was a major target. We mainly focused on the damages induced on the DNA structure, as it is essential for bacteria survival and replication. Critical modifications in DNA structures were observed and were enhanced in the presence of AuNPs as a consequence of the synergetic effect of N-TiO2 photocatalyst with AuNPs. AuNPs appear to improve the photocatalytic efficiency of N-TiO2 by stabilizing charge carriers on gold surface then preventing electron–hole pairs recombination. Further studies are ongoing to deepen the observed phenomena and identify key factors effectors of the improved photocatalytic activity.

P-148
Comparison of Isolated and Live Nuclei with AFM-Microrheology

Ellen Juel Pørtner[1]

[1] University of Southern Denmark

The cell nucleus is the site of many biological functions such as cell division, DNA replication and repair. It is also subject to various mechanical forces applied by the cytoskeleton and neighboring cells, particularly in cells in tissue exposed to mechanical stress. The nuclear mechanics of cancer cells are of particular interest, as the relatively large size and rigidity of the nucleus limits the cancer cells' invasive abilities. In some experiments, isolated nuclei are used as model systems for live nuclei. However, it is not clear whether isolated nuclei are an appropriate model for live cell nuclei. The isolation process of nuclei typically involves detergents, or mechanically rupturing the cell membrane, and it is unknown whether these processes induce changes in the mechanically dominant nuclear structures. Here we compare isolated and live nuclei from the same breast cancer cell type. Material properties, such as viscosity and elasticity, of single nuclei can be measured with atomic force microscope microrheology. Preliminary data indicates that isolated nuclei are more elastic and viscous than living nuclei in intact cells. This can f.x. result in a wrong estimation of cancer cells' migratory abilities if they are estimated based on viscoelastic properties of isolated nuclei.

P-149
Peptide Wars: Revenge of the AMPs (Antimicrobial peptides); a new strategy against colorectal cancer

Mina Raileanu[1], Mihaela Bacalum[1]

[1] Horia Hulubei National Institute for R&D in Physics and Nuclear Engineering (IFIN-HH), Romania

Cancer is a multifaceted health issue that affects people globally and it is considered to be one of the leading causes of death with a high percentage of victims worldwide. In recent years, research studies have uncovered great advances in the diagnosis and treatment of cancer. But there are still major drawbacks of the conventional therapies used including severe side effects, toxicities, and drug resistance. That is why it is critical to develop new drugs that have advantages like low cytotoxicity and no treatment resistance of the cancer cells. Antimicrobial peptides (AMPs) have recently attracted attention as a novel therapeutic strategy for the treatment of various cancers, targeting tumor cells with less toxicity to normal tissues. In this study, we present the results of using various antimicrobial peptides (melittin, cecropin A and the hybrid cecropin A – melittin) on spheroids made out of human colorectal cancer cell lines (HT-29 and HCT-116). We observed, that by using different concentrations of these compounds the structure of the spheroids is highly compromised and also certain mechanisms that are activated lead to the death of these cancer cells.

Poster Presentations
– Session 1 –

P-150
The Effects of State-Selective Binding on Photoswitchable Protein Thermal Reversion
Paul Max Reed[1], Ryan Woloschuk[1], Jaewan Jang[1], G. Andrew Woolley[1]
[1] University of Toronto, Canada

Light-switchable proteins are widely used as tools in synthetic biology. Typically, these proteins have a thermally stable dark-state and convert to a "light" state upon irradiation. The light-state then thermally reverts to the dark-state. One important parameter of any light-controlled system is the half-life of the light-state; this dictates the time it takes to thermally revert to the "dark" state, and hence the persistence of the effect of light. An interesting aspect of such systems is the degree to which light-induced protein- protein association affects this half-life. In some cases, we found that the association of a photoswitchable protein and a binder selective for the light-state can increase the half-life by more than 10-fold. Therefore, it is of interest to study the general relationships between light-dependent binding events and changes in photoswitchable protein half-lives. We have demonstrated that, for two unrelated systems, binders selective for the light-state increase the half-life while binders selective for the dark- state decrease it. Literature suggests that this is part of a larger framework for understanding the effects of state-selective binding on the thermal half-lives of photoswitchable proteins.

P-151
Structural dynamics basis of function in the SecB chaperone
Amal Saidi[1], Guillaume Roussel[1], Jochem Smit[1], Lily Karamanou[1], Tassos Economou[1]
[1] KU Leuven, Department of Microbiology and Immunology, Rega Institute for Medical Research, Laboratory of Molecular Bacteriology, Belgium

How chaperones recognize and act on their clients remains a major universal unresolved. Some of them are proposed to act as 'holdases' that tightly bind to polypeptides, and others as 'foldases' that provide an active contribution to the folding process. In the secretory pathway, the role of chaperones is critical as secretory polypeptides need to remain non-folded for several seconds in the cytoplasm prior to translocation. Applying advanced methods, it was possible to demonstrate that SecB, a bacterial chaperone belonging to the Sec pathway, possesses at least 3 activities as it acts on its client. First it 'denatures' folding intermediates to expanded states, then it 'holds' onto the expanded client for several minutes during a highly dynamic on-and-off interaction and, finally, the client is released in a way that it can follow a distinct refolding pathway towards the native state. The C-tail of SecB has been shown to be involved in preventing the aggregation of preproteins in the cytosol and in ensuring their functional secretion. The project is embedded in the effort of the lab to understand how chaperones modulate secretory protein (non)folding. Using (pro)MBP as a model for SecB clients, this project will: characterize in vivo/in vitro the functional role of SecB C-tail in preprotein secretion, probe the role of the C-tail of SecB in interacting with clients and in the intrinsic disorder of SecB, and identify features of the C-tail that are important for SecB function.

P-152
Two photon lithography for in situ encapsulation of multicellular biosystems
Barbara Schamberger[1], Mohammadreza Taale[1], Fereydoon Taheri[1], Yasmin Antonelli[1], Aldo Leal-Egaña[1] Christine Selhuber-Unkel1
[1] Institute for Molecular Systems Engineering and Advanced Materials (IMSEAM), Heidelberg University, Im Neuenheimer Feld 225, 69120 Heidelberg, Germany

In cancer research, spheroids are a model for studying cellular behavior in a multicellular system. However, adapting the mechanical microenvironment in an in vivo setup is technically challenging. A promising tool is high resolution 3D printing using 2-photon polymerization. So far there is only a limited amount of suitable biocompatible materials available. This project aims to encapsulate spheroids in a hydrogel and allow only directed migration. For this purpose, MCF7 breast cancer spheroids were mounted on glass-clover slides and encapsulated using a gelatin based ink cross-linked by Nanoscribe Photonic Professional GT2. After the printing the spheroids were tested for cell viability. We show that the cells of the encased spheroids were vital and escaped the 3D printed envelope only in a predefined direction. Also, we show that the cells exposed to both laser light and printing material were dead after 1d were as the material or the laser light alone does not show toxic effect towards the cells. The presented technique paves the way towards new strategies in cancer research adapting it to organ on a chip or other screening methods.

P-153
Dynamic assembly of the Hsp90 chaperone machinery investigated by multi-colour single-molecule FRET
Julia Schimpf[1,2], Leonie Vollmar[1,2], Thorsten Hugel[1,3]
[1] Institute of Physical Chemistry, University of Freiburg, Freiburg, Germany , [2] Spemann Graduate School of Biology and Medicine (SGBM), University of Freiburg, Freiburg, Germany, [3] BIOSS and CIBSS Signalling Research Centres, University of Freiburg, Freiburg, Germany

Being one of the most abundant proteins in the cytosol, Hsp90 forms various chaperone machineries with different cochaperones for a plethora of clients. Here, we determine the assembly of some Hsp90-based machineries and the dynamics of the interactions within. We wish to clarify whether the proteins involved have random encounters and form complexes by chance or if there is an underlying, sequential mechanism that includes one protein recruiting another and then the next and so on. Specifically, we are studying the interaction between the yeast proteins Hsp90, the cochaperones Cdc37 and Aha1 as well as the client kinase Ste11 in real time with multi-colour single-molecule FRET. In our prism-TIRF setup, we can use up to four lasers in alternating excitation mode to observe the dynamic behaviour of surface-immobilised Hsp90 and its interaction partners. To overcome their low mutual affinities, linked protein fusions with orthogonal labelling sites, that ensure specific fluorescent labelling, are used. Three-colour single-molecule traces already show interactions between Hsp90, Cdc37 and Ste11 in our in vitro conditions and we currently quantify their dynamic assembly. Our first results for the interaction between Hsp90 and the cochaperones Aha1 and Cdc37 point towards a highly dynamic recruiting process by Hsp90. I will show that the currently best explanation is a combination of random and sequential interactions, which is also energetically most efficient. In summary, the developed multi-colour single-molecule FRET method enables us to directly observe and quantify complex assembly pathways.

Poster Presentations
– Session 1 –

P-154
Electrostatics in Antibacterial Resistance: Investigating Active Site Electric Fields as the Basis for Catalysis in TEM β-Lactamases
Mirjam Schulz[1]
[1] Freie Universität Berlin, Germany

Tackling the emergence of broad-spectrum beta-lactamases is one of the greatest challenges in the field of the evolution of antibiotic resistances. It is, therefore, important to obtain a detailed understanding of the enzymatic mechanisms improving reactivity of beta-lactamases against novel antibiotics that are under constant development. Previously, experimental studies utilizing the concept of the vibrational Stark effect have indicated that TEM beta-lactamases take advantage of the phenomenon of electrostatic catalysis. This effect improves the hydrolytic activity by specifically lowering the transition state energy and, thus, the energy barrier of the reaction via preorganized electric fields at the active site amounting to up to -160 MV/cm to facilitate catalysis. However, the experimental data was complicated by various spectral contributions, hindering the clear understanding of the underlying physical origins of the explored effects. Here, we use molecular dynamics simulations using fixed-charge and polarizable fore fields (AMOEBA) to reconstruct the electric field changes proposed by the experimental results. By this, we aim to provide a clear assignment for the observed IR spectral features and give insight into the molecular principles and physical origins that may help predicting how enzymes adapt to new antibiotics.

P-155
Effect of Erbb2 Missense Mutations on Dimer Formation
Tayde Gabriela Serrano Cano[1], Atena Yasari[2], Irene Tiemann-Boege[2], Péter Nagy[1]
[1] Department of Biophysics and Cell Biology, University of Debrecen, Debrecen, Hungary, [2] Institute of Biophysics, Johannes Kepler University, Linz, Austria

ErbB2 can be activated by heterodimerization with ligand-activated ErbB1, also known as EGFR or homodimerization at high membrane concentration. ErbB2 overexpression is caused by ErbB2 gene amplification linked to aggressive cancer. The R678Q, R143Q, and V842I missense mutations are three recurrent ErbB2 mutations in sperm cells that occur more frequently than expected for de novo germline mutations, and their effect on cell signaling or potentially activating functional significance has yet to be elucidated. To achieve this, CHO cells were transfected with ErbB2 variants and co-transfected with EGFR to measure ErbB2 homodimerization and its heterodimerization with EGFR using Förster resonance energy transfer (FRET). Additionally, receptor mobility was analyzed with fluorescence recovery after photobleaching (FRAP). All ErbB2 variants showed low levels of homoclustering and somewhat higher levels of basal heterodimerization with EGFR without stimulation. EGF stimulation increased the level of heteroassociation between wtErbB2 and EGFR, but the three investigated variants did not show a higher interaction with EGFR after EGF binding, similar to the constitutively active ErbB2 mutant. The mobility of the R143Q, V842I, and R678Q mutants and the constitutively active ErbB2 version was significantly enhanced in single transfected cells, but all mutant versions responded with reduced mobility after EGF stimulation in cells co-expressing EGFR. While the experiments reveal a complex relationship between receptor activation and biophysical properties, the dimerization and diffusional properties of the mutations isolated from sperm samples resemble the constitutively active version, implying that the mutations might result in a gain of function.

P-156
A novel biophysical-based approach to understand the stabilisation and suppressed aggregation of protein therapeutics in ionic liquid formulations
Talia A. Shmool[1], Jason P. Hallett[1]
[1] Imperial College London, Department of Chemical Engineering, United Kingdom

Over 96 percent of drug development fails, largely due to the inherently high aggregation propensity of protein therapeutics in aqueous formulations. Understanding protein folding in different environmental conditions is fundamentally important for predicting protein structures and developing innovative stabilising therapeutic formulations. Ionic liquids (ILs) are comprised of cations and anions and have been found effective for suppressing the aggregation of diverse protein therapeutics (Shmool et al. Chem. Sci. 2021, Shmool et al. JACS Au 2022). Nonetheless, the mechanisms of protein stabilisation and prevention of aggregation by ILs remains largely unknown. The focus of this research is to demonstrate a biophysical-based approach for the design of IL containing formulations and the guided evaluation for formulation optimisation. To date, strategic formulations of ILs and excipients, such as sugars and amino acids, and systematic analysis enabled: (i) Extending the shelf-life of RNA- based vaccines; (ii) Improving the thermal stability of virus-like particles to eliminate the costly cold chain; (iii) Suppressing aggregation and enhancing the therapeutic delivery in vivo; and (iv) Directing controlled self-assembly of lipid nanoparticles and injectable hydrogels for drug delivery. Computational and thermodynamic studies, which will be shown, revealed insight into protein aggregation processes, confinement effects, the conformational stability and dynamics of proteins which can be modulated by judicious selection of the excipients and ILs. Ultimately, we provide unique insight into the complex process of protein folding and innovative pharmaceutical development based on the distinctive properties of IL containing formulations.

P-157
In-cell folding stability of the highly conserved Cold Shock domain of Y-Box binding protein depends on DNA/RNA binding and phosphorylation: a key factor for regulating gene expression
Puja Shrestha[1,2], Simon Ebbinghaus[1,2]
[1] Institute of Physical and Theoretical Chemistry, TU Braunchweig, Rebenring 56, D-38106 , [2] Braunschweig Integrated Centre of Systems Biology (BRICS), 38106 Braunschweig, Germany

Y-Box Binding protein (YB1) has multiple functions including the ability to bind to DNA/RNA thereby regulating transcription and translation. In various cancers, YB1 expression is upregulated and translocated into the nucleus, a process suggested to be mediated by phosphorylation of serine at position 102, thus seen as a promising candidate for therapeutic targets. A previous in vitro study suggested that the cold shock domain (CSD52 -129; CSD52-140 (CSDex)) is marginally stable and stability as well as DNA/RNA binding processes are interconnected, being additionally regulated by S102 phosphorylation. So far, the stability of YB1-CSD inside cells has not been studied. Moreover, it remains unclear which conformational state (folded, partially folded or unfolded) interacts with DNA/RNA, how binding affects the stability and how phosphorylation mediates this process. Here we build a FRET-based folding sensor to investigate how RNA/DNA affects the stability of CSD and the function of S102 phosphorylation in this process directly inside cells. We measured the stability of both CSD and CSDex using Fast relaxation imaging (FReI). So far, our results not only confirmed that the CSDex (Tm=36°C) is more stable than the CSD (Tm =27°C) but also suggest that both proteins are significantly unfolded at physiological conditions. In addition, our phosphomimetic mutant S102D is less stable in-cell than the wt (Tm= 4°C lower) suggesting that S102 might not be phosphorylated in the cytoplasm. Overall, our results provide functional insights into the roles of ligand-binding and PTM in YB1 function, which might be fundamental considering YB1 as a therapeutic model.

Poster Presentations
– Session 1 –

P-158
Biophysical characterization of dengue virus capsid protein interaction with nucleic acids
Nelly M. Silva[1], Ana S. Martins[1], Nina Karguth[1], Francisco J. Enguita[1], Roland G. Huber[2], Nuno C. Santos[1], Ivo C. Martins[1]
[1] Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, 1649-028 Lisbon, Portugal , [2] Bioinformatics Institute (BII), Agency for Science, Technology and Research (A*STAR), 138671 Singapore, Singapore

Dengue virus (DENV) is a mosquito-borne flavivirus, which transmission has been dramatically increasing. Its nucleocapsid is formed by the single-stranded RNA genome condensed with multiple copies of the capsid (C) protein. This is an essential protein, which is involved in key steps of the viral life cycle, namely encapsidation and viral assembly. A key step, essential for viral replication, requires DENV C specific binding to lipid droplets (LD), an interaction previously characterized by us and other authors. Those findings led to the development of pep14-23, a patented peptide able to inhibit DENV C binding to LDs. To characterize DENV C interactions with the viral RNA, we started examining locations within the viral RNA to which the protein has higher affinity, with specific RNA sequences being identified. Circular dichroism and fluorescence spectroscopies data show that some analogous single-stranded DNA sequences used as proxies of selected RNA sequences interact with DENV C, causing changes in the protein secondary structure and suggesting the formation of large complexes. These data may help developing inhibitors against this essential interaction as well as to construct a model of flaviviruses C proteins biological activity. These methodologies may be applied to related flaviviruses, as well as other human viral pathogens.

P-159
Biophysical Features and Local Early Conformational Propensities in Intrinsically Disordered Regions of Rhabdoviral Glycoproteins
Nikolay Simankov[1,2], Rachid Tahzima[1,3], Hélène Soyeurt[2], Sébastien Massart[1]
[1] Laboratory of Plant Pathology – TERRA - Gembloux Agro-BioTech – University of Liège (ULiège) - 5030 Gembloux, Belgium, [2] Statistics, Computer Science and Modeling applied to bioengineering (SIMa) – TERRA – University of Liège (ULiège) 5030 Gembloux, Belgium, [3] Flanders Research Institute for Agriculture, Plant Sciences, Fisheries and Food (ILVO), 9820 Merelbeke, Belgium

Most harmful rhabdoviruses cause disease that is invariably lethal to humans, animals and plants. Throughout viral infection, protein refolding is a complex process critical to both receptor recognition and membrane-interacting fusion domains mediated by transmembrane rhabdoviral glycoprotein (Gp). We hypothesized that the early stages of context-sensitive structural transitions of the Gp involve local interactions between residues situated in intrinsically disordered regions (IDRs). This study investigates the host-specific biophysical (Bp) IDRs-determinants encoded in the primary amino acid sequence of rhabdoviral Gp, which are predicted to modulate early conformational events. This can be related to host- specific Bp features of the local backbone and secondary structure propensities close to or in IDRs of Gps. While showing striking differences between IDRs of N- and C-termini, our results give direct insights into the Bp folding signals located in these regions and are in congruence with independent experimental observations. In conclusion, the accurate connection of Bp structural features with both IDRs conformational propensities and context-sensitive folding data suggests their statistically significant role in local transition with lasting effects on subsequent conformational states during virus-host interactions and disease-related pathogenicity outcomes.

P-160
Amyloid fibers in biofilms: structure and adaptation from environmental cues
Macarena Siri[1], Agustin Mangiarotti[1], Monica Vazquez-Davila[1], Cecile Bidan[1]
[1] Max Planck Institute of Colloids and Interfaces, Germany

Biofilms consist of bacteria embedded in a self-produced matrix mainly made of proteins and polysaccharides. Biofilms are not only resistant but also adaptable to environmental conditions (e.g., temperature, humidity), making them an interesting source of biomaterials. Although their extracellular matrix is considered to be an important cause of biofilm properties, it remains unclear how they acquire such resilience. Within their molecular organization, proteins can be found in the form of amyloid fibers, which provide structure, rigidity and protection. They are also contributing factors in the morphogenesis and physico-chemical properties of biofilms. Understanding how environmental cues affect the molecular structure of biofilms and their resulting properties, would greatly clarify the molecular structure/macroscopic behavior of biofilms beneficial to, for instance, the emerging field of engineered living materials. We studied the structure and function of purified curli fibers from E. coli biofilms grown on substrates with different water contents using electron microscopy, infrared and fluorescence spectroscopy. By microindentation experiments we correlated the fibers physico-chemical properties and their contribution in the biofilm stiffness. Our multimodal analysis revealed differences in the yield of curli fibers purified from the E. coli biofilms, in the packing of these fibers, their hydrophobicity and their chemical stability. Most interestingly, the biofilm stiffness was shown to correlate with the structure of the fibers. The findings reported provide valuable knowledge regarding the structure – function relationship that spans biofilm scales from the molecular to the tissue level, and give insights into the adaptation response of biofilms in specific environments.

P-161
Indirect optical manipulation for building simple multicellular systems
Cyril Slabý[1], Jana Kubacková[2], Viktória Pevná[1], Veronika Huntošová[3], Gergely T. Iványi[4], Alena Strejčková[5], Gaszton Vizsnyiczai[4], Lóránd Kelemen[4], Zoltán Tomori[2], Gregor Bánó[1]
[1] Department of Biophysics, Faculty of Science, P. J. Šafárik University in Košice, Jesenná 5, 041 54 Košice, Slovakia , [2] Department of Biophysics, Institute of Experimental Physics, Slovak Academy of Sciences, Watsonova 47, 040 01 Košice, Slovakia, [3] Center for Interdisciplinary Biosciences, P. J. Šafárik University, Jesenná 5, 041 54 Košice, Slovakia, [4] Biological Research Centre, Institute of Biophysics, ELKH, Szeged, Hungary, [5] Department of Chemistry, Biochemistry and Biophysics, University of Veterinary Medicine and Pharmacy in Košice, Komenského 68/73, 041 81 Košice, Slovakia

When moving from single cell studies to tissue conditions, multicellular systems that allow for direct mechanical and chemical interactions between nearest cell neighbors are required. Spheroids formed by 100-100.000 cells are typically used. Our goal is to create low-number cell systems containing 2-10 cells to facilitate cell-cell interaction studies under the most basic conditions possible. Two-photon polymerization direct laser writing was used to create optically trappable microstructures. A dual optical trap manipulates the structures that work in a bulldozer-like regime and is used to push individual cells into containers that restrict further cell motion. Linear cell chains are formed in this manner. A cancer cell line is used for proof-of-concept experiments. This work was supported by the Slovak Research and Development Agency, grant APVV-21-0333, and by the grant agency of the Ministry of Education, Science, Research and Sports of the Slovak Republic, grant VEGA 2/0101/22.

Poster Presentations
– Session 1 –

P-162
Tackling the Lindemann criterion in protein physics
Daniele Sonaglioni[1,2], Simone Capaccioli[1,2], Valeria Libera[3], Elpidio Tombari[4], Judith Peters[5,6,7], Alessandro Paciaroni[3]
[1] CISUP, Centro per l'Integrazione della Strumentazione dell'Università di Pisa, Lungarno Pacinotti 43, 56126, Pisa, Italy, [2] Dipartimento di Fisica "E. Fermi", Università di Pisa, Largo Pontecorvo 3, 56127, Pisa, Italy, [3] Dipartimento di Fisica e Geologia, Università di Perugia, Via Alessandro Pascoli, 06123 Perugia, Italy, [4] Consiglio Nazionale delle Ricerche, Istituto per i Processi Chimico-Fisici (CNR-IPCF), Via Moruzzi 1, 56124 Pisa, Italy, [5] Université Grenoble Alpes, CNRS, LiPhy, Saint-Martin-d'Hères, 38400, France, [6] Institut Laue Langevin, Grenoble Cedex 9, F-38042, France, [7] Institut Universitaire de France

Fast fluctuations are central in protein physics and are coupled to the external environment: yet their role in protein stability and unfolding remains elusive. Recent progresses proposed the existence of a critical dynamical regime at the thermal unfolding, suggesting a Lindemann-like criterion. Combining Elastic Incoherent Neutron Scattering (EINS) and differential scanning calorimetry, we studied one protein for each class, i.e. mesophilic, thermophilic and intrinsically disordered (IDP) proteins, in three different glass-forming deuterated environments, investigating their dynamic and thermodynamic properties. Different solvents lead to different dynamical and thermodynamical behaviours. A common dynamical regime can be found, especially for mesophilic and thermophilic proteins, mirrored by thermodynamics, with similar unfolding temperatures. The only exception is represented by the IDP, having a unique behaviour from both points of view. A thorough comparison of EINS data, in the whole available temperature and exchanged momentum range, has allowed a better understanding of the interplay between the solvent and the protein intrinsic properties. In conclusion, our multi-technique approach builds a bridge between dynamics and thermodynamics in protein physics, confirming old results and raising new questions: the Lindemann-like criterion was extended to thermophilic proteins, but does not fully apply for the IDP.

P-163
Increased obesogenic action of palmitic acid during the early stage of adipogenesis
Ewa Stanek[1,2], Krzysztof Czamara[2], Agnieszka Kaczor[2,3]
[1] Jagiellonian University, Doctoral School of Exact and Natural Sciences, 11 Lojasiewicza Str., 30-348 Krakow, Poland, [2] Jagiellonian Centre for Experimental Therapeutics, Jagiellonian University, 14 Bobrzynskiego Str, Krakow, Poland, [3] Faculty of Chemistry, Jagiellonian University, 2 Gronostajowa Str., 30-387 Krakow, Poland

Background: Adipocyte development is substantially sensitive to lifestyle and dietary habits. Overconsumption of products rich in fat, especially saturated fatty acids like palmitic acid (PA), is associated with the development of obesity. Although the action of PA on the adipose tissue is well described, there is a lack of data on the chemical alterations during different stages of adipogenesis. Therefore, this work aimed to verify the PA- induced effect on primary adipocytes as well as the adipocytes derived from the cell culture. Methods: The study was conducted on epididymal white adipose tissue (eWAT) isolated from C57Bl/6J male mice at the age of 10 and 20 weeks. Studied cells were exposed to deuterium-labelled palmitic acid (D31-PA) for 24 hours and measured using the confocal Raman WITec system equipped with a 532 nm laser. Results: The most significant changes in the lipid composition occurred in preadipocytes. High accumulation of D31-PA in these cells resulted in altered morphology, elevated formation of triacylglycerols, and decrease in lipid unsaturation degree of lipid droplets. Conclusion: Palmitic acid has a significant impact on cells in the early stage of adipocyte development. Interestingly, the less mature the cells, the more chemical composition is changed.

P-164
Structural dynamics of the intrinsically disordered SNARE protein SNAP25a in its pre-fusion conformation
Tobias Stief[1,2], Katharina Vormann[1,2], Lothar Gremer[1,2], Ralf Biehl[3], Ángel Pérez-Lara [4], Nils-Alexander Lakomek[1,2]
[1] Forschungszentrum Jülich, Structural Biochemistry (IBI-7), Germany, [2] Heinrich Heine University of Düsseldorf, Physical Biology, Germany, [3] Jülich Centre for Neutron Science (JCNS-1) and Institute for Biological Information Processing (IBI-8), Forschungszentrum Jülich, Germany, [4] Dept. of Physical Chemistry, University of Granada, Campus Universitario de Cartuja, Spain

The fusion of synaptic vesicles with the plasma membrane at the neuronal synapse in the process of neuronal exocytosis is a key requirement for the release of neurotransmitters. Here, the assembly of the so-called SNARE (soluble N-ethylmaleimide-sensitive-factor attachment receptor) complex plays a central role by providing the energy necessary for membrane fusion. At the molecular level, important structural transitions of the SNARE proteins and their membrane interactions remain not well understood. The neuronal SNARE protein SNAP25a (isoform 2) is an indispensable component in the assembly process of the SNARE complex triggering neuronal exocytosis. While the post-fusion cis-SNARE complex is relatively well understood, few details are known about the pre-fusion conformation of SNAP25a. We investigated the secondary structure and backbone dynamics of SNAP25 by three-dimensional NMR backbone assignment experiments and NMR relaxation measurements, as well as further biophysical methods, such as SAXS and CD spectroscopy. We found that large regions of SNAP25a are highly disordered and exhibit high internal flexibility, while the N-terminus of SNAP25a is α-helical. We speculate that this region acts as a nucleation site for initiating the so-called SNARE zippering process.

P-166
Uncovering Novel Inhibition Mechanisms of Insulin-Regulated Aminopeptidase through Allosteric Sites and Conformational Restriction
Anastasia Mpakali[1,2], Petros Giastas[3], Efstratios Stratikos[1,2]
[1] National and Kapodistrian University of Athens, Greece [2] National Centre for Scientific Research Demokritos, [3] Agricultural University of Athens

Inhibition of Insulin-Regulated Aminopeptidase is being actively explored for the treatment of several human diseases and several classes of inhibitors have been developed although no clinical applications have been reported yet. Here, we combine enzymological analysis with x-ray crystallography to investigate the mechanism employed by two of the most studied inhibitors of IRAP, an aryl sulfonamide, and a 2-amino-4H- benzopyran. Although both compounds have been hypothesized to target the enzyme's active site by competitive mechanisms, we find that they instead target previously unidentified allosteric sites and utilize non-competitive and uncompetitive inhibition mechanisms. X-ray crystallographic analysis demonstrated that the aryl sulfonamide stabilizes the closed, more active, conformation of the enzyme whereas the benzopyran locks the enzyme in a semi-open, but less active, conformation. The benzopyran inhibitor potency is highly substrate-dependent and fails to effectively block the degradation of the physiological substrate cyclic peptide oxytocin. Our findings demonstrate alternative mechanisms for inhibiting IRAP through allosteric sites and conformational restricting and suggest that the benzopyran inhibitor's usefulness is more clinically limited than initially considered. Such conformation-specific interactions between IRAP and small molecules can be exploited for the design of more effective second- generation inhibitors.

Poster Presentations
– Session 1 –

P-167
Characterization of biophysical properties of staphylokinase variant selected by ribosome display
Monika Štulajterová[1], Mária Tomková[2], Ľuboš Ambro[2], Erik Sedlák[2,3]
[1] Department of Biophysics, Faculty of Science, P.J. Šafárik University, Slovakia[2] Center for Interdisciplinary Biosciences, Technology and Innovation Park, P. J. Šafárik University, [3] Department of Biochemistry, Faculty of Science, P.J. Šafárik University

Thrombosis, the blockage of blood vessels by clots, can lead to acute myocardial infarction, ischemic stroke, and other medical complications leading to death. Unfortunately, commonly used thrombolytics have some drawbacks, such as high production cost, non-fibrin specificity, allergic reaction or systemic bleeding complications and there is a need to investigate new treatment options. Bacterial staphylokinase (SAK) is a small-size plasminogen activator, expressed by lysogenic strains of Staphylococcus aureus. SAK forms a productive equimolar (1:1) complex with plasmin. Created complex can activate inactive zymogen plasminogen to its active form, plasmin. SAK has the potential to become a promising third-generation thrombolytic agent with properties such as low production cost, improved efficacy and fibrin specificity with minimal bleeding tendencies. However, for such utilization, an improvement of the SAK affinity and selectivity is inevitable in order to increase its residence time on plasmin, thus reducing the severity of side effects. Directed evolution is a powerful approach to tailor protein properties toward new or enhanced functions. We employed ribosome display (RD), in vitro selection and evolution technology, to improve SAK affinity for plasmin (affinity maturation). After 6 rounds of RD more than 90% of selected SAK variants harboured one particular mutation. Here, we present comparison of biophysical and functional properties of the SAK variant with this specific mutation with SAK wild-type.

P-168
Optimization of nanodevice geometry for improved molecular-motor transport in biocomputing applications
Pradheebha Surendiran[1], Marko Ušaj[2], Till Korten[3], Alf Månsson[2], Heiner Linke[1]
[1] NanoLund and Solid State Physics, Lund University, Lund, Sweden, [2] Department of Chemistry and Biomedical Sciences, Linnaeus university, Kalmar, Sweden, [3] B CUBE - Center for Molecular Bioengineering, Technische Universität Dresden, D-01307 Dresden, Germany

Network-based Biocomputation (NBC) is a novel parallel computing paradigm that encodes combinatorial problems into a nanofabricated device's graphical network of channels, enabling cytoskeletal filaments propelled by molecular motors to explore the solution space and overcome the exponential time complexity of conventional serial computation. NBC promises to require significantly less energy than traditional computers due to the high energy efficiency of molecular motors. However, high error rates associated with the pass junction crossing, main path-regulating geometry, pose a challenge for scaling up this technology. This study addresses this issue by optimizing the geometry of the pass junction, varying features such as the nanochannel width, junction crossing area, and angles. Sixteen different pass junction designs were developed, and their error rates were evaluated using experimental and simulation methods. The resulting optimized design exhibited a reduced error rate of less than 1% and can be employed for future NBC devices, which is crucial for scaling up the technology. This research highlights the importance of systemic control of nanodevice features for the manipulation and controlled guidance of molecular motors in various nanotechnological applications, potentially leading to significant advancements in molecular transport and computing applications.

P-169
Viscosity changes of amphiphilic phosphorous dendrimers under various conditions of induced shear rate
Šimon Šutý[1], Patrick Mydla[1], Martin Kopáni[2], Pavol Vitovič[3], Dzmitry Shcharbin[4], Maksim Ionov[5], Jean-Pierre Majoral[6], Maria Bryszewska[5], Iveta Waczulíková[1]
[1] Faculty of Mathematics, Physics and Informatics, Comenius University in Bratislava, [2] Institute of Medical Physics, Biophysics, Informatics and Telemedicine, Faculty of Medicine, Comenius University, Bratislava, Slovakia, [3] Department of Simulation and Virtual Medical Education, Faculty of Medicine, Comenius University, Bratislava, Slovakia, [4] Institute of Biophysics and Cell Engineering of NASB, Minsk, Belarus , [5] Faculty of Biology and Environmental protection, Department of General Biophysics, University of Lodz, Poland, [6] Laboratoire de Chimie de Coordination Du CNRS, Toulouse, France

For tumor drug delivery, intravenously administered nanoparticles circulate in the bloodstream and must be small enough to escape circulation through tumor microvasculature. However, events such as a premature release of the drug from nanovehicle still may occur, which results in unintended interactions with blood components. Blood compatibility and safety of any nanoformulation for medical use must therefore be carefully evaluated before. In our study we used a first (DD1) and a second generation dendrimer (DD2) at concentrations of 0, 2, 10 and 30 μM which had already been tested in cell studies. Since blood is considered a non-newtonian fluid, we focused on the flow behaviour and viscosity changes upon interaction of blood with DD1 and DD2. We used a rotational modular rheometer in the range of shear rate of 1 to 1000 s-1 to mimic circulation in various in vivo conditions. We observed an increase in viscosity at all concentrations of both dendrimers. These effects are correlated with hemocoagulation parameters presumably without physiological significance. However, higher doses may pose increased risk under in vivo conditions ranging from problems with perfusion in body tissues to thrombosis and embolism.

P-170
Protein Charge Transfer Spectra (ProCharTS): A label-free probe to track protein unfolding and aggregation
Rajaram Swaminathan[1]
[1] Indian Institute of Technology Guwahati, Assam, INDIA

Intrinsic electronic absorption and luminescence arising from non-aromatic groups in proteins has attracted attention in recent times. In this context, work from our lab [Chem. Sci. 8, 5416, 2017] and our collaborator's lab [JPCB 123,10967, 2019] has shown that charged amino acid sidechains of Lys, Glu, Asp, Arg and His along with polypeptide backbone can participate as Donor/Acceptor in photoinduced electron transfer yielding charge transfer (CT) absorption spectra among proteins in the UV-Vis region (250—800 nm). Interestingly, the molar extinction coefficient in such a CT absorption spectrum or its associated steady state luminescence intensity [JPCB 124, 2731, 2020] were shown to be clearly dependent on the spatial proximity among charged atoms in the 3D fold of the protein. Here, we report applications of ProCharTS absorption/luminescence spectra as label-free spectral probes to monitor dynamic biochemical events. We track the equilibrium unfolding of charge-rich proteins like human serum albumin using ProCharTS absorption spectra to show that disruption of tertiary contacts among charged residues occurs at much lower denaturant concentrations compared to loss of secondary structure. Further aggregate growth kinetics of Abeta (16 -22, KLVFFAE) derived switch-peptides were sensitively tracked using ProCharTS absorption and luminescence. Increasing proximity between Lys/Glu charged headgroups in the oligomer during progress of the early stages of Abeta aggregation was picked up by rise in ProCharTS absorption and luminescence. In summary, we show that ProCharTS absorption/luminescence can be a sensitive probe for detecting changes in protein structure which alter the proximity among charged groups in the protein.

Poster Presentations
– Session 1 –

P-171
Adhesive and biomechanical properties of skin cells under static and flow conditions
Renata Szydlak[1], Marcin Luty[1], Ingrid H. Oevreeide[2], Victorien E. Prot[3], Joanna Zemła[1], Bjørn T. Stokke[2], Małgorzata Lekka[1]
[1] Institute of Nuclear Physics, Polish Academy of Sciences, PL-31342 Krakow, Poland , [2] Biophysics and Medical Technology, Department of Physics, NTNU The Norwegian University of Science and Technology, NO-7491 Trondheim, Norway, [3] Biomechanics, Department of Structural Engineering, NTNU The Norwegian University of Science and Technology, NO-7491 Trondheim, Norway

Melanoma is a skin cancer originating from a malignancy of melanocytes growing between keratinocytes. Oncogenically altered cells are characterized by different biomechanical properties, enormous mutations, and molecular changes on the cell surface, including glycosylation patterns. These changes, including glycans expression and composition, are crucial for cancer pathology and have enormous potential as diagnostic and prognostic markers. Various techniques use lectins to detect different glycans present on the cellular surface exploiting specificity of lectin- glycan interactions hosted by various lectins. Keratinocytes and melanocytes are two basic skin forming cells, where the latter transforms into melanoma (the most frequent skin cancer) during cancer progression. Here, we characterize the biophysical properties of melanoma cells originating from different stages of cancer progression (WM35 from the radial growth phase, WM115 from the vertical growth phase, and WM266-4 from metastasis to skin) and keratinocytes (HaCaT cells). Their mechanical properties and adhesion to surfaces coated with selected lectins were quantified in static and flow conditions. The presence or absence of lectin-specific glycans on the surface of the investigated cells was confirmed using fluorescently labeled lectins. Biomechanical parameters such as cell size and deformation were determined from images taken during the flow of cells through a channel (height 200 μm, width 5000 μm and length 50 mm). The obtained results showed that cancer cells differ in mechanical properties and also adhere to other lectins compared to normal cells.

P-172
Time-resolved studies on conformational changes of α-synuclein by a micro-stopped-flow grating system
Masahide Terazima[1], Shunki Takaramoto[1], Yusuke Nakasone[1]
[1] Kyoto University, Japan

For understanding molecular mechanism of protein functions, the reaction dynamics should be elucidated. In many cases, spectroscopy has been used. However, the absorption or emission spectrum refracts only changes around chromophores and any conformation change far from the chromophore could be spectrally silent. We have investigated the reaction dynamics from a view point of diffusion coefficient (D) using the transient grating (TG) method, which can detect global conformational changes of protein moiety. In this study, we developed a novel TG method combined with a stopped-flow (SF-TG) method. This system expands the applicability of the TG detection considerably, and we applied this novel SF-TG technique to conformation dynamics of α-Synuclein (αSyn). αSyn is a protein localized at the presynaptic terminals of neurons, and is a principal component of Lewy bodies, which are accumulated in the brains of patients with Parkinson's disease. The protein is classified as an intrinsically disordered protein. The conformation of αSyn depends on the environment. While the structure of αSyn has been studied, the process of structural change from the denatured state to the folded state remains unclear. The interaction dynamics between αSyn and SDS micelles were investigated using our developed SF-TG. It was interesting to find that D decreased in an intermediate state and then increased to the final state. We also carried out SF-kinetic measurements of CD and intramolecular fluorescence resonance energy transfer. Based on these results, the formation of a compact structure derived from the helix bending on the micelle was elucidated.

P-173
Structural characterization of Prolyl endopeptidase-like, a mitochondrial protein involved in congenital myasthenic syndrome-22
Anastasia Theodoropoulou[1], Edoardo Cavani[1], Yenthe Monnens[2], Maria Marcaida[1], John Creemers[2], Matteo Dal Peraro[1] [1] EPFL, Switzerland [2] KU Leuven

PREPL (Prolyl endopeptidase-like) is a protein involved in mitochondrial homeostasis. In humans, PREPL deficiency and point mutations result in mitochondrial dysfunction and cause a recessive metabolic disorder called congenital myasthenic syndrome-22 (CMS-22). PREPLwas added to the prolyl oligopeptidase subfamily based on sequence similarity and predicted structural homology with prolyl endopeptidase (PREP). However, despite its homology with peptidases, PREPL has (thio)esterase activity. In our work, we are studying the underlying molecular reasons for it being an esterase. In this context, we are performing X-ray crystallography and single particle cryoEM of PREPL bound to different substrates, in order to elucidate the mechanism of substrate recognition and hydrolysis in PREPL. In parallel, we are studying how the point mutations present in CMS-22 patients lead to a loss of function of PREPL and what is their effect on substrate binding. For that purpose, we are performing biophysical characterization of the mutants and binding assays to study their interaction with different ligands. Our work provides new clues towards the comprehension on a molecular level of the compound phenotype of patients with CMS22.

P-174
Different regions of a-Synuclein N-terminus regulate the interaction with amyloid fibrils or biomolecular condensates.
Rebecca Thrush[1], Devkee Vadukul[1], Sian Allerton[1], Marko Storch[1], Francesco Aprile[1]
[1] Imperial College London, United Kingdom

Accumulation of proteinaceous inclusion inside the brain is the hallmark of several neurodegenerative diseases, including Parkinson's disease. The main component of these inclusions is amyloid fibrils, formed by the self-association of α-synuclein monomers. α-Synuclein can undergo several post-translational modifications including N-terminal truncation. This modification is of great interest as α-synuclein's N-terminus has been linked to both normal and toxic protein function. Following development of a novel method to generate highly pure, pathologically relevant N-terminally truncated α-synuclein, we aimed to study the modifications effect on two different fibrilization pathways. Under the classical pathway monomers self-associate to form small oligomers which then grow via multiple processes into long fibrils. Alternatively, the monomers can liquid-liquid phase separate to form liquid droplets which then undergo a liquid to solid transition, eventually leading to fibrils. Biophysical characterization by Thioflavin-T fluorescence, microscopy and TEM identified a region that is necessary for processes mediated by interaction with preformed fibrils but not for those mediated by biomolecular condensates. These findings highlight the importance of both the N-terminus and the phase-separation pathway as targets for disease therapeutics.

Poster Presentations
– Session 1 –

P-175
The Trigger Factor Chaperone compacts nascent chains and stabilizes folded structures
Katharina Till[1]
[1] AMOLF, Netherlands

Conformational control of nascent polypeptides is poorly understood. It has been shown that chaperones can stabilize, collapse, unfold and disaggregate polypeptides away from the ribosome. In comparison, much less is known about such elementary mechanisms of conformational control by chaperones at the ribosome. Yet, proteins encounter major folding and aggregation challenges during translation, while chaperones are also increasingly found to engage co-translationally. Using optical tweezers and selective ribosome profiling, we show that the Escherichia coli chaperone trigger factor (TF) stimulates the compaction of nascent chains, and hence directly promotes tertiary structure formation during translation, using the protein dihydrofolate reductase (DHFR) as a model system. The TF-mediated nascent chain compactions lead to compact partially folded structures that are stabilized by TF against unfolding. This stabilization of folded structures also depends on translation stage; specifically on the emergence of a peptide segment that is part of the core beta-sheet structure of DHFR. Consistently, TF binding is also stabilized by nascent chain folding, as shown by simultaneous single-molecule fluorescence detection. The results suggest that the confining surfaces provided by the bound and cradle-shaped TF can enhance polypeptide collapse, as has recently been observed for GroEL-ES, which has implications for understanding de-novo folding, interplay with DnaK, and co-translational protein assembly.

P-176
Linking the Diffusive Dynamics of Bacterial Proteome to Cell Metabolism and Death
Stepan Timr[1,2,3], Daniele Di Bari[4,5,6], Marianne Guiral[7], Marie-Thérèse Giudici-Orticoni[7], Tilo Seydel[6], Christian Beck[6], Caterina Petrillo[5], Philippe Derreumaux[2,3,8], Simone Melchionna[9,10], Fabio Sterpone[2,3], Judith Peters[4,6,8], Alessandro Paciaroni[5]
[1] J. Heyrovsky Institute of Physical Chemistry, Czech Academy of Sciences, Czech Republic, [2] Laboratoire de Biochimie Théorique (UPR9080), CNRS, Université de Paris Cité, France, [3] Institut de Biologie Physico-Chimique, Fondation Edmond de Rothschild, France, [4] Université Grenoble Alpes, CNRS, Laboratoire Interdisciplinaire de Physique, France, [5] Università degli Studi di Perugia, Dipartimento di Fisica e Geologia, Italy, [6] Institut Laue-Langevin, France, [7] Laboratoire de Bioénergétique et Ingénierie des Protéines, BIP, CNRS, Aix-Marseille Université, France, [8] Institut Universitaire de France, France, [9] ISC-CNR, Dipartimento di Fisica, Università Sapienza, Italy, [10] Lexma Technology, USA

Temperature variations have a big impact on bacterial metabolism and death, yet an exhaustive molecular picture of these processes is still missing. Here, by combining multiscale molecular simulations of the E. coli cytoplasm with neutron scattering measurements, we clearly show that only a minor fraction of the proteome unfolds at the cell death. Moreover, we prove that the dynamical state of the E. coli proteome is an excellent proxy for temperature-dependent bacterial metabolism and death. The proteome diffusive dynamics peaks around the bacterial optimal growth temperature, and a dramatic dynamical slowdown starts just below the cell's death temperature. We show that this slowdown is caused by the unfolding of a small fraction of proteins, which establish an entangling interprotein network, dominated by hydrophobic interactions. Finally, the deduced progress of the proteome unfolding and its diffusive dynamics are both key to correctly reproduce the E. coli growth rate.

P-177
Droplet microfluidics and deep learning for label-free analysis of single-cell bacterial growth and lysis
Anuj Tiwari[1], Vasilis Anagnostidis[1], Nela Nikolic[1], Fabrice Gielen[1]
[1] University of Exeter, United Kingdom

Bacteria identification and counting is important to many applications in food safety industry, diagnostics of infectious diseases and in the study and discovery of novel antimicrobial compounds. However, most of the traditional and computer aided counting techniques are slow and laborious. There is still a lack of fast and accurate methods to count small populations of cells at the single-cell level and no methods to date have been reported to count individual motile bacterial cells freely swimming in microfluidic anchored droplets. In this study, we present a method of counting single cells using the YOLOv4 (You Only Look Once version 4) deep learning framework for cell detection. To do that, we trap Escherichia coli cells in the presence or absence of T7 lytic bacteriophages and acquire bright field images using an automated, Z-stack mechanism. We report an average detection precision (AP) of 83.6% for correctly identifying cells in-plane. This technology enables us to accurately track individual cell division and cell lysis events at different temperatures. It also aids in studying phenotypic heterogeneities among individuals starting from initial populations as low as one cell and identify cells resisting antimicrobial treatment. This droplet-based method to study bacteria-bacteriophage interactions allows us to obtain detailed growth dynamics over timescales of hours to days, paving the way towards deciphering phage life cycle in confined environment. We aim to develop this method further to study the interaction of polymicrobial-phage interactions involving clinically relevant strains such as Pseudomonas Aeruginosa and Staphylococcus Aureus.

P-178
Thermodynamic properties of the F type ferryl intermediate of the cytochrome c oxidase
Adriana Tomkova[1], Tereza Sztachová[1], Marian Fabian[2], Daniel Jancura[1]
[1] Department of Biophysics, Faculty of Science, P.J. Safarik University in Kosice, Slovakia[2] Center for Interdisciplinary Biosciences, Technology and Inovation Park, P.J. Safarik University in Kosice

The reduction of O2 to H2O in cytochrome c oxidases (CcO) occurs through a sequence of several oxy- intermediates of the binuclear catalytic center (BNC). From these intermediates, two ferryl states, P and F, are involved in the proton pumping. The reduction of F by one electron transfer into BNC leads to the formation of the oxidized CcO (O). The energy released at this transformation should be sufficient to pump minimally one proton through the membrane and transfer one proton into BNC. However, the energy liberated at this stage was estimated until now only from the ATP-dependent reversal process, the O to F transition in mitochondria. In this work, we determined the amount of energy released during the forward transition F-to-O. The one electron reduction of the F by ferrocyanide was monitored by UV-Vis spectrometry and in parallel measurement, the enthalpy changes of this reaction were registered by isothermal titration calorimetry. The results showed that the midpoint potential of F/O couple is Em = + 550 mV, if water, plausibly produced at the BNC during this transition, is not released. In the case that water is released, Em should be close to + 630 mV (pH 8.0, 5 °C). Consequently, the change of Gibbs energy associated with a transfer of electron from c2+ to the F state is, dependent on the presence of water in BNC, either 6.7 or -8.5 kcal/mol.

Poster Presentations
– Session 1 –

P-179
Substrate selectivity of Sulfotransferase Isoenzymes, results based on Molecular Dynamics and Virtual Screening

Daniel Toth[1,2], Balint Dudas[2,3], David Perahia[3], Maria Miteva[2], Erika Balog[1]
[1] Semmelweis University, Hungary, [2]Université Paris Cité, France, [3] Laboratoire de biologie et pharmacologie appliquee, Ecole Normale Superieure Paris-Saclay, France

Sulfotransferase enzymes (SULTs) are a family of cytosolic globular proteins in the chain of metabolism. By catalysing a sulfate transfer from their co-factor, 3′-Phosphoadenosine 5′-Phosphosulfate (PAPS), they eliminate a large variety of small molecules like drugs, hormones and neurotransmitters. Even though the tertiary structure across the family is very similar, their substrates vary considerably in size and complexity. The aim of our project is to better understand the reasons of selectivity between the different SULT isoenzymes, by comparing the broad targeting hepatic detoxifier SULT1A1, and the ileum located, dopamine selective SULT1A3. Based on our previous results and Molecular Dynamics (MD) and Molecular Dynamics with excited Normal Modes (MDeNM), an extended conformational space of the PAPS-bound SULT1A1 was explored. Further developments of our method utilising ensemble docking with categorised ligands, a method known as Virtual Screening was achieved. Moreover, we have broadened our scope to use the same approach for the SULT1A3. Based on our new results, we identified the key differences, that are responsible for changing the protein dynamics and binding mechanisms, by opening the binding pocket to an unfavourable conformation for the most common ligands of 1A1, thus acting as efficient selectors. These results can be helpful in the future to develop an algorithm for machine learning, that could differentiate and even predict new substrates of the different isoforms, thus helping in the development of ADME-Tox profiling of novel drug candidates and xenobiotics.

P-180
Effects of dapagliflozin therapy in type I diabetes mellitus patients: nanomechanical and morphological studies of erythrocyte properties

Patrycja Twardawa[1,2], Bartlomiej Matejko[3], Agata Kubisiak[1,2], Katarzyna Cyranka[3,4], Tomasz Klupa[3,4], Marta Targosz-Korecka[1]
[1] Jagiellonian University, Faculty of Physics, Astronomy and Applied Computer Science, M. Smoluchowski Institute of Physics, Krakow, Poland, [2] Jagiellonian University, Doctoral School of Exact and Natural Sciences, Krakow, Poland, [3] University Hospital in Krakow, Krakow, Poland, [4] Jagiellonian University Medical College, Department of Metabolic Diseases, Krakow, Poland

The term “diabetes mellitus” includes a group of metabolic diseases characterized by various clinical presentations and pathogeneses, with hyperglycemia being a common symptom. Flozins are medications commonly used in the treatment of type 2 diabetes (T2DM) that function as inhibitors of sodium-glucose transporters (SGLT2) – receptors responsible for renal resorption of glucose. Increased activity of SGLT2 has been observed in people with diabetes. The cardioprotective effect of flozins has been confirmed, including reduction of blood pressure and body mass, however its exact mechanism remains unknown. Although flozins are not typically used in type 1 diabetes (T1DM) patients, they could potentially be beneficial due to the increased cardiovascular risk in these patients. The aim of our study was to investigate the mechanism of the cardioprotective effect of flozins in T1DM patients. Nanomechanical and morphological parameters of red blood cells were measured before and after one month of dapagliflozin therapy using atomic force microscopy (AFM). Morphological analysis was performed on optical microscope images. A semi-automatic approach was employed for obtaining cell parameters in optical image analysis. The presented results include a preliminary report along with the presentation of data analysis methods, where morphological and nanomechanical properties of erythrocytes are compared.

P-181
Single-molecule FRET mapping of intrinsically disordered regions in the key melanoma regulator MITF

Matti Már Valdimarsson[1], Hong Nhung Vu[1], Heinz Arnheiter[2], Eiríkur Steingrímsson[1], Pétur Orri Heiðarsson[1]
[1] University of Iceland, Iceland, [2] National Institute of Neurological Disorders and Stroke: Bethesda, Maryland, US

Transcription factors translate signals to transcriptional changes that shape how cells react to their environment. Transcriptional effects are mediated by elements embedded in long intrinsically disordered regions (IDRs). How the structure and dynamics of these IDRs are affected by DNA binding is ill-described and may play a key role in regulation of DNA or co-factor binding and therefore on the transcriptional changes mediated by the TF. The microphthalmia-associated transcription factor (MITF), a key regulator of metastasis and proliferation in melanoma, has two IDRs flanking a basic helix-loop-helix zipper DNA binding domain. Using a combination of smFRET and in-vivo experiments we map the structure, dynamics, and DNA binding of MITF. Not only do the flanking IDRs of MITF adopt different conformations from each other in the free state, they also react differently to DNA binding. The N-terminal transcriptional effector IDR undergoes substantial conformational re- arrangement, while the C-terminal IDR reacts only to phosphorylation which induces nuclear export. Truncation of the C-terminus causes constitutive nuclear localization and rescues dominant loss of function mutations in mice. Finally, we applied coarse-grained MD to produce an ensemble description of the MITF dimer off and on DNA, providing insight into the “structure”-function relationship of TF IDRs.

P-182
When does the Hsp90 machinery cycle?

Leonie Vollmar[1,2], Julia Schimpf[1,2], Thorsten Hugel[2,3]
[1] Spemann Graduate School of Biology and Medicine (SGBM), University of Freiburg, Freiburg, Germany, [2] Institute of Physical Chemistry, University of Freiburg, Freiburg, Germany, [3] BIOSS and CIBSS Signalling Research Centres, University of Freiburg, Freiburg, Germany

Together with its cochaperones, the molecular chaperone Hsp90 processes many clients (e.g. kinases) and exhibits dynamics on a wide range of timescales. On the seconds and minutes time scale, Hsp90 shows large conformational changes of N-terminal opening and closing, which are not coupled to ATP hydrolysis, but show different affinities for cochaperones and clients. Therefore, many cycles for successive conformational changes, binding and release of proteins to Hsp90 have been proposed. But how does Hsp90 proceed through such cycles? This is a fundamental question, as circular processes can only have a direction in a non-equilibrium system, i.e. if there is a constant energy supply (e.g. ATP) driving it forward. Single-molecule experiments are ideal to investigate such non-equilibrium systems. Here, we use single-molecule FRET in a TIRF setup in combination with maximum likelihood analysis, to quantify Hsp90-cochaperone-client systems. First, we have probed the Hsp90-Cdc37-Ste11 interactions. We show that various dynamic complexes can be formed, but surprisingly largely independent from ATP (hydrolysis), i.e. we still see no directionality in Hsp90’s cycle, hence, the system is close to equilibrium. However, when adding three cochaperones to the system (Cdc37, Aha1 and Sba1), directionality can be observed. Thus, Aha1 and Sba1 convey the energy of ATP hydrolysis for the assembly of the Hsp90 machinery. In contrast to current models, we argue that an upstream equilibrium of sub-complexes followed by a directed selection of the functional complex is the most likely and most energy efficient pathway for kinase maturation.

Poster Presentations
– Session 1 –

P-183
Integrated Structural Biology as a Toolbox to Dissect Viral Entry
Eleonore von Castelmur[1], Marion Pichon[1], Anna Wehlin[1], Jacqueline Staring[2], Ling Zhu[3], Thijn Brummelkamp[2,4], Anastassis Perrakis[2,4], Elizabeth Fry[3], David Stuart[3]
[1] Linkoping University, Linköping, Sweden, [2] Oncode Insitute, Amsterdam, The Netherlands, [3] Oxford University, Oxford, United Kingdom, [4]Netherlands Cancer Institute, The Netherlands

While the conserved genome organization is one of the defining features of picornaviruses, the 2A proteins are amongst the most divergent. To date there are at least 5 different types identified, with varying roles during infection. Remarkably, there is a group of picornaviruses, including Aichi Virus (AiV) and Parechovirus A (HPeV), whose 2AH/NC proteins share conserved H-box and NC-motifs with the human protein PLAAT3, which we previously identified as a picornavirus host factor. These residues are essential for enzymatic activity for the cellular enzyme as well as for the reported enzymatic role of the viral 2A proteins in RNA replication. We set out to discover whether the 2A proteins are active as lipid modifying enzymes, and have been acquired to allow AiV and HPeV to become independent from the cellular host factor. The crystal structures of the AiV-2A and HPeV1-2A proteins show a similar topology to PLAAT3 in the N-terminus, but diverge in the C-terminal half. AiV-2A preserves the active site configuration, but is inactive as a phospholipase. Remarkably, a topological rearrangement of the C-terminus in HPeV1-2A results in a conformation incompatible with catalysis, despite the conservation of the H-box and NC-motif; consistently the HPeV1-2A protein is inactive as a phospholipase too. Intriguingly, however, HPeV1 is independent of PLAAT3 for cellular entry, through an as yet unidentified mechanism. Further work is required and ongoing to trace 2AH/NC proteins' potential role during virus entry and how might they have been repurposed to fulfil new functions in the viral replication cycle.

P-184
Structure of Aβ42 oligomers studied with isotope-edited FTIR spectroscopy
Faraz Vosough[1], Andreas Barth
[1] Stockholm University, Sweden

Amyloid beta (Aβ) peptides have been studied as the main culprits behind the neuropathological changes in Alzheimer's disease for over three decades. More recently, the soluble aggregates of Aβ, better known as Aβ oligomers, have drawn much attention as the major neurotoxic assemblies of the peptide. Despite their clinical relevance, the structural information on Aβ oligomers is limited, largely due to their metastable and heterogeneous nature. Such characteristics makes them difficult to study with conventional structural biology methods. Fourier-transform infrared spectroscopy (FTIR) is an alternative biophysical technique capable of providing valuable data on amyloid proteins, including Aβ aggregates. We have applied FTIR spectroscopy in combination with isotope labeling to study the molecular structure of Aβ oligomers prepared in presence and absence of the detergent molecule SDS. Based on the predictions from available molecular models for Aβ oligomers, which suggest a hairpin-like structure, residues F20, A30, I32 and F20-A30 in Aβ42 were 13C, 15N-labeled and the oligomers were studied with FTIR spectroscopy. The results indicate that in detergent-free Aβ42 oligomers, all residues F20, A30 and I32 are located within β-strands, with some sort of intramolecular contacts between F20 and A30. This is in partial agreement with a number of available molecular models for Aβ oligomers. By contrast, in SDS- stabilized oligomers, F20 residue is not stably located in β-strands. The obtained results suggest that the structures of Aβ42 oligomers formed in presence and absence of SDS are different in terms of the hairpin-like conformation and the contacts between the residues.

P-185
Multi-target, 3D and live STED microscopy using renewable fluorophore labels
Dongni Wang[1], Marius Glogger[1], Julian Kompa[2], Ashwin Balakrishnan[1], Julien Hiblot[2], Hans-Dieter Barth[1], Kaarjel K. Narayanasamy[1,3], Aleksandar Stojic[3], Thomas Kuner[3], Kai Johnsson[2], Mike Heilemann[1]
[1] Institute of Physical and Theoretical Chemistry, Goethe-University Frankfurt, Frankfurt, Germany, [2] Department of Chemical Biology, Max Planck Institute for Medical Research, Heidelberg, Germany, [3] Department of Functional Neuroanatomy, Institute for Anatomy and Cell Biology, Heidelberg University, Heidelberg, Germany

Super-resolution imaging of multiple targets offers exciting possibilities to explore biomolecular structures, functions and interactions. One prominent technique is stimulated emission depletion (STED) microscopy. However, as a high-performance method, STED microscopy suffers from photobleaching, and the number of targets that can be visualized simultaneously relates to and requires balancing of available excitation/emission wavelengths. One way to overcome these challenges is by utilizing non-covalent, weak-affinity ("exchangeable"/"renewable") fluorophore labels that can reversibly bind and unbind to a target molecule. A constant exchange is achieved when a photobleached fluorophore unbinds from the target and is replenished by an intact one from a reservoir (the imaging buffer), which reduces photobleaching. Sequential imaging of many targets is possible by multiple rounds of exchange of fluorophore labels by introducing washing steps, which helps to overcome the "spectral barriers". Here, we combine multiple weak-affinity labels (DNA-PAINT, PAINT, HaloTag-PAINT) and synergize the use of these different labeling strategies for STED imaging of cells and tissues. We achieve multi-target 2D and volumetric 3D STED imaging of single cells and 3D multicolor imaging of rat brain tissues. In addition, we demonstrate live-cell imaging with extended observation times. Our results propose weak- affinity labels as valuable extension for STED microscopy of biological samples.

P-186
Exploring the Structural and Functional Evolution of Picornavirus 2A Proteins
Anna Wehlin[1], Alexandra Ahlner[1], Eleonore von Castelmur[1]
[1] Linköping University, Sweden

Picornaviruses are a large and diverse family of biomedically important non-enveloped, RNA viruses and to date no antiviral treatments are available. Attempts to identify potential drug targets for antiviral therapies identified PLAAT3 as a common host factor for several enteroviruses, enabling genome transfer to the cytoplasm prior to clearance via autophagy. PLAAT3 is part of a human phospholipid-modifying enzyme family of five members, PLAAT1-5, which all have a conserved H-box/NC-motif forming the active site of these enzymes. The H-box/NC-motif is also to be found in the 2A locus of some picornaviruses, suggesting that these viruses might have acquired the protein through horizontal gene transfer to become independent of the human host factor. By studying the structures and functions of representative 2AHbox/NC proteins from different picornavirus genera, we aim to identify different steps of evolutionary repurposing. This will help us understand their role(s) in the viral lifecycle and determine the molecular mechanism allowing them to by-pass PLAAT3 as a host factor. Here we have used NMR to generate a structural model of TuAsV 2A3, a 2AHbox/NC protein from an avian picornavirus. The structure reveals a rearranged topology compared to the PLAAT-family, with the conserved residues far apart in a conformation incompatible with catalysis. Consistently, TuAsV 2A3 is inactive as a phospholipase and must have developed other functions. Further work is ongoing to identify what new functions TuAsV 2A3 has evolved to fulfil the viral replication cycle.

Poster Presentations
– Session 1 –

P-187
Development of a high-throughput fluorescent assay to study protein assemblies
Camille Wernersson[1], Signe Christensen[1], Ingemar André[1]
[1] Department of Biochemistry and Structural Biology, Lund University, Lund, Sweden

Protein assemblies are crucial to many biological processes. However, recombinant production is challenging due to many issues. To optimize production, monitoring expression via a high-throughput in vivo fluorescence assay is beneficial. Usually, expression reporters are directly fused to the protein of interest, but this approach could prevent assembly. To counter this, operons are often used, which only monitor transcription. I designed several constructs to develop a fluorescent reporter that follows expression without hindering assembly and found that translational coupling systems seem to monitor best. Expression monitoring is key to develop a high-throughput fluorescent assay that studies protein assemblies. Another building block is an assay to measure oligomer stability, something we demonstrated for monomeric proteins. The fluorescent reporter was under a promoter induced by heat shock. With the screening of a deep mutational scanning library, we identified stabilizing mutations with a minimal false positive rate. Together these reporter systems are the basis for a high-throughput screening assay of assemblies.

P-188
Field theoretic model of phase behaviours of 20-amino-acid-type disordered protein sequences
Jonas Wessén[1], Tanmoy Pal[1], Suman Das[1], Hue Sun Chan[1]
[1] University of Toronto, Canada

The spatial organization of biomolecules through liquid-liquid phase separation (LLPS) is critical for cellular function, but simulating such systems poses significant challenges due to the large number of molecules and interaction types involved. Field theory simulations (FTS) offer a promising computational approach to studying LLPS, but previous FTS models have mainly focused on long-range electrostatic forces, neglecting short-range interactions such as pi-pi, cation-pi, and hydrophobic interactions. Here, we describe a novel approach to include short-range residue-residue specific interactions in FTS. The method relies on an eigenvalue decomposition of a 20-by-20 matrix of pairwise contact energies, amounting to one field per eigenvalue with residue-specific charges contained in the associated eigenvectors. Excellent numerical accuracy is achieved by including only the few most dominant eigenvalues allowing for computationally efficient simulations. The theory is explored using an approximate analytical approach where energy parameters adapted from several molecular dynamics force fields are used to calculate [protein]-[salt]-temperature phase diagrams for wild type Ddx4 and 3 mutant variants. Moreover, FTS of chains of hydrophobic/polar (HP) residues indicate that micro-phase separated substructures within condensates exhibit a high degree of sensitivity to variations in the HP sequence. The results are validated by coarse-grained molecular dynamics simulations and our method presents an important step towards accurate simulations of LLPS in biologically relevant systems.

P-190
Quantitative Mechanical and Electrical Mapping during the Contraction of hiPSC-derived Cardiomyocytes
Xinyu Zhang[1], Nafsika Chala[2], Jens Duru[1], Carla Cofiño-Fabres[3], Robert Passier[3], Tomaso Zambelli[1]
[1] ETHz D-ITET, Switzerland, [2] ETHz D-MAVT, [3] University of Twente

The contraction of cardiomyocytes (CMs) is a fascinating biochemical reaction in which the flow of ions is transformed by cells into mechanical contractions, representing the physiological state of the CMs. The dysregulation of this process is associated with cardiac diseases, making it critical to quantitatively measure contractile activity for a better understanding and characterization of CMs contraction. Isolated CMs from adult rodents represent the gold standard for in vitro models, but they quickly degrade after a few hours, limiting the time window of the experiments. On the other hand, human induced pluripotent stem cells (hiPSCs) offer a valid alternative source for studying cardiac cell biology, drug development, and cardiac therapies with the advantage of long-term culture capability. We are employing several techniques to monitor the activity of CMs in terms of electrochemical reaction and mechanical contraction. By combining FluidFM and Traction Force Microscopy (TFM), we can obtain a 3D time- resolved force map of the contraction of CMs with nano-Newton sensitivity, across the vertical and horizontal direction. Additionally, this versatile approach enables the application of local chemical or electrical stimuli. Moreover, high-resolution CMOS-based microelectrode arrays (MEAs) are used to record the spontaneous and stimulation-induced electrical activity of CMs. Combining both techniques, we are able to study the CM contraction mechanically and electrically, revealing the cluster automaticity of hPSCs-CMs. By mapping the in-plane and out-of-plane contraction forces and the electrical activity of CMs, we are able to analyze and compare the activity of CMs following an extracellular electrical or chemical stimulus.

P-191
Structural and binding studies of a new lytic polysaccharide monooxygenase from the marine bacterium Vibrio campbellii
Yong Zhou[1], Jeerus Sucharitakul[2], Robert C. Robinson[1], Wipa Suginta[1]
[1] School of Biomolecular Science and Engineering (BSE), Vidyasirimedhi Institute of Science and Technology (VISTEC), Rayong, Thailand, [2] Department of Biochemistry, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand

Vibrio spp. play a crucial role in the global recycling of biomass in marine ecosystems and utilize it as their major carbon source. Here, we report the first crystal structures of a complete four-domain chitin-active AA10 lytic polysaccharide monooxygenase from Vibrio campbellii. The crystal structures of apo- and copper-bound VhLPMO10As were resolved as homodimers including a AA10 catalytic domain, a GbpA_2 domain, the Module X domain, and the C-terminal CBM73, while SEC and SAXS analysis confirmed that VhLPMO10A existed as a monomer in solution. The active site of VhLPMO10A is located on the surface of the catalytic domain, with three conserved residues (His1, His98 and Phe170) forming the Cu (II)-binding site. Metal-binding studies gave consistently strong Cu(II) signals and ITC showed VhLPMO10A could bind various divalent cations, but most strongly to Cu(II) ion (Kd =110 nM). By contrast, a Kd (1.9 nM) was estimated for Cu(I) from redox potential measurements. The presence of AscA is crucial to produce H2O2 in VhLPMO10A reaction. MALDI-TOF MS identified VhLPMO10A as a C1-specific LPMO, generating oxidized chitooligosaccharide products with different degrees of polymerization (DP2ox - DP8ox). This new member of chitin-active AA10 LPMOs could serve as a powerful biocatalyst in biofuel production from chitin biomass.

Poster Presentations
– Session 1 –

P-192
Phytophthora parasitica NLP shares three-dimensional fold and plant plasma membrane receptor with other Nep 1-like Proteins
Nika Žibrat[1], Marjetka Podobnik[1], Gregor Anderluh[1]
[1] National Institute of Chemistry, Slovenia

Necrosis- and ethylene-inducing 1-like proteins (NLPs) constitute a superfamily of proteins found in diverse phyla of plant-associated microorganisms. Numerous NLPs are cytotoxic and facilitate infections in a wide range of crops. The evidence of NLPs being toxic towards both monocot and dicot plants is inconclusive. The target for interaction with the plant plasma membrane is a sphingolipid glycosyl inositol phosphoceramide (GIPC). Studies of NLPPya from Phytium aphanidermatum highlight important residues for interaction with the terminal hexose unit of GIPC and a unique mode of membrane damage was observed. Despite 1,700 identified homologues, 3D structures of only 3 NLPs are solved. Considering their wide taxonomic distribution and crucial role in pathogenesis, structural information on NLPs is critically lacking. Studies of NLPs from evolutionary distant organisms are essential to understand the evolution and mechanistic action of these proteins. We study structural and functional characteristics of NLPPp from Phytophthora parasitica, an important plant pathogen. Results of Nicotiana tabacum infiltration assays indicate NLPPp causes necrosis and ion leakage of plant tissue. The protein binds to vesicles consisting of both mono- and dicot derived GIPC, and binds to terminal hexose unit found on GIPC polar head. Despite NLPPp is exhibiting less profound binding and plant tissue damage in comparison to model NLPPya, modes of action seem to be similar. In addition, we determined the crystal structure of NLPPp at 1.7 Å resolution, revealing this protein shares structural characteristics with other NLPs, with a central β□sandwich flanked by α□helices.

P-193
Sweet and sour transport: simulation insights into H+/sucrose symport in plants
Lorena Zuzic[1], Laust Bavnhøj[1], Jan Heiner Driller[1], Amanda Dyrholm Stange[1], Birgit Schiøtt[1], Bjørn Panyella Pedersen[1]
[1] Aarhus University, Denmark

Sugars play an important role in the regulation of plant growth and development. Sucrose has a multifaceted role in plants, as it serves as a transformable form of stored energy, an osmolyte, and a signaling molecule that is a carrier of shoot-to-root information important for responding to the environmental conditions. Sugar carriers (SUC) use proton-driving force to transport sucrose from an acidic apoplast into the cytoplasm against its concentration gradient. The newly discovered crystal apo structure of SUC1 is examined in ~34 μs of atomistic molecular dynamics (MD) simulations. The simulations with differing protonation states of an Asp residue located in the central cavity demonstrate spontaneous sucrose binding when Asp is protonated. Increased sucrose binding stability is linked to the protonated Asp as compared to the deprotonated systems. We demonstrate the crucial role of this residue in sucrose stabilisation mediated by its direct interaction with the glycosyl moiety of the substrate and describe how protonation and sucrose binding are strongly coupled. This research showcases a new model for proton-driven symport in the context of a low-affinity substrate, and defines a pH-dependent, stepwise process that is sucrose binding to the SUC binding site.

P-194
Mitochondrial Dynamics in an Alzheimer's Disease Mouse Model Fed with Membrane Lipid Precursors
Devrim Öz-Arslan[1,2], Elif Nedret Keskinoz[3], Musa Celik[2], Berkcan Koç[2]
[1] Acibadem Mehmet Ali Aydinlar University, School of Medicine, Department of Biophysics, Turkey [2] Acibadem Mehmet Ali Aydinlar University, Institute of Health Sciences, Department of Biophysics, [3] Acibadem Mehmet Ali Aydinlar University, School of Medicine, Department of Anatomy

Mitochondrial function has been thought to be significantly disrupted during the earliest stages of Alzheimer's disease. The majority of the synaptic membrane develops during the prenatal and early postnatal periods. We aimed to investigate the impact of uridine monophosphate (UMP), choline, and fish oil given as membrane precursors during prenatal and lactation periods on mitochondrial dynamics in 5XFAD transgenic mice as an Alzheimer disease model during development. Mitochondrial fusion, fission, and mitophagy protein levels were investigated by western blot analysis in the hippocampal tissue of 5XFAD and control mice fed with or without a diet. We observed that Mfn2, Drp1, and Pink1 protein levels were altered during the developmental process. Additionally, we assessed mitochondrial superoxide production, mitochondrial mass, and mitochondrial membrane potential in peripheral blood mononuclear cells by flow cytometry using mitochondrial-specific fluorescent dyes. We observed that the MitoSox level in response to a complex I inhibitor in the lymphocyte population of transgenic mice was lower than that of non- transgenic mice, indicating an impairment in the complex I level in transgenic mice. Our results will contribute to mechanistic insight into AD's progression and the development of preventive dietary supplements for neurodegenerative diseases. This study is supported by TUBITAK (219S307).

Poster Presentations
– Session 2 –

P-300
Exploration of lipid-membrane protein interactions a study of an evolutionary deprived in silico designed protein
Mia L Abramsson[1], Robin A Corey[2], Phillip J Stansfeld[3], Michael Landreh[1]
[1] Karolinska Institutet, Sweden[2] University of Oxford, [3] University of Warwick

Membrane proteins (MPs) are a major focus for drug discovery due to their importance in cellular function and survival, but also complicated targets due to their complex interactions with the surrounding lipid environment. We use an in silico designed protein called TMHC4_R as scaffold-free from evolutionary constraints (Lu, et al., Science, 2018) to establish first principles for specific lipid recognition and lipid-mediated stabilization. By introducing point mutations designed with the help of artificial intelligence predictions and coarse-grained molecular dynamics simulations, we create a specific binding site for structural lipids. However, capturing the interactions between MPs and lipids is challenging for most biophysical approaches. We therefore turned to native mass spectrometry (MS) combined with ion mobility spectroscopy (IM), to monitor lipid binding and how it affects the oligomeric state and conformational stability of TMHC4_R. The MS read-out enables us to identify sequence features that contribute to specific lipid recognition. Using coarse-grained simulations, we identified a specific binding site for cardiolipin (CDL). Reverse engineering the sit revealed a lipid-activated salt bridge switch that increased the stability of the TMHC4_R protomer. These findings form a suitable basis for targeted mutational studies that connect CDL recognition and conformational stabilization. In conclusion, our approach to identify the first principles of lipid interactions can open new avenues for studying the interplay between lipids and MPs.

P-301
Calcium as a Protein-Free Fusogen in GUVs: An Elusive
Effect Matilde Accorsi[1], Naresh Yandrapalli[1], Shreya Pramanik[1], Rumiana Dimova[1]
[1] Max Planck Institute of Colloids and Interfaces, Germany

Studies in the 1980s have established calcium as a membrane fusogen. This property has been associated to the interaction of calcium ions with molecules present on the membrane, triggering proteins that are involved in membrane fusion. Furthermore, calcium has also been observed to induce liposome fusion in the absence of proteins, interacting with phospholipids, specifically in the case of negatively-charged large unilamellar vesicles (LUVs). The fusogenic properties of calcium in pure, protein-free, phospholipid membranes on the micrometric scale as in giant unilamellar vesicles (GUVs) have not been widely explored. Using confocal microscopy and through the aid of microfluidic devices, we explored protein-free fusion by subjecting negatively-charged GUVs of different compositions to various calcium concentrations and osmolarity gradients across the membrane. Amongst the calcium concentrations that we explored, lipid-mixing between GUVs was observed in the presence of 4mM Ca2+ or more. While lipid-mixing is oftentimes a step towards fusion, we remark that pure phospholipid GUVs rarely surpass this step to achieve full fusion, with the most common outcome being adhesion or rupture. Regardless of osmolarity conditions and membrane composition, we find that protein-free GUV-GUV fusion using calcium as the sole fusogen is elusive, and our work is a contribution towards understanding how vesicle size affects the outcomes of calcium-induced fusion.

P-302
Development of biosensors based on ion channel and calmodulin complex structures for the discovery of new therapeutic drugs
Sara Alicante [1,2], Eider Nuñez [2], Arantza Muguruza [1], Alvaro Villarroel[1]
[1] CSIC, [2] University of the Basque Country, Spain

Calmodulin recognizes more than 300 different targets with very little sequence similarity. Those targets control key events on human physiology with great therapeutically potential. We are working on creating a library of biosensors with the aim of capturing the key molecular rearrangements that take place in different full-length ligands, an connecting those events to easily detectable fluorescent signals amenable of high throughput screening. As a proof of concept, we will present the development of a biosensor that recapitulates the most essential effects of Riluzole, the only drug available for treatment of amyotrophic lateral sclerosis.

P-303
Impact of lipid polyunsaturation on dopamine D2 receptor activation and signaling
Isabel Alves[1], Rim Baccouch[1], Marie-Lise Jobin[1,2], Véronique De Smedt-Peyrousse[2], Joyce Heuninck[3], Silvia Sposini[4], Ramon Guixa-Gonzalez[5], Jana Selent[5], Thierry Durroux[3], David Perrais[4], Pierre Trifilieff[2]
[1] 1CBMN, CNRS UMR 5248, Univ. Bordeaux, Allée Geoffroy St Hilaire, 33600 Pessac, France, [2] 2NutriNeuro, INRA UMR 1286, 146 rue Léo Saignat, 33000 Bordeaux, France, [3] 3IGF, CNRS UMR 5203, INSERM U1191, U. Montpellier 1 et 2, 34094 Montpellier, France, [4] 4IINS 146 Rue Léo Saignat, 33000 Bordeaux, [5] 5IMIM, Barcelona Biomedical Research Park, Doctor Aiguader, 88, 08003 Barcelona

Increasing evidence supports a relationship between lipid metabolism and mental health. In particular, the biostatus of polyunsaturated fatty acids (PUFAs) correlates with some symptoms of psychiatric disorders, as well as the efficacy of pharmacological treatments. Recent findings highlight a direct association between brain PUFA levels and dopamine transmission, a major neuromodulatory system implicated in the etiology of psychiatric symptoms. However, the mechanisms underlying this relationship are still unknown. Here we demonstrate that membrane enrichment in the n-3 PUFA docosahexaenoic acid (DHA), potentiates ligand binding to the dopamine D2 receptor (D2R), suggesting that DHA acts as an allosteric modulator of this receptor. Molecular dynamics simulations confirm that DHA has a high preference for interaction with the D2R and show that membrane unsaturation selectively enhances the conformational dynamics of the receptor around its second intracellular loop. We find that membrane unsaturation spares G protein activity but potentiates the recruitment of β-arrestin in cells. Furthermore, in vivo n-3 PUFA deficiency blunts the behavioral effects of two D2R ligands, quinpirole and aripiprazole. These results highlight the importance of membrane unsaturation for D2R activity and provide a putative mechanism for the ability of PUFAs to enhance antipsychotic efficacy.

POSTER #2

Poster Presentations
– Session 2 –

P-304
Structural characterization of pH- and calcium-modulated open and closed states of the pentameric ligand-gated ion channel, DeCLIC

Olivia Andén[1], Urška Rovšnik[1], Marie Lycksell[1], Marc Delarue[2], Rebecca J Howard[1], Erik Lindahl[1]
[1] Stockholm University, Science for Life Laboratory, Box 1031, 17121 Solna, Sweden, [2] Unit of Architecture and Dynamics of Biological Macromolecules, CNRS UMR 3528, 25-28 rue du Docteur Roux, Institut Pasteur, Paris, France

Pentameric ligand-gated ion channels are critical mediators of electrochemical signal transduction. Biophysical and pharmacological development in this family relies heavily on high-quality structural data in multiple distinct functional states. Bacterial pLGICs can provide valuable insight into fundamental mechanisms of gating, as they often desensitize to a lesser extent than eukaryotic channels, while retaining sensitivity to modulatory conditions. Recently, a new prokaryotic family member (DeCLIC) was identified, including a previously uncharacterized N-terminal domain. The channel was found to be modulated by calcium ions, and with Ca2+ present it crystallized with a dramatically expanded pore. Here, we used cryo- electron microscopy to identify multiple conformations of the channel under acidic conditions. When Ca2+ was present, two classes of particles were identified, one class was similar to the crystallographic closed state, while the second represented an apparent open state notably divergent from the open X-ray structure. When Ca2+ was absent, the predominant class had a similar open pore, while a second class had a closed pore and evidence for a highly dynamic N-terminal domain. These data provide new insight into pH modulation and the under-characterized open state of pentameric channels, as well as a novel mechanism of dynamic ion-channel regulation via an N-terminal module.

P-305
Using integrative structural biology for the identification of novel aerolysin-like pore forming toxins for nanopore sensing

Jana Susanne Anton[1], Fernando Meireles[1], Juan F. Bada[1], Maria J. Marcaida[1], Matteo Dal Peraro[1]
[1] École polytechnique fédérale de Lausanne, Switzerland

Pore-forming toxins (PFTs) have emerged as a valuable tool for nanopore sequencing. These toxins are secreted as soluble monomers and only upon membrane binding oligomerize into transmembrane pores. One of the well characterized PFTs used for nanopore sequencing is aerolysin. However, pores with different characteristics from aerolysin such as size, oligomeric state and charge might be more suitable for other applications. Currently, most pores, including aerolysin, are characterized under non-physiological conditions using detergents to stabilize the pore. Lipid nanodisc present a suitable alternative, which allows to characterize membrane proteins in their natural lipid environment. Here, we used the Alphafold database to identify new pore candidates with unknown properties by structural search. Pore candidates are further expressed and characterized using various biophysical methods including mass photometry and single bilayer measurements. Moreover, their lipid preferences are being analyzed. In a last step, we structurally characterized a few pores in polymer lipid nanodiscs by cryo-EM and substantially increased the current resolution of aerolysin. This opens the door for the structural characterization of the new pore candidates under natural conditions for biotechnological application such as nanopore sensing.

P-306
Decoding Ligand-Induced Oligomerization State of c-Met Receptor using Molecular Dynamics and FRET Experiments

Serena Maria Arghittu[1,2], Yunqing Li[3], Mike Heilemann[3], Roberto Covino[1]
[1] Frankfurt Institute for Advanced Studies, Frankfurt am Main, Germany, [2] International Max Plank Research School on Cellular Biophysics, Frankfurt am Main, Germany, [3] Goethe-Universität, Frankfurt am Main, Germany

Single pass plasma membrane receptors regulate cellular processes by acting as a gateway for cellular signals. The information transduction is generally triggered via ligand binding that in turn induces conformational changes in the receptors' extracellular and cytosolic chains, and their oligomerization. Due to the high complexity of these systems, the activation mechanism of many receptors is still elusive. This is the case of the human Tyrosine Kinase Receptor MET (c-Met or HGFR), that plays a crucial role in epithelial tissue development, healing and, when deregulated, in carcinogenesis and bacterial invasion. By exploiting molecular dynamics simulations integrated with Föster resonance energy transfer (FRET) experiments, we investigated the ligand-induced oligomerization state of the c-Met extracellular domain. To perform the FRET measurements on in vivo cells, we used a non-native ligand, the Listeria M.'s invasion protein InlB. Abundant experimental evidence supports InlB's capacity to activate native signaling pathways of the c-Met receptor. Therefore, we can reasonably assess general mechanistic hypotheses of the c-Met's activation based on our results. By fitting MD simulations to experiments, we identified two possible homodimeric models, in strong analogy with the native c-Met dimer for which two different dimeric structures exist. Further analysis will establish the relative populations of this dimers and provide key insight to understand the conformational rearrangements leading to c-Met-mediated signalling.

P-307
Phospholipids diffusion on the Surface of Model Lipid Droplets

Shima Asfia[1], Ralf Seemann[1], Jean-Baptiste Fleury[1]
[1] Department of Experimental Physics and Center for Biophysics, Saarland University, Germany

Lipid droplets (LD) are organelles localized in the membrane of the Endoplasmic Reticulum (ER) that play an important role in metabolic functions. They consist of a core of neutral lipids surrounded by a monolayer of phospholipids and proteins resembling an oil-in-water emulsion droplet. Many studies have focused on the biophysical properties of these LDs. However, despite numerous efforts, we are lacking information on the mobility of phospholipids on the LDs surface, although they may play a key role in the protein distribution. In this article, we developed a microfluidic setup that allows the formation of a triolein–buffer interface decorated with a phospholipid monolayer. Using this setup, we measured the motility of phospholipid molecules by performing Fluorescent Recovery After Photobleaching (FRAP) experiments for different lipidic compositions. The results of the FRAP measurements reveal that the motility of phospholipids is controlled by the monolayer packing decorating the interface.

Poster Presentations
– Session 2 –

P-308
Using Yeast Phospholipid Extracts in Planar Membrane Mimics to Investigate Mitochondrial Cell Death
Sophie Ayscough[1,2], Jörgen Åden[3], Tamas Nagy[3], Sebastian Köhler[4], Gerhard Gröbner[3], Hanna Wacklin-Knecht[1,2]
[1] European Spallation Source, Sweden [2] Division of Physical Chemistry, Lund University, [3] Department of Chemistry, Umeå University, [4] LINXS, Lund

Mitochondria play a crucial role in apoptosis, a programmed cell death mechanism. This process is tightly regulated by proteins at the mitochondrial outer membrane (MOM). The MOM has a unique lipid composition that primarily differs from other cellular membranes by the presence of cardiolipin (CL). CL plays an important role during apoptosis in recruiting the pro-apoptotic Bax protein to the MOM, where it induces membrane leakage. In healthy cells, Bax is neutralized by the anti-apoptotic Bcl-2 residing in the MOM. The complexity of the MOM system makes it difficult to obtain an overall picture of its organisation. However, preparation of planar lipid bilayers and analysis with neutron reflectivity (NR) can provide this information by employing hydrogen/deuterium labeling to create contrast between the lipid and protein components. Using a stepwise approach, we have increased the complexity of the lipid membranes, moving from investigating simple commercial lipids (POPC and CL) to investigating polar yeast lipid extracts which have a very similar lipid composition to that of the MOM. The lipid extraction, separation and quantification steps have been carried out at DEMAX, the ESS deuteration facility. Our NR results show that a higher cardiolipin content in the lipid membrane increases the rate at which Bax assembles at the bilayer interface and increases the amount of lipid removed from the membrane, both in simple and complex lipid mixtures.

P-309
Cationic peptides and membrane interactions in antitumor therapy
Mihaela Bacalum[1], Mina Raileanu[1], Bogdan Zorila[1]
[1] Horia Hulubei National Institute of Physics and Nuclear Engineering, Romania

Cationic peptides represent emerging class of peptides derived from antimicrobial peptide, which are an ancient innate defense system of the living organism against a wide variety of pathogens, especially bacteria. However, cationic peptides have also displayed a high specificity for cancer cells due to the differences found between the membrane of the normal and cancer cells. The increase of the anionic molecules like phosphatidylserine in the membrane of cancer cells can facilitate peptide-membrane interaction. Up until now, based on the peptides structures, a few models were proposed to describe the peptide-membrane interactions: barrel-stave model, detergent like model and toroidal pores formation. However, their action mechanisms can be influenced by changes in membrane properties: different composition, changes in membrane fluidity or charges.
Considering that the plasma membrane has a protective role for cells, but also represents the first gate for the peptides or other antitumor agents to enter the cells, fully understanding the way the peptides interact and affect them is essential in helping the researchers to develop more efficient treatments in the future. Fluorescent lipid membrane probes (i.e. Laurdan) can be used to probe different regions in the lipid membranes and can monitor fluidity and lipid order changes induced by cationic peptides. Finally, the fluorescent studies can be correlated with the cellular biology studies in order to give a more detailed picture of the cationic peptide activity against cancer cells.

P-310
Lipid composition effects on membrane damage inflicted by alpha hemolysin from Staphylococcus Aureus
Rūta Bagdonaitė[1], Gintaras Valinčius
[1] Vilnius University, Lithuania

Alpha hemolysin (αHL) is a pore forming toxin, secreted by one of the most common human pathogens Staphylococcus aureus. Membrane bound αHL monomers assemble into heptameric channels disrupting cell integrity in a species and cell type-dependent manner. The aim of this study was to assess the impact of membrane dielectric environment on αHL incorporation by changing the lipid composition. We employed an artificial membrane model – tethered bilayer lipid membranes (tBLMs) formed on self-assembled monolayer modified gold surface and measured their properties using electrochemical impedance spectroscopy. Pure dioleoylglycerophosphocholine (DOPC) phospholipid containing bilayers displayed high resistance to αHL, while addition of cholesterol, which as we determined lower the dielectric constant within the hydrophobic sheet of the bilayer, amplified pore forming ability. To further confirm the importance of the dielectric environment we also tested tBLMs composed of diphytanoylphosphocholine (DPhPC), which exhibits lower dielectric constant as compared to pure DOPC tBLMs and found that it was readily damaged at notably lower toxin concentration. Thus, dielectric environment seems to be an important factor for αHL incorporation and damage to lipid bilayers.

P-311
Enhancing Amphiphilic Peptide Transport Across Cell Membranes Using Transmembrane Proteins
Ladislav Bartoš[1], Adelheid Hanáčková[1], Martina Drabinová[1], Robert Vácha[1]
[1] Masaryk University, Czech Republic

Cell membranes consist of a double layer of phospholipids, with transmembrane proteins embedded within. Among these proteins are scramblases, which possess the ability to facilitate the movement of phospholipids between the individual leaflets of the membranes. We hypothesize that scramblases and similar proteins could also improve the transport of other amphiphilic molecules through the membrane, including antimicrobial or cell-penetrating peptides. We used molecular dynamics simulations supplemented by in vitro experiments to identify the properties of transmembrane proteins that maximize the enhancement of peptide translocation across phospholipid membranes. Optimal translocation- enhancing proteins contain hydrophilic residues forming a continuous patch, charged residues (preferably in the protein center), and aromatic residues. Moreover, our results demonstrate that the enhancement of translocation originates from both membrane disruption caused by the protein and stabilizing enthalpic interactions between the protein and the translocating peptide. Our findings have significant implications, as they suggest that naturally occurring scramblases, as well as de novo designed proteins or peptides, could be utilized to facilitate more efficient transport of amphiphilic peptides into cells.

Poster Presentations
– Session 2 –

P-312
Antimicrobial peptides: leaky fusion and lipid clustering in model membranes
Katharina Beck[1], Janina Nandy[2], Maria Hoernke[3]
[1] University of Freiburg, [2] Research Center Borstel - Leibniz Lung Center, [3] University of Halle, Germany

The constantly growing antibiotic resistance emphasises the importance of alternatives to classical antibiotics, such as membrane-active antimicrobial peptides (AMPs). We examine the mechanism of action of the antimicrobial trivalent cyclic hexapeptide cR3W3. More precisely, we investigate the effects of the peptide on binary model membranes containing various anionic and zwitterionic phospholipids. Isothermal titration calorimetry (ITC) measurements show binding selectivity for negatively charged membranes over zwitterionic membranes. This agrees with the observed selectivity of the peptide for bacteria over mammalian cells. Differential scanning calorimetry (DSC) and Laurdan fluorescence spectroscopy reveal the influence of cR3W3 on the thermotropic membrane behaviour, such as lipid chain melting. Our findings enable the detection of electrostatic lipid clustering in saturated and unsaturated lipid membranes. We use the self-quenching dye Calcein to quantify vesicle membrane leakage induced by the peptide. On closer inspection, leakage turns out to be mainly caused by leaky fusion. Yet, the biological relevance of this mechanism should be carefully assed. In conclusion, binding of cR3W3 to model membranes induces various effects: electrostatic lipid clustering, membrane fusion, vesicle aggregation, and vesicle leakage.

P-313
Investigating the proton translocation mechanism of complex I from the bottom-up
Adel Beghiah[1], Grant Kemp[1], Patricia Saura[1], Hyunho Kim[1], Johan Berg[1], Dirk B. Auman[1], Ville R. I. Kaila[1]
[1] Department of Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden

NADH:ubiquinol oxidoreductase or Complex I is a key enzyme of the cellular respiratory chain. It catalyzes proton coupled-electron transfer (PCET) reactions that contribute to the creation of a proton gradient across the membrane, powering ATP production via ATP synthase. Despite decades of biochemical, biophysical, and structural studies, the PCET mechanism of Complex I remains unclear and highly debated. Moreover, malfunction of the enzyme result in several diseases, such as Leigh syndrome, Alzheimer's, and Parkinson's disease, and deciphering the mechanism of the Complex I is thus also critical for curing those diseases which are a major challenge to human health. To understand the proton pumping principles of Complex I, we isolate and study here individual antiporter-like subunits using a combination of biophysical assays in proteoliposomes and multi-scale simulations. Our combined findings illustrate key proton pathways across the membrane, functional elements that control the rate of proton conduction across the membrane, and that all antiporter-like subunits can transfer protons across the membrane. Our findings provide a blueprint for understanding the long-range proton translocation mechanism in Complex I.

P-314
Integrating simulations and cryo-EM reveal lipid binding sites in a ligand-gated ion channel
Cathrine Bergh[1,2], Urska Rovsnik[2,3], Rebecca Howard[2,3], Erik Lindahl[1,2,3]
[1] KTH Royal Institute of Technology, [2] Science for Life Laboratory, [3] Stockholm University, Sweden

Ligand-gated ion channels are necessary for transmitting electrochemical signals in neurons and excitable cells. Apart from canonical ligands, phospholipids can bind and occasionally modulate several types of channels, but structural details have not yet been fully characterized due to limited resolution in experimental structures. Here, we discovered multiple lipid interactions in the GLIC ion channel by integrating cryo-EM and large-scale molecular dynamics simulations. We found 25 bound lipids in the GLIC closed state, a conformation where none, to our knowledge, were previously known. In this state, both simulations and cryo-EM revealed, for each subunit, three lipids in the inner leaflet and two in the outer leaflet. Additionally, simulations were employed to predict lipid sites in the open state, resulting in six lipids. Noteworthy interactions include one inner leaflet lipid with its tail wedged deeply into the subunit interface, where tail saturation changed the binding pose, and where a single mutation disrupted the long interaction times. Another lipid of the open state revealed state-dependent interactions, indicating a potential role in stabilizing this state. This study provides molecular details of GLIC-lipid contacts, particularly in the relatively unexplored closed state, testable hypotheses for state-dependent binding, and a multidisciplinary approach for modeling protein-lipid interactions.

P-315
Fibroblast Growth Factor 2 oligomerization is essential for direct translocation through PI(4,5)P2 containing lipid bilayers
Daniel Beyer[1], Fabio Lolicato[1], Walter Nickel[1]
[1] Heidelberg University Biochemistry Center, Germany

Fibroblast Growth Factor 2 (FGF2) follows a type I unconventional secretory pathway to directly translocate across the plasma membrane. After recruitment to the membrane, binding to phosphatidyl-4,5-bisphosphate (PIP2) leads to oligomerization and membrane insertion. Translocation is completed by extracellular heparan sulfate proteoglycans outcompeting PIP2. Though the hallmarks of FGF2 translocation are well understood, the molecular mechanism by which FGF2 forms highly dynamic oligomers capable of inserting into the membrane remains unclear. Therefore, we employed atomistic and coarse-grained molecular dynamics (MD) simulations to shed light on FGF2 oligomerization and the role of PIP2 in the pore formation process. Our findings demonstrate that the clustering of PIP2 is influenced by its concentration, cholesterol level, and pore size.
Similarly, we investigated the diffusion of membrane-bound FGF2 in a preformed pore. Our simulations revealed that a rearrangement of FGF2 occurs when a toroidal pore contains at least two molecules. This process exposes the PIP2-binding site to the outer membrane leaflet, allowing for its extraction by heparan sulfate chains. The clustering of PIP2 induced by FGF2 oligomerization is a crucial step in membrane insertion as it causes stress on the membrane, forming pores that facilitate FGF2 translocation.

Poster Presentations
– Session 2 –

P-316
Determining the nanoscopic membrane environment of transmembrane proteins in live-cells with protein micropatterning
Marina Bishara[1], Veronika Brumovska[1], Andreas M. Arnold[2], Barbora Kalouskova[1], Gergö Fülöp[1], Gerhard J. Schütz[1], Eva Sevcsik[1]
[1] Institute of Applied Physics, TU Wien, Vienna, Austria, [2] National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD, USA

The plasma membrane of eukaryotic cells hosts a plethora of chemically distinct lipids and proteins which may interact with structural elements of the cytoskeleton. Studying their organization as well as their interactions therefore becomes particularly challenging. Often model systems, e.g. functionalized lipid bilayers or giant unilamellar vesicles, are used to assess the behaviour of lipids and proteins in the plasma membrane, but may not account for certain, likely relevant aspects of plasma membrane function, i.e. membrane heterogeneity and asymmetry, cytoskeleton interactions, etc. We use a micropatterning approach to determine the immediate membrane environment of transmembrane proteins in living cells. Here, GFP-tagged proteins are selectively immobilized in micropatterns posing as obstacles for freely diffusing lipids in the plasma membrane. By tracking fluorescently labelled lipids (tracers) and comparing their mobility in- and outside of created protein micropatterns the immediate nano-environment of studied proteins can be assessed. Particularly, this approach allows to discern if a given protein of interest is surrounded by nanofeatures such as more viscous membrane phases or annular lipids as they would alter the diffusion of the tracer lipid. We found no evidence of such nanofeatures for all studied transmembrane proteins.

P-317
A novel mechanism for membrane voltage sensing by dipolar peptides
Konark Bisht[1], Michael Lomholt[1], Himanshu Khandelia[1]
[1] PhyLife, Department of Physics, Chemistry and Pharmacy, University of Southern Denmark

Membrane voltage sensing is essential for a wide range of biological processes, including neuronal signalling, muscle contraction, and ion channel gating. While voltage-sensing domains have been extensively studied in various membrane proteins, the mechanisms underlying their function are not well understood. In this work, we propose a novel mechanism for sensing membrane voltage that involves the reorientation of alpha-helices present in transmembrane proteins in response to changes in the membrane voltage. To investigate this mechanism, we performed coarse-grained molecular dynamics simulations on model dipolar peptides embedded in a lipid bilayer. We studied the effect of variation of membrane voltage on the tilt angle of the peptide and ascertained the optimal parameters for designing a sensitive membrane voltage sensor. Our results show that the voltage sensitivity of the sensor increases with the number of charged residues and peaks at an optimum hydrophobic length for peptides with a strong electrical dipole. We developed a theoretical model for the system based on phenomenological energies to further explore the parameter space and establish the dependence of voltage sensitivity on the design parameters. Overall, our study suggests that dipolar peptides may represent a promising platform for developing novel voltage-sensing technologies.

P-318
Towards predicting the outcome of disease-related genetic variants
Rikard Blunck[1,2,3,4], Michael Morin[2,4]
[1] Department of Pharmacology and Physiology, Université de Montréal, Canada [2] CUBiQ, Consortium universitaire en biophysique du Québec, [3] CIRCA, Centre interdisciplinaire de recherche sur le cerveau et l'apprentissage, [4] Department of Physics, Université de Montréal

Voltage-gated potassium (Kv) channels are responsible for the repolarization of neuronal and cardiac action potentials and adjust membrane excitability. But their expression is not restricted to muscular and neuronal cells. Instead, they are expressed throughout the human body. Accordingly, many genetic variants identified in patients have been linked to neurological, cardiac and other diseases. To establish causality between a genetic variant and disease development, it is necessary to study the electrophysiological properties of the channels. Characterization of genetic variants is time consuming and will lead to delays in the treatment of the patients. This raised the question whether it will be possible to predict the electrophysiological profile of genetic variants based on previous results. We present our preliminary findings in the development of a deep-learning algorithm that takes into account previous biophysical studies as well as the atomic-scale structures of Kv channels. In order to do so, we developed a database of the biophysical studies of Kv channels known to date. The long-term aim of this algorithm is to predict the outcome of a genetic variant on the biophysical properties of Kv channels in order to then analytically calculate the cellular and organ response.

P-319
Amphiphilic Au nanoparticles and cholesterol-containing liposomes serving as minimal tunable fusion machinery
Davide Bochicchio[1], Ester Canepa[1], Giorgia Brosio[1], Paulo Henrique Jacob Silva[2], Francesco Stellacci[2], Silvia Dante[3], Giulia Rossi[1], Annalisa Relini[1]
[1] University of Genova, [2] EPFL, [3] Istituto Italiano di Tecnologia, Italy

Membrane fusion is indispensable for various biological functions, such as neurotransmitter release and intracellular trafficking. In vivo, this intricate, multi-step process is regulated by specialized proteins, which cooperate with specific lipid compositions and ionic environments. Synthetic approaches for controlled membrane fusion have been recently endeavored and will be crucial for next-generation biomedical applications. However, controlling fusion rates in lipid membranes is challenging and requires a profound comprehension of the molecular mechanisms involved in the distinct fusion steps. Integrating experimental techniques and coarse-grained molecular dynamics (MD) simulations, we demonstrate that amphiphilic gold nanoparticles (Au NPs) combined with cholesterol-containing liposomes serve as minimal tunable fusion machinery. The cooperation of Au NPs, ions, and cholesterol governs the fusogenic activity of this system. While divalent ions (Ca^{2+} or Mg^{2+}) unspecifically trigger the fusion events, the fusion rates vary with the different liposome cholesterol content. In particular, a higher cholesterol content enhances liposome-liposome fusion. Thanks to the high spatiotemporal resolution provided by MD, we provide a molecular interpretation: cholesterol enriches the stalk region over the NP, stabilizing this intermediate fusion step. Our research has significant implications for developing cargo loading and targeted drug delivery applications. By providing a detailed understanding of the mechanisms entailed in the NP-induced fusion process, our findings pave the way for the design of synthetic nanomaterials able to provoke and control fusion events.

Poster Presentations
– Session 2 –

P-320
Prediction of skin permeability coefficient by means of coarse grained molecular dynamics simulations
Marine Bozdaganyan[1], Philipp Orekhov[1], Anqi Yang[1]
[1] Shenzhen MSU-BIT University, China

Transdermal delivery plays a crucial role in both medical and cosmetic industries. However, experimentation on human skin is costly and often impractical, while substitutes such as pig skin, monolayer cells, or chemical biofilms do not perfectly mimic human skin. Molecular simulations of model skin cell membranes can provide useful predictions for experimental parameters such as permeability coefficients. This study focuses on the interactions between different chemical compounds and model skin bilayers. The MARTINI force field and Gromacs software are used to simulate skin cell membranes with a 1:1:1 ratio of ceramide, cholesterol, and free fatty acid, which are equilibrated at 310 K for 0.5 microseconds. The potential of mean force (PMF) between the membrane and coarse-grained beads is calculated, followed by estimations of logKp. Additionally, PMFs and logKp values are calculated for selected compounds from CPE-DB database and analyzed for correlation with experimental values. Overall, this research provides valuable insights for the development and screening of new chemicals for transdermal drug delivery. By utilizing molecular simulations, it is possible to predict the permeability of different compounds through skin, without costly experimentation on human skin. The project is supported by the National Natural Science Foundation of China, grant #32250410316.

P-321
Piezo1 channel investigation in cardiac fibrosis precursor cells mechano-physiology
Nicoletta Braidotti[1,2], Giorgia Demontis[1,2], Laura Andolfi[2], Orfeo Sbaizero[3], Dan Cojoc[2]
[1] Department of Physics, University of Trieste, Via A. Valerio 2, 34127 Trieste, Italy, [2] Institute of Materials, National Research Council of Italy (CNR-IOM), Area Science Park Basovizza, Strada Statale 14, Km 163,5, 34149 Trieste, Italy, [3] Department of Engineering and Architecture, University of Trieste, Via A. Valerio 6/A, 34127 Trieste, Italy

Cardiac fibrosis consists in a structural myocardium remodeling and its progressive functional impairment. In fibrosis, cardiac fibroblasts (CF) undergo a phenotypic transition into muscle-like cell type, namely myofibroblasts, which are responsible for the progressive tissue stiffening. A positive feedback loop for myofibroblasts sustainment is already established, but the leading pathophysiological fibrotic mechanisms are still not fully understood. The present work deals with the possible involvement of Piezo1 channel in fibrosis. Therefore, the response of primary rat CF to mechanical stimulations was investigated. Forces from 10 pN to 350 nN were applied by exploiting Optical Tweezers and Atomic Force Microscopy, while monitoring calcium mobilization. 24-hours cultured cells showed an increased response at the higher applied pressure (from 11% at 3.8 kPa to 60% at 8.9 kPa), meanwhile 48-hours cultured CF displayed a reduction in responding cell number, possibly associated to CF phenotypical changes. The stiffness of CF at 24h and 48h did not change significantly and as a result the cell mechanics is not directly involved in the different mechanotransduction behavior. To evaluate the involvement of Piezo1 in the response to the applied pressure, cells were Yoda-1 treated (1, 5, 10 M) for 24h. Preliminary experiments showed an increment in responsive Yoda-1 treated cells in the pressure range of 3-9 kPa. Piezo1 at 24h/48h culturing and Yoda-1 co-culturing is now investigated by qPCR and immunofluorescence imaging. Altogether, our results suggest a Piezo 1 channel principal role in the response of primary CF to microenvironment mechanical variations.

P-322
Towards the design of fusogenic nanoparticles: mechanisms of nanoparticle-induced stalk formation
Giorgia Brosio[1], Davide Bochicchio[1], Giulia Rossi[1]
[1] Physics department - University of Genoa, Italy

Membrane fusion is crucial for eukaryotic cells. In vivo, it is a multi-step process involving the concerted action of fusogenic proteins. In vitro, learning to use artificial fusogenic agents to control fusion processes is critical for drug delivery applications and for realizing artificial vesicles with controlled composition. Gold nanoparticles (Au NPs) functionalized with amphiphilic ligands can facilitate membrane fusion, but the mechanisms are still unclear. Molecular Dynamics simulations, combined with advanced sampling techniques, can address this challenge. Here we adapt the definition of a collective variable, recently proposed to study stalk formation between two model membranes, to the case in which a membrane- embedded Au NP mediates stalk formation. We apply our method to NPs of different diameters (2 and 4 nm), revealing that smaller NPs lead to a lower free energy barrier for stalk formation and better stalk stability. We identify the origin of this size effect with the higher conformational freedom of charged ligands on the smaller NPs, in turn deriving from their larger curvature. We also demonstrate the interplay between the membrane lipid composition and the NP physical features, showing that cholesterol-containing membranes are especially prone to undergo NP- mediated fusion. Our study provides precious, general insights into the molecular mechanisms underlying NP-mediated membrane fusion, suggesting new routes for the design of synthetic, fusogenic NPs.

P-323
Droplet-based synthetic cells provide fine-tuned biophysical cues for T cell expansion
Anna Burgstaller[1,2,3], Nils Piernitzki[1,2,3], Marcus Koch[4], Hermann Eichler[5], Eva Schwarz[6], Michael Dustin[7], Franziska Lautenschläger[3], Oskar Staufer[1,2,3,8]
[1] Immuno-Materials Group, Leibniz Institute for New Materials, Germany, [2] Helmholtz Institute for Pharmaceutical Research, Germany , [3] Center for Biophysics, Germany , [4] Physical Analysis Group, Leibniz Institute for New Materials, Germany, [5] Institute of Clinical Hemostaseology and Transfusion Medicine, Saarland University and Saarland University Medical Center, Germany , [6] Department of Biophysics, School of Medicine, Saarland University, Germany , [7] Kennedy Institute of Rheumatology, Nuffield Department of Orthopedics, Rheumatology and, Musculoskeletal Sciences, University of Oxford, UK , [8] Max Planck Bristol Centre for Minimal Biology, Cantock's Close, United Kingdom

Ex vivo T cell activation and expansion is crucial for effective immunotherapy. In this context, synthetic cell-mimics, acting as artificial antigen presenting cells (aAPCs) in vitro, are a promising approach. However, mimicking the natural biochemical and biophysical cues required for a reliable expansion of high-fidelity T cells is still challenging. Therefore, we designed a synthetic cell assembly strategy to form dispersed droplet- supported lipid bilayers (dsLBs) based on oil droplets. The synthetic cells were designed to precisely emulate the natural lipid membrane characteristics of APCs, including stiffness, lateral ligand mobility and density. The dsLB potential to activate and stimulate human primary T cells was evaluated. The lipid bilayer formation was assessed by cryoTEM and environmental SEM, while the bilayer structure was analyzed by NBD assays and fluorescence recovery after photobleaching. Nanoindentation analysis and real-time deformation cytometry was applied to probe the synthetic cells stiffness. Finally, T cell activation and differentiation was characterized by flow cytometry. Our results demonstrate that dsLB-based synthetic cells are an advanced cell differentiation technology for therapeutic T cells. Further, our study suggests that considering lateral ligand mobility as an additional biophysical cue results in differentiation of less exhausted T cell phenotypes.

Poster Presentations
– Session 2 –

P-324
Role of Receptor-Receptor Interaction as Checkpoint in Immune Signaling

Rainer Böckmann[1,2], Matthias Pöhnl[1], Marius Trollmann[1,2], Cristian Popov[1], Michaela Seeling[3], Falk Nimmerjahn[3]
[1] Computational Biology, Department of Biology, Friedrich-Alexander-Universität Erlangen-Nürnberg, 91058 Erlangen, Germany, [2] Erlangen National High Performance Computing Center (NHR@FAU), Friedrich-Alexander-Universität Erlangen-Nürnberg, 91058 Erlangen, Germany, [3] Division of Genetics, Department of Biology, Friedrich-Alexander-Universität Erlangen-Nürnberg, 91058 Erlangen, Germany

Fc receptors are single-pass transmembrane proteins, expressed on the surface of innate immune effector cells, that bind the so-called Fc portion of antibodies. Immune effector cells are regulated by a precise interplay of activating and inhibitory receptors, which, upon ligand binding, contribute either a positive or a negative signal to the pro-inflammatory response. Malfunctioning of these mechanisms can lead to overactive immune cells and the destruction of healthy tissue known as autoimmune response. In human, the only inhibitory Fcγ receptor (Fc receptors that bind specifically to immunoglobulin G (IgG)) is FcγRIIb whose binding to IgG prevents an inflammatory response. Here, we used combined coarse- grained and atomistic molecular dynamics simulations and experimental studies to investigate IgG binding to Fc γRIIb. We show that the Dectin-1 receptor modulates the capacity of FcγRIIb to interact with IgG via rearranging FcγRIIb conformation and membrane clustering, suggesting that Dectin-1 acts as a co-inhibitory checkpoint modulating FcγRIIb inhibitory function. Furthermore, we investigate how competitive binding of the Fcε R1γ-dimers to activating Fc receptors may modulate immune cell activation, and decipher the role of the lipid nano-environment for immune cell activation.

P-325
Calcium transient alterations in cardiac myocytes of voluntarily running rats

Michal Cagalinec[1,2], Iuliia Baglaeva[1], Alexandra Zahradníková[1], Bogdan Iaparov[1], Ivan Zahradník[1], Alexandra Zahradníková jr[1]
[1] Dept. of Cellular Cardiology, Institute of Experimental Endocrinology, Biomedical Research Center, Slovak Academy of Sciences, Dúbravská cesta 9, 845 05, Bratislava, Slovakia, [2] Centre of Excellence for Advanced Material Application, Slovak Academy of Sciences, Dúbravská cesta 9, 845 11 Bratislava, Slovakia

Physical exercise is known to improve cardiac performance. In rodents, the slight increase of cardiomyocyte contractility induced by training was suggested to be due to an increase in myofilament Ca2+ sensitivity. This study aimed to estimate whether calcium transients in ventricular myocytes of young rats are affected at the early stage of training by voluntary running. Experiments were performed on left ventricular myocytes isolated from hearts of 12-week old rats. The myocytes were field stimulated and calcium transients were recorded by laser scanning confocal microscopy and analysed by in-house software. The calcium transient amplitude in trained rats was significantly increased by 13 % in comparison with the control group. This indicates that in young rats, even two weeks of training affect the amplitude of calcium transients in ventricular myocytes and implicates a positive effect of regular mild exercise on calcium signaling.

P-326
Coarse-grained simulations unveil the interactions of metal oxide nanoparticles with biological systems

Sonia Cambiaso[1], Julia Bassila[1], Enrico Lavagna[1], Davide Bochicchio[1], Giulia Rossi[1]
[1] University of Genoa, Italy

The interactions between metal oxide nanoparticles (NPs) and the biological environment are interesting from a toxicological and a biotechnological perspective. On the one hand, metal oxide NPs are often a component of advanced nanocomposite materials, and due to possible routes of environmental exposure, their toxicity should be carefully addressed. On the other hand, metal oxide NPs may also be exploited as biomedical nanoagents in diagnostics and therapy, and achieving control of their biological activity is crucial since their design stage. Studying metal oxide NPs in biological environments at fully atomistic resolution is computationally challenging due to intrinsic time and length scale limitations. Less detailed, coarse-grained approaches have the potential to overcome these difficulties but face the challenge of effectively modeling the peculiar, highly reactive nature of the metal oxide interface in aqueous environments. Here we present the development of coarse-grained models of ZnO and SiO2 NPs functionalized by hydrophilic organosilanes within the framework of the popular Martini model. We used Molecular Dynamics (MD) simulations and advanced sampling techniques to validate the models based on the available physicochemical characterization of their interface. Eventually, we simulated their interactions with model lipid membranes and highlighted which molecular mechanisms can lead to the alteration of the membrane's physical properties. MD simulations are thus envisaged as one of the steps of a multiscale approach to establish Safe and Sustainable by Design strategies for developing new multi-component nanomaterials.

P-327
How to make use of time-resolved fluorescence from tryptophan in your peptide

Iulia Carabadjac[1], Jessica Steigenberger[1], Yentl Verleysen[2], Vic De Roo[2], Niels Geudens[2], Penthip Muangkaew[2], José C. Martins[2], Heiko Heerklotz[1,3]
[1] Institute of Pharmaceutical Sciences, University Freiburg, Germany [2] Department of Organic and Macromolecular Chemistry, Ghent University, BE, [3] Leslie Dan Faculty of Pharmacy, UofT, CA

Time-resolved (TR) emission spectral shift can provide information about the polarity, order and dynamics of the fluorophore's environment. This information is essential for understanding the mechanism of action of antimicrobial membrane-active peptides, aiming at designing new types of antibiotics in times of antibiotic resistance crisis. To obtain this information, the usual way is to label the peptide or the membrane with solvatochromic fluorophores. However, the fluorophore itself already alters the properties of its vicinity, changing the very system we want to observe. An elegant way to solve this problem is to use intrinsic fluorophores - fluorophores that are already present in the system of interest. They often have less-than-ideal, complex fluorescence behavior, making analysis and understanding of the data difficult. The aromatic amino acid tryptophan (Trp) is one of such fluorophores and is commonly found in hydrophobic, transmembrane, or membrane-active peptides. The aim of this project is therefore to learn more about the TR fluorescence behavior of Trp in peptides. To this purpose, we measure and analyze TR emission spectra, TR anisotropy, TR quenching, and steady-state spectra of four different peptide analogs in which one hydrophobic amino acid of the natural variant is exchanged for Trp, in POPC membrane and compare these data with MD simulations. In this way, we get, interpret and understand data on the impact of the Trp's position in the peptide, the incorporation of the peptides into the membrane, their incorporation depth and orientation in the membrane and their effects on the membrane structure.

Poster Presentations
– Session 2 –

P-328
Revealing the mechanism of the lipid storage disorder Niemann-Pick C by combining multi-omics and biophysical imaging

Pablo Carravilla[1], Ugne Ceplaite[1], Christian Sommerauer[2], Raül García Veiga[2], Erdinc Sezgin[1], Claudia Kutter[2]
[1] Department of Women's and Children's Health, Karolinska Institute, Science for Life Laboratory, Sweden, [2] Department of Microbiology, Tumour, and Cell Biology, Karolinska Institute, Science for Life Laboratory, Sweden

Lipids are essential for cell homeostasis and cells tightly regulate their lipid metabolism. Niemann-Pick Type C (NPC) is a rare neurodegenerative disorder caused by mutations in the NPC intracellular cholesterol transporters 1 and 2 that result in cholesterol and sphingolipid accumulation in the lysosome. The subsequent pathogenic cascade responsible for the broad range of NPC symptoms remains unknown. We hypothesise that such an unbalanced lipid distribution in the cell would alter the biophysical properties of cellular membranes, which may have a pathological implication. Using advanced imaging, we quantify the lipid order and tension of multiple organelles in fibroblasts obtained from NPC patients, healthy volunteers and mice. Our analyses confirm that indeed NPC mutations induce changes in the biophysical properties of the cell membrane. To investigate the metabolic response mechanisms to such imbalance, we performed transcriptomic and lipidomic analyses and identified dysregulated pathways involved in lipid metabolism. In future experiments, we will assess whether by interfering with such pathways we can revert the NPC phenotype and find potential treatments for this fatal lipid storage disorder.

P-329
Lateral diffusion of lipids in supported bilayers in polymeric microcorrals

Nuria Carrillo Godoy[1,2,3], Julia Rubio Loscertales[4,5], Victor Galileo Almendro Vedia[1,2,3,6], Jose Requejo Isidro[4,5], Iván López Montero[1,2,3]
[1] Departamento Química Física, Universidad Complutense de Madrid, Avda. Complutense s/n 28040 Madrid, Spain, [2] Instituto de Investigación Hospital Doce de Octubre (imas12), Avenida de Córdoba s/n, 28041 Madrid, Spain, [3] Instituto Pluridisciplinar, Universidad Complutense de Madrid, Ps. Juan XXIII, 1 28040 Madrid, Spain, [4] Centro Nacional de Biotecnología CNB-CSIC, C/Darwin, 3 28049 Madrid, Spain, [5] Unidad de Nanobiotecnología, CNB-CSIC-IMDEA Nanociencia Associated Unit, 28049 Madrid, [6] Sección Departamental Farmacia Galénica y Tecnología Alimentaria, Universidad Complutense de Madrid, Av. Puerta de Hierro s/n28040 Madrid, Spain

Lateral organization of membranes depends on the transport properties of proteins and lipids. Lipids undergo lateral diffusion in the plane of the membrane whereas the transverse movement across the membrane is not favored. In this work, we study the lateral movement of lipids within supported lipid bilayers composed of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) embedded in polymeric microcorrals stamped on glass cover slides. The lateral diffusion of the fluorescent lipid analogues was assessed by two independent methods, including Image Correlation Spectroscopy (ICS) and Single Molecule tracking (SMT). The diffusion coefficient of lipids was determined from the fluorescence intensity time traces or individual trajectories from movies recorded at high acquisition rate, respectively. A comparative analysis of obtained diffusion coefficients with methodologies and experimental conditions is presented.

P-330
Long-range proton channel communication in Paracoccus denitrificans cytochrome c oxidase

Metehan ÇELEBİ[1], Ulrike ALEXIEV[1], Jens BALKE[1], Patricia SAURA[2], Ville KAILA[2]
[1] Freie Universität Berlin - Department of Physics - Germany, [2] Stockholm University - Department of biochemistry and biophysics - Sweden

In the respiratory chain, cytochrome c oxidase (CcO) is an essential membrane protein that catalyzes the reaction of molecular oxygen to water and pumps protons from the N- to the P-side of CcO. Proton uptake takes place via two channels (D- and K-channel). Only the D-channel pumps protons. Helix-6 harbors the catalytic Y280 and the essential E278 being involved in directing the pumped and chemical protons. We aim at revealing (i) a communication between the D- and K-channel by using the mutant D124N (D-channel block) and the protein dynamics reporter site at the N-side end of helix 6 (K-channel entrance). (ii) The communication between the P-side and the helix 6 reporter site is investigated by the P- side surface mutant D156N. By using time-resolved fluorescence anisotropy experiments and molecular dynamics simulations we find long-range effects both between the two channels and from the P-side to the K-channel.

P-331
Cooperative effort of sodium ions and water molecules facilitates lipid mobility in model cell membranes

Madhurima Chattopadhyay[1], Emilia Krok[1], Hanna Orlikowska-Rzeznik[1], Lukasz Piatkowski[1]
[1] Poznan University of Technology, Poland

Ions present around the biomembranes interact with the hydration layer of the lipid headgroups. This hydration layer also significantly modulates lipid dynamics in the membrane. Using fluorescence recovery after photobleaching (FRAP) technique we unravel that the cooperative effort of Na+ ions and water is crucial to maintain PC lipid mobility when the direct hydration layer of lipids is perturbed. At fully hydrated condition, Na+ ions have hardly any effect on lipid mobility, but the significance of Na+ ions facilitating lipid diffusion emerges after the removal of bulk water. The lipid diffusion ceases in the absence Na+ ions at water-depleted conditions. We demonstrate that Na+ and K+ ions bound to the phosphate oxygen of phosphocholine moiety of lipid headgroup, strengthen the water clathrate cage structure around it, which in turn facilitates lipid diffusion. In contrast, Mg2+ and Ca2+ ions do not support lipid mobility in low water availability conditions. We demonstrate that not the charge or charge density, but the competition between the lipid binding affinity of the cations vs. their hydration energies act as the determining factor here. Our results have important implications revealing the importance of ionic compositions in biological processes involving transient dehydrations, e.g. cell fusion, neurotransmission, viral entry.

Poster Presentations
– Session 2 –

P-332
Mechanism of Ligand-dependent G-protein-coupled Receptor Activation Reveled by Free-energy Landscapes
Yue Chen[1,2], Jens Carlsson[1,3], Lucie Delemotte[1,2]
[1] Science for Life Laboratory, Sweden [2] KTH Royal Institute of Technology, [3] Uppsala University

G-protein-coupled receptors (GPCRs) respond to a variety of extracellular stimuli to mediate diverse cellular signals. Despite the high similarity of their orthosteric binding sites and endogenous ligand chemistry, GPCRs are selectively activated by specific ligands/drugs. Thus, understanding and predicting the underlying mechanisms for ligand-dependent signaling responses is critical for the development of efficient drugs, which remains a major challenge. Here we used enhanced sampling molecular dynamic (MD) simulations to disclose the activation mechanism for D2 dopamine receptor (D2R) in the presence of agonists with similar chemical structures but various efficacies. Free energy landscapes projected along functional microswitches located from ligand-binding to G-protein binding sites revealed agonist-induced conformational transitions between the inactive and the active states. Further analyses of the correlation between ligand efficacy and the outward movement of transmembrane helix 6 (TM6) identify that D2R exists in a ligand efficacy-dependent equilibrium between an inactive, partially active, and fully active conformation. Together, these findings provide mechanistic insights into the dynamic activation of GPCRs and useful information for designing specific GPCR
ligands with desired activity profile.

P-333
The Photoreaction of the Proton-Pumping Rhodopsin 1 from the Maize Pathogen Basidiomycete Ustilago maydis
Jheng-Liang Chen[1], Mariafrancesca La Greca[1], Luiz Schubert[1], Jacek Kozuch[1], Tim Berneiser[1], Joachim Heberle[1], Ramona Schlesinger[1]
[1] Free University Berlin, Germany

Microbial rhodopsins have recently been discovered in pathogenic fungi and have been postulated to be involved in signaling during the course of infection. Here, we report on the spectroscopic characterization of a light-driven outward proton pump rhodopsin (UmRh1) from the smut pathogen Ustilago maydis, the causative agent of tumors in maize plants. Time-resolved UV/Vis and vibrational spectroscopy indicate a pH- dependent photocycle. The photocycle upon pH change in UmRh1 is significantly different from the well- known bacteriorhodopsin from archaea (HsBR). We applied site-directed mutagenesis on UmRh1 based on the structural model and sequence alignment, in order to understand this different behaviour of proton pumping. We also characterized the impact of the auxin hormone indole-3-acetic acid (IAA) that was shown to influence the pump activity of UmRh1 on individual photocycle intermediates. A facile pumping activity test was established of UmRh1 expressed in Pichia pastoris cells, for probing proton pumping out of the living yeast cells during illumination. We show similarities and distinct differences to HsBR and discuss the putative role of UmRh1 in pathogenesis.

P-334
Interpretation of STED-FCS diffusion law plots for nanoscopically heterogeneous membranes
Barbora Chmelová[1,2], Alena Koukalová[3], Martin Hof[1], Erdinc Sezgin[4], Radek Šachl[1], Hans Blom[5]
[1] J. Heyrovský Institute of Physical Chemistry of the Czech Academy of Sciences, Dolejškova 3, 182 23 Prague, Czech Republic, [2] Faculty of Mathematics and Physics, Charles University, Ke Karlovu 5, 121 16 Prague, Czech Republic, [3] Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Flemingovo náměstí 542/2, 160 00 Praha 6, Czech Republic, [4] Karolinska Institutet, Department of Women's and Children's Health, 171 77 Stockholm, Sweden, [5] Science for Life Laboratory, Department of Applied Physics, Royal Institute of Technology, Solna, Sweden

Stimulated emission depletion combined with fluorescence correlation spectroscopy (STED-FCS) is one of the few techniques that can nowadays detect nanoscopic membrane heterogeneities in biological membranes by the analysis of so-called diffusion law plots. These plots relate in- membrane probe diffusion coefficients to the size of the observation spot. Despite their frequent use in membrane biophysics, the interpretation of these dependencies is often ambiguous without exploiting their full potential. In this contribution, we show that a quantitative analysis of STED- FCS diffusion law plots provides unique information about the mobility of nanoscopic membrane heterogeneities as well as elucidates the diffusivity of individual lipids within these nanodomains. We demonstrate the applicability of this approach by performing STED-FCS experiments on nanoscopically heterogeneous membranes of giant lipid vesicles that contain ganglioside nanodomains of controllable size and membrane surface concentration. The final interpretation of experimental diffusion law plots corresponding to dynamics of model membrane is accomplished by their comparison with in-silico simulations of lipid probe diffusion in heterogeneous bilayers. By this combinatory approach we get better insight into the dynamics of nanoscopically heterogeneous membranes.

P-335
DAG activates TRPC3 through direct interaction with pore-lining residues
Amy Clarke[1], Klaus Groschner[2], Thomas Stockner[1]
[1] Medical University of Vienna, Austria [2] Medical University of Graz

Calcium signalling is an essential part of the cellular signal transduction machinery, and regulates an array of processes in humans, including muscle contraction, neuronal signal transmission, and fertilisation. Of central importance to calcium signalling is a superfamily of calcium- permeable ion channels, the transient receptor potential (TRP) channels, which respond to a variety of signals to allow Ca2+ influx. The transient receptor potential canonical channel 3 (TRPC3) is activated by the lipid diacylglycerol (DAG). Recent combined computational and experimental work has identified that DAG binds to the L2 lipid interaction site, located in the extracellular leaflet with a lateral fenestration pointing towards the pore of the channel. However, although the site of DAG binding has now been elucidated, the determinants of its interaction with TRPC3 have not. Here we present data from a multiscale molecular dynamics approach, showing that DAG interacts with key pore-lining residues of the S6, and that the presence of DAG at the L2 binding site alters the dynamics of the selectivity filter. Our results represent the first time that an all-atom approach has been used to explore the interaction of TRPC3 with its lipid activator DAG, and suggest a mechanism of channel activation through which DAG directly interacts with the pore-lining machinery of TRPC3.

Poster Presentations
– Session 2 –

P-336
Electrostatic Switch Mechanism in Ion Pump Function
Ronald Clarke[1], Bogdan Lev[2], Milna Chennath[3], Toby Allen[2], Flemming Cornelius[4]
[1] University of Sydney, Australia, [2] RMIT University, [3] University of Technology Sydney, [4] University of Aarhus

Prior to the publication of any crystal structures, mutagenesis studies suggested that regulation of the Na^+-pump might involve the formation and breakage of a salt bridge between Lys30 of the N-terminus and Glu233 in the first cytoplasmic loop of the alpha subunit. In X-ray crystallographic studies of the Na^+-pump, the N-terminus could never be resolved, probably because it is too mobile on the time scale of structure determination.
Nevertheless, after theoretically predicting the N-terminus conformation, and adding it to the crystal structure, we found that the distance between
residues Lys30 and Glu233 makes it unlikely that a direct interaction between them is involved in pump function. Instead, we propose an interaction of the positively charged lysine residues of the N-terminus with negatively charged lipid headgroups (notably phosphatidylserine) on the cytoplasmic surface of the surrounding membrane. Such an interaction could be regulated by an electrostatic switch mechanism, whereby serine and tyrosine residues of the N-terminus are phosphorylated by protein kinases. Phosphorylation of these residues would decrease the positive charge of the N-terminus, allowing its release from the membrane.

P-337
A High-Throughput Screen to Identify Modifiers of KCNQ1 Trafficking
Katherine Clowes[1], Mason Wilkinson[1], Katherine Stefanski[1], Charles Sanders[1]
[1] Department of Biochemistry, Vanderbilt University, Nashville, TN, USA

Loss-of-function (LOF) mutations in the voltage gated potassium channel KCNQ1 cause Type 1 Long QT Syndrome (LQT1), a cardiac disorder. Previous studies found that only ~20% of expressed WT KCNQ1 successfully traffics to the plasma membrane, and that many LQT1-associated mutations in KCNQ1 destabilize the protein and decrease trafficking efficiency further. Protein mistrafficking is a common mechanism of several diseases and has been found to be rescuable with small molecules. This led us to hypothesize that fold-stabilizing small molecules can increase the trafficking efficiency of KCNQ1. To test this hypothesis, we developed an immunofluorescence-based high-throughput screen to identify compounds that alter the trafficking of KCNQ1 in cells. While ongoing, screening has identified several hits that alter KCNQ1 expression and trafficking efficiency. Follow up experiments will determine whether these compounds bind and stabilize KCNQ1 directly. These studies will contribute to our larger hypothesis that misfolding induced mistrafficking is a common, rescuable, mechanism of KCNQ1 LOF in LQT1.

P-338
Membrane permeabilization in pyroptosis at the single-molecule level
Katia Cosentino[1], Eleonora Margheritis[1], Shirin Kappelhoff[1]
[1] University of Osnabrück, Germany

Plasma membrane (PM) permeabilization is a critical step in the execution of pyroptosis, a highly inflammatory cell death program with important implications for infection, immunity, and associated diseases. In pyroptosis, membrane perforation is executed by proteins of the pore-forming Gasdermin (GSDM) family via a mechanism that remains poorly understood. By combining biophysical tools and advanced high-resolution fluorescence microscopy, we have gained detailed molecular insights into the mechanism of pore formation by the Gasdermin D (GSDMD) family member. By stoichiometry analysis of individual fluorescently labeled GSDMD oligomers in mimetic membrane systems, we have identified dimers and trimers as the building blocks of GSDMD oligomerization and the minimal oligomers that can insert into the membrane. In addition, by cysteine mutation analysis, we have uncovered new structural elements that enable the initial assembly of GSDMD into dimers and trimers and revealed how the mechanism of high-order oligomer formation occurs. Furthermore, by combining DNA-PAINT microscopy with a newly developed approach called polymer-supported plasma membranes (PSPMs) we have resolved the structural details of GSDMD oligomers directly at the PM of pyroptotic cells. Using this approach, we demonstrated that GSDMD forms heterogeneous structures at the PM and identified the structures relevant to the execution of pyroptosis.

P-339
Noncanonical electromechanical coupling paths in cardiac hERG potassium channel
Flavio Costa[1], Carlos A. Z. Bassetto Jr[2], Carlo Guardiani[1], Francisco Bezanilla[2], Alberto Giacomello[1]
[1] Sapienza University of Rome, Italy [2] The University of Chicago

The human ether-à-go-go–related gene (hERG) codes for a voltage-gated potassium channel that plays a key role in the heart mechanics. Indeed, during the cardiac action potential, this channel rapidly recovers from inactivation, to cease the action potential. Interestingly, hERG operates seamlessly without a covalent interaction between the voltage sensor (VSD) and the pore (PD), suggesting the presence of an alternative communication between VSD and PD that excludes the S4-S5 linker. Such mechanism has been previously shown in the domain-swapped Shaker potassium channels. Here, we used a combination of mutagenesis and molecular dynamics simulations/network analysis to identify a chain of residues coupling the VSD to PD that involves S4\S1 and S1\S5 subunit interfaces. The role of each residue in the identified noncanonical paths was quantified by performing betweenness centrality analysis and tested using electrophysiological assays combined with leucine-scanning mutagenesis. To provide unequivocal evidence of the noncanonical path and the agreement between the computational prediction and experimental data, we plotted the betweenness centrality and the minimal distance of each mutant along the noncanonical paths against the free- energy perturbation of activation and of inactivation. These plots showed a good agreement between computational and experimental results, demonstrating that the residues identified are part of the noncanonical electromechanical coupling in hERG. Altogether, our work identifies the presence of allosteric, noncanonical gating paths in the hERG channel and shed new light on the elusive activation and inactivation mechanisms of non-domain-swapped channels.

Poster Presentations
– Session 2 –

P-340
Probing the gating cycle of KcsA potassium channel using advanced homo-FRET approaches
Ana Coutinho[1,2], Clara Díaz-García [1], M. Lourdes Renart[3], Ana M. Giudici[3], José A. Poveda[3], José M. González-Ros[3], M. N. Berberan-Santos[1], Manuel Prieto[1]
[1] iBB and Associate Laboratory i4HB, Instituto Superior Técnico, Universidade de Lisboa, Portugal, [2] Dep. de Química e Bioquímica Faculdade de Ciências, Universidade de Lisboa, Portugal, [3] Instituto de Investigación, Desarrollo e Innovación en Biotecnología Sanitaria de Elche, Universidad Miguel Hernández, Elche, Spain

Potassium channels are integral membrane proteins ubiquitously found among prokaryotic and eukaryotic organisms that play an essential role in several vital physiological processes. Understanding the molecular mechanisms that underlie their functional properties is crucial given their association with numerous ion channel disorders. The prokaryotic potassium channel KcsA was used here as a case study to illustrate how homo-Förster Resonance Energy Transfer (homo-FRET)-based measurements can uncover the structural plasticity of these membrane proteins. The intrinsic fluorescence properties of the channel (W67 emission) were first used to characterize the influence of the degree of ion occupancy on the conformational dynamics of its pore-helices interconnected to the selectivity filter (SF) (outer gate) by using time-resolved homo-FRET measurements. A homo-FRET based sensor sensitive to the pH-induced gating of KcsA was then engineered by conjugating the tetramethylrhodamine (TMR) dye at the opposite G116C position of W67 KcsA (inner gate). By combining absorption, steady-state and time- resolved fluorescence anisotropy measurements of our double reporter system (TMR-G116C W67 KcsA labelled at variable dye:protein molar ratios), we were able to monitor the protonation-sensitive conformational switch of the activation gate of KcsA and confirm its allosteric communication with the selectivity filter.

P-341
Structure and dynamics of differential ligand binding in GABAA receptors
John Cowgill[1,2], Chen Fan[1,2], Nandan Haloi[1,3], Victor Tobiasson[1,2], Yuxuan Zhuang[1,2], Rebecca Howard[1,2], Erik Lindahl[1,2,3]
[1] Scilifelab, [2] Stockholm University, Sweden [3] KTH Royal Institute of Technology

P-342
Design of novel chimeric G-Protein Coupled Receptors (GPCRs): Identification of equivalent regions at the GPCR sequence, structural and biophysical level
Charlotte Crauwels[1,2], Wim Vranken[1,2]
[1] Structural Biology Brussels, Vrije Universiteit Brussel, Brussels, Belgium, [2] Interuniversity Institute of Bioinformatics Brussels, Vrije Universiteit Brussel - Université Libre Bruxelles, Brussels, Belgium

Currently, 60% of the druggable G-Protein Coupled Receptors (GPCRs), proteins involved in a broad range of diseases, remain untargeted due to the lack of insights at their sequence and structural level. Chimeric GPCRs, typically the merger of well- and not well-characterized GPCRs, can help to increase GPCR understanding by revealing novel 3D structures, biological function, and ligand binders. However, designing chimeras is challenging as equivalent regions need to be merged to ensure expression and function of the resulting chimera. Correct sequence alignment of the GPCRs is thus crucial. Therefore, we evaluated the state-of-the-art Multiple Sequence Alignment (MSA) of GPCRs by studying the predicted biophysical properties of 312 aligned class A GPCR sequences. Unexpected shifts in predicted biophysical behaviors were observed for GPCRs known to be phylogenetically related. Based on an AlphaFold2-guided structure-based MSA, an improved MSA was obtained where specific residues known to be equivalent were aligned, and where biophysical behaviors of related GPCRs were better matched. This work will lead to proposing novel chimeric designs and exploring a method to improve sequence alignment directly using the predicted biophysical behavior.

P-343
Characterizing the interaction of detergents and liposomes with Laurdan and Nile Red using time-correlated single photon counting
Sarah Crocoll[1], Iulia Carabadjac[1], Jesús Sot[2], Félix M. Goñi [2], Heiko Heerklotz[1,3]
[1] Institute of Pharmaceutical Sciences, University of Freiburg, Germany, [2] Institute of Biophysics and Department of Biochemistry of University of the Basque Country, Spain, [3] Leslie Dan Faculty of Pharmacy, University of Toronto, Canada

Interactions of detergents with lipid bilayers play an important role in many fields, for example delivery of drugs or cellular processes. The well- known three-stage model describes these interactions and distinguishes between stage I (detergent binding), stage II (lamellar-micellar phase transition) and stage III (mixed micelles). The main aim of our study is to compare the information provided about such systems from time- resolved fluorescence studies of the two membrane probes Laurdan and Nile Red. Samples of lipid and Triton X-100 in all three stages were characterized by time-resolved emission spectra (TRES) and time-resolved anisotropy measurements (TR anisotropy). While TRES gives information on the presence and mobility of water close to the fluorophore, TR anisotropy provides insights into the speed and limitation of its angular motions. Therefore, both methods provide information on the order and dynamics of lipid bilayers. Our data demonstrate that Nile Red is a little sensitive to Triton-induced changes in the order and dynamics of bilayers – effects that are revealed by Laurdan. This focus on topological changes rather than general disordering renders Nile Red superior for the detection of onset and completion of solubilization. That means that for an overall description of the lipid-detergent system, it is advantageous to consider both probes in parallel.

Poster Presentations
– Session 2 –

P-344
Blockage of Aquaporin-3 by gold compounds affects the biophysical properties of the membrane, impairing melanoma progression

Inês V. da Silva[1,2], Inês Paccetti-Alves[1,2], Catarina Pimpão[1,2], Sophie R. Thomas[3], Angela Casini[3], Graça Soveral[1,2]
[1] Universidade de Lisboa, Portugal, [2] Universidade de LIsboa, Portugal, [3] University of Munich, Germany

Aquaporin-3 (AQP3) is a water channel that also facilitates glycerol and H2O2 permeation across cell membranes and is aberrantly expressed in melanoma. The contribution of AQP3 to melanoma relates with its crucial role in cell adhesion, migration, and proliferation. AQP3 activity is modulated by gold compounds with reduced associated toxicity, suggesting these molecules constitute promising cancer therapy drugs. Here, we assessed the inhibitory effect of a new series of organogold compounds (compounds 1 - 3) derived from Auphen, a potent AQP3 inhibitor, over AQP3. The screening of the gold compounds was first performed in red blood cells (RBCs), that highly express AQP3, and then in MNT-1 and A375 human melanoma cells with distinct levels of AQP3 expression and malignancies. Additionally, the effect of gold complexes on the biophysical properties of the membrane was evaluated on cell adhesion, proliferation, and migration. The first screening in RBCs unveiled two organogold compounds as promising blockers of AQP3 permeability. Although no inhibition in water permeability was observed, a moderate reduction of glycerol and a drastic impairment of H2O2 permeability was monitored for compounds 1 and 2 in both human melanoma cell lines. Moreover, compounds 1 and 2 affected cell adhesion, proliferation, and migration. Our results indicate that the suppression of AQP3-H2O2 permeability by gold compounds strongly impacts in melanoma progression. Concluding, the present data reinforces gold compounds as promising AQP3 inhibitors with implications in melanoma progression, unveiling their potential as anticancer drugs against melanoma and other AQP3-overexpressing tumors.

P-345
Height mismatch auxiliary molecules significantly reduces binding affinity on cell contacts and influences protein organization

Tommy Dam[1], Manto Chouliara[1], Victoria Junghans[2], Ana Mafalda Santos[3], Simon J. Davis[3], Peter Jönsson[1]
[1] Division of Physical Chemistry, Department of Chemistry, Lund University, Lund, Sweden, [2] CAMS Oxford Institute and Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom, [3] Radcliff Department of Medicine, University of Oxford, Oxford, United Kingdom

The adhesion pair CD2/CD58 facilitates interactions between T cell receptors (TCRs) and peptide-bound major histocompatibility complexes (pMHCs) by positioning the interacting cells at an optimal distance. But not all vital adhesion molecules are of similar height as TCR/pMHC. How a height mismatch influences the binding kinetics of TCR/pMHC is largely unknown. Here the two-dimensional dissociation constant (2D Kd) for TCR/pMHC was measured in the presence of adhesion molecules of different height and molecular density. Labeled L3-12 TCR was anchored on a supported lipid bilayer (SLB) and bound pMHC (HLA-DQ8-glia-α1) expressed on Jurkat cells. Rat CD48 mutant T92A was expressed on the cells and would bind either of three variants of rat CD2 in the SLB, that had different heights but identical binding region. Notably, a height mismatch of ~4 nm increased the effective 2D Kd of TCR/pMHC by an order of magnitude compared to the corresponding value for the individual interaction. The height-matched wild type rCD2/rCD48T92A always colocalized with TCR/pMHC and gave a similar 2D Kd value as without adhesion molecules at moderate TCR/pMHC densities, but a 2.6 times higher value at low TCR/pMHC densities. Altogether this demonstrates how height and molecular density differences can significantly influence binding affinity.

P-346
Nature of the interaction between ssDNA-loaded cationic Lipid Nanoparticles (cLNPs) and Giant Unilamellar Vesicles (GUVs) in Membrane Fusion

David Davidović[1], Nestor Lopez Mora, Ye Zeng, Panagiota Papadopoulou, Alexander Kros, Martin Hof, Radek Šachl
[1] J. Heyrovsky Institute of Physical Chemistry, Czech Republic

Lipid nanoparticles (LNPs) have shown great potential as nucleic acids delivery vehicles, but with limited efficacy. In order to successfully deliver cargo to the cytosol, LNPs need to overcome the challenge of membrane fusion-mediated escape from endosomal compartments. However, the mechanism that drives this process is not yet fully grasped. In this work, the interaction of cationic lipid-containing LNPs with the negatively charged lipids on the surface of the endosomal-mimicking giant unilamellar vesicles (GUVs) was studied employing a wide variety of Fluorescence methods, both Microscopy and Spectroscopy. We investigated the extent and kinetics of membrane fusion between the two structures as a function of LNPs with various helper lipid contents. We characterized the internal structure of novel formulations of LNPs and we showed the importance of the LNPs' lipid composition on their fusogenic properties. By studying this model system, we gain insights into how LNPs are trapped and processed at the anionic endosomal membrane prior to nucleic acids release. This could foster the identification of new strategies to improve the efficacy of LNP-based gene delivery systems.

P-347
The functional interplay of the ABC transporter Pgp with its lipid substrates

Dario De Vecchis[1], Lars Schaefer[1]
[1] Ruhr University Bochum, Theoretical Chemistry Department, Bochum, Germany

The multidrug efflux pump P-glycoprotein (Pgp) is an ABC transporter which hydrolyzes ATP to energize the translocation of hydrophobic compounds through the plasma membrane, is overexpressed in a variety of cancer and associated with multidrug resistance. Pgp is located in highly specialized membranes often rich in cholesterol and sphingolipids. However, at the atomic level, the link between this consistent lipid environment and Pgp structural dynamics and mechanism remains elusive. We investigated the structure and dynamics of human Pgp employing all-atom and coarse-grained molecular dynamics simulations in asymmetric multi-component bilayers that mimic the hepatocyte membrane where Pgp is expressed. We explored and compared the dynamics of two human Pgp inward-open structures, which were previously solved in detergent and nanodiscs and greatly differ in their proposed portal helices. The simulations visualize preferential interactions with sphingolipids at the portal helices and show how cholesterol and different lipid species wedge, snorkel, and enter within the substrate cavity of Pgp solved in detergent. However, in Pgp solved in nanodiscs the access to the cavity is restricted, suggesting that this structure is a likely post substrate-bound state. Furthermore, the volume and dynamics of this cavity largely differ between the two Pgp structures, and are modulated by removal of cholesterol and the presence or absence of ATP. Our study emphasizes the importance of the lipid environment for Pgp dynamics and investigates the functional role of the two different inward-open states in the context of the overall mechanism of this transporter.

Poster Presentations
– Session 2 –

P-348
Architecture of a bacterial signalosome revealed by magic-angle spinning NMR-based integrative structural biology
Loïc Delcourte[1], Corinne Sanchez[2], Mélanie Berbon[1], Alons Lends[1], Asen Daskalov[1], Fatjona Xhango[1], Amandine Jalbert[1], Birgit Habenstein[1], Brice Kauffmann[1], Axelle Grélard[1], Estelle Morvan[1], Virginie Coustou[1], Sven Saupe[1], Antoine Loquet[1]
[1] CNRS, [2] Université de Bordeaux, France

Signalosomes are large protein machineries associated with innate immunity functions in plants, bacteria, fungi and mammals. Using a combination of magic-angle spinning NMR combined with solution NMR, X-ray cristallography and electron microscopy, we investigate the molecular architecture of the signalosome from Streptomyces olivochromogenes. Taking advantage of a divide-and-conquer strategy, we first solved the high resolution structure of the globular effector domain called BELL and compared its architecture to reported fungal signalosome effectors to reveal structural divergence. Next, we showed that a domain of the signalosome can self- assemble to form homogenous filaments. X-ray diffraction was employed to determine the cross-beta nature of the filament fold, and chemical shift analysis using magic-angle spinning NMR revealed the secondary structure of this functional amyloid domain in isolation. We show no structural homology to known amyloid fold reported by solid-state NMR for signalosomes in fungi and mammals. Magic-angle spinning NMR was then used to study filamentous assemblies formed by a protein construct encompassing the globular effector and the functional amyloid domain, to explore the scaffold role of the amyloid fold in bacterial signalosomes. Our study paves the way to provide a transkingdom comparison of functional amyloid domains in signalosome machineries involved in regulated cell-death processes.

P-349
Investigating the Regulation of Sortilin by NSG1 and NSG2 Using in silico Methods
Lasse Desdorf[1], Malene Overby[1], Julie Aae Lorentsen[1], Heidi Kaastrup Müller[1], Birgit Schiøtt[1], Nils Anton Berglund[1]
[1] Aarhus University, Denmark

The pathological hallmark of Alzheimer's disease is the presence of amyloid plaques in the brain and recent studies have found fragments Sortilin (SORT1) within these plaques. Two novel interaction partners, from the neuron-specific gene family, have been found to increase/decrease the cleavage of SORT1 (NSG1/NSG2). Neuronal membrane composition changes due to ageing and cholesterol is depleted, this is known to affect protein conformations and dynamics. In this project, we want to study the interaction of SORT1 with NSG1 and NSG2 to gain further insight into the regulatory process. Molecular dynamics was used to study the dimerization process, Markov state models provided kinetic information about the binding conformations of the dimers. Enhanced sampling was used to map the energy landscape of the dimer conformations. NSG1 and NSG2 were found to compete for the same binding interface with SORT1. The SORT1 and NSG1 dimer was largely unaffected by the change in membrane composition however the SORT1 and NSG2 dimer was heavily affected when cholesterol was depleted. An increase in SORT1 cleavage is correlated with the decrease of binding affinity towards NSG2 and the subsequent increase in binding affinity towards NSG1.

P-350
A molecular imprint of intracellular Amyloid-β-ApoE interaction correlates with toxicity
Arpan Dey[1], Aditi Verma[1], Bidyut Sarkar[2], Uchit Bhaskar[3], Mamata Kallianpur[1], Vicky Visvakarma[1], Kanchan Garai[4], Odity Mukherjee[3], Kunihiko Ishii[2], Tahei Tahara[2], Sudipta Maiti[1]
[1] Tata Institute of Fundamental Research, Mumbai, India, [2] Molecular Spectroscopy Laboratory, RIKEN, Wako, Saitama, Japan, [3] Institute of Stem Cell Science and Regenerative Medicine, Bangalore, India, [4] Tata Institute Of Fundamental Research, Hyderabad, India

Apolipoprotein E (e4 isoform of ApoE) is the strongest genetic risk related to Alzheimer's disease (AD), which is initiated by the aggregation of the amyloid beta (Aβ) peptide. A molecular-level understanding of how ApoE is involved in AD pathology, and how it may interact with Aβ may lead us to effective therapies. Here, we employed a fluorescence lifetime-based assay, and found a specific ApoE-dependent modification of Aβ40 in solution. We also explored this phenomenon in several types of cells using fluorescence lifetime imaging. We observed that the change of lifetime of Aβ40 oligomers correlated with their relative ApoE content, with the highest effect displayed by dissociated wild-type rat astrocytes. This ApoE- induced modification also produced distinct species of Aβ40 oligomers (measured using single molecule photobleaching (smPB) and 2-dimensional fluorescence lifetime correlation spectroscopy (2D FLCS)), which showed an increased affinity to artificial lipid bilayers. A known peptide inhibitor of Aβ-ApoE interaction, LVFFA, reduced both the lifetime modification and toxicity of Aβ40 oligomers in a dose-dependent manner. The correlation between the short lifetime component and Aβ40 toxicity was also corroborated by experiments using two nontoxic Aβ variants. Finally, neuronal stem cells derived from AD patients also showed the short-lifetime component, the changed oligomeric state, and the increased membrane affinity observed in the other cells. Therefore, our findings regarding ApoE induced modifications of Aβ40 are likely to be relevant for AD, and can potentially provide a quantitative cellular assay for AD drug discovery.

P-351
Building an in vitro model for GABAA-Receptor Organization
Lara Dohmen[1], Sinika Kahl[1], Claudia Steinem[1,2]
[1] Georg-August-Universität, Institute of Organic and Biomolecular Chemistry, 37077 Göttingen, Germany, [2] Max-Planck-Institute for Dynamics and Self-Organization, 37077 Göttingen, Germany

The assembly of the GABAergic post-synapse is a key step in synaptogenesis and crucial for the regulation of neuronal communication. Its formation is dependent on the interplay between a variety of proteins. Owing to its complexity, the regulation of the GABAergic inhibitory post- synapse is extremely prone to disfunctions causing diseases, such as anxiety disorders or epilepsy. Attributed to their interactions, the proteins Collybistin 2 (CB2) and Neuroligin 2 (NL2) play key roles in GABAergic synapse regulation. Here, we present an in vitro system allowing the examination of this protein-protein interaction located directly at a lipid monolayer. Upon formation of a solid-supported membrane, a section of the cytosolic loop of NL2 is attached to the membrane via an effective Michael-click addition of a cysteine thiol-residue to maleimide-functionalized lipids. The successful fixation of the construct was proven by reflectometric interference and ATR-FTIR spectroscopy. Membrane-linkage of CB2 will be mediated by the receptor lipid PIP2 and the influence on NL2 on CB2 binding will be evaluated.

Poster Presentations
– Session 2 –

P-352
Combining polyphenols with chemotherapeutic treatment : effect of Resveratrol and Xanthohumol on the mechanical properties of colorectal cancer cells measured by Atomic Force Microscopy

Alysson DUCHALET[1,2], Cecile FEUILLIE[1], Hassan EL-BTAOURI[2], Laurent MARTINY[2], Michael MOLINARI[1]
[1] Univ. Bordeaux, CNRS, Bordeaux INP, CBMN, UMR 5248, F-33600 Pessac, France, [2] CNRS UMR7369 MEDyC, Université de Reims Champagne- Ardenne, Reims, France

WHO classifies colorectal cancer (CRC) as one of the deadliest and most common cancers in Europe due to acquisition of therapeutic resistance leading to metastasis. The use of chemotherapeutic drugs, like 5-Fluorouracil (5-FU), is often linked to the development of chemoresistance mechanisms due to tumor aggressiveness. Polyphenols like resveratrol (RSV) and flavonoids like xanthohumol (XN) have shown interesting properties when used in combination with chemotherapeutic treatments by enhancing their efficiency while minimally affecting surrounding healthy cells. However, how these compounds affect membrane dynamics and cell mechanic is still poorly understood. Thanks to Atomic Force Microscopy (AFM), which allows nanoscale mechanical measurements on soft samples in physiological conditions, we aim to investigate the effects of 5-FU treatment with/without RSV or XN on live CRC cells mechanical properties. We performed PeakForce QNM imaging and force spectroscopy on CRC cell lines with different chemoresistance (SW480, SW620, HT29), in order to evaluate changes in elasticity 24 hours after adding the treatment. We were able to focus on the cell membrane notably by monitoring lipid packing through Laurdan fluorescence with Confocal microscopy. We also analysed the membrane composition of the different cell lines through lipidomic measurements and prepared lipidic bilayers, that we analysed by AFM to try to better understand in real time the effect of RSV and XN on membrane elasticity. Overall, our results will allow a better understanding of the impact of natural molecules on CRC cells and their mechanical behaviour in response to treatment.

P-353
Exploring the conformational landscape of the Insulin Receptor using advanced sampling methods

Amanda Dyrholm Stange[1], Kasper Busk Pedersen[1], Miłosz Wieczór[2], Nils Anton Berglund[1], Birgit Schiøtt[1]
[1] Aarhus University, Denmark, [2] IRB Barcelona, Spain

The Insulin Receptor (IR) plays a crucial role in the regulation of blood sugar levels and is activated when insulin is bound. IR can bind up to four insulins at a time, and with two distinct binding sites, Site 1 and Site 2. The last few years have seen an increase in high quality cryo-EM structures of IR at different binding conformations, however, the mechanisms of these transitions are still unknown. We have applied different computational methods such as Molecular Dynamics (MD) at different resolutions and enhanced sampling methods such as GO discrete MD to investigate the transitions between the different conformations. We have found that the system and the transitions are too large to resolve using only unbiased atomistic MD simulations due to the high computation demand of the system size and the timescale of the transition. However, the use of coarse- grained force fields and enhanced sampling methods were able to uncover the transitions between the different states and allow for the analysis of important contacts during the transitions. More work is needed to validate these transitions using both atomistic representation and experiments.

P-354
Low-intensity ultrasound only affects cells and tissue through shear

Thomas Combriat[1], Petter Angell Olsen[2], Fabien Barras[1], Sverre Holm[3], Anders Malthe-Sørenssen[1,4], Stefan Krauss[2], Dag Kristian Dysthe[1]
[1] University of Oslo, Department of Physics, Njord Centre, P.O. Box 1048 Blindern, 0316 Oslo, Norway, [2] University of Oslo, Hybrid Technology Hub, Institute of Basic Medical Sciences P.O. Box 1110 Blindern, 0317 OSLO, Norway, [3] University of Oslo, Department of Physics, Electronics section, P.O. Box 1048 Blindern, 0316 Oslo, Norway, [4] University of Oslo, Center for Computing in Science Education, P.O. Box 1048 Blindern, 0316 Oslo, Norway

Low-intensity ultrasound (LIUS) has generated much interest as a possible non-intrusive mechanical stimulus to, among others, treat cancer and direct stem cell differentiation. In order to determine the mechanism that LIUS has on living cells we devised an instrument that can 1) produce longitudinal ultrasonic waves and 2) produce a varying intensity of shear flow in the liquid surrounding adherent cells. We simultaneously measure the shear strain of the cells and live Ca2+ fluorescence imaging before, during and after the ultrasound/shear pulses. We find that during LIUS, Ca2 + release into HeLa, NHFL and BJ cells is proportional to the shear strain magnitude and independent of the pressure. The result has a simple explanation in the huge difference between the bulk and shear moduli of cells and tissue. Our results explain the low reproducibility of studies reporting the biological effects of LIUS: shear motion induced by LIUS that produces the biological effects has not been reported and is difficult to predict.

P-355
Lipid bilayer binding and remodeling characterized by dual-color fluorescence cross-correlation spectroscopy and fluorescence lifetime measurements

Jan Ebenhan[1], Sebastian Daum[1], Caroline Haupt[1], Kirsten Bacia[1]
[1] University of Halle, Germany

Nanodiscs formed by polymers like SMA and DIBMA acting on lipid bilayers are often depicted as structurally similar to those formed with a membrane-scaffold protein. We use dual-color fluorescence cross-correlation spectroscopy (FCCS), single-color FCS and TCSPC-FRET to study solubilization of liposomes by DIBMA. FCCS confirms polymer nanodisc size heterogeneity and exchange of lipids. The sensitivity of the techniques allowed extending the solubilization diagram to lower concentrations than before. We find particle morphologies to be determined not only by polymer-to-lipid ratios, as published for higher concentrations, but also by absolute concentrations. Furthermore, FRET between two lipid dyes is reduced at polymer-to-lipid ratios below those needed for liposome solubilization, suggesting that DIBMA becomes part of the lipid membrane. FCCS is also a useful technique for increasingly quantitative binding analyses of proteins interacting with lipids or membrane receptors.

Poster Presentations
– Session 2 –

P-356
The Function of Rhomboid Intramembrane Protease GlpG is Dependent on the Membrane Environment.
Oskar Engberg[1], David Ulbricht[1], Viola Döbel[1], Verena Siebert[2], Christian Frie[3], Anja Penk[1], Marius K. Lemberg[2,3], Daniel Huster[1]
[1] Institute for Medical Physics and Biophysics, University of Leipzig, Leipzig, Germany, [2] ZMBH Research Group, Ruprecht Karls University Heidelberg, Heidelberg, Germany, [3] Center for Biochemistry, Medical Faculty, University of Cologne, Cologne, Germany

The influence of the membrane environment on large transmembrane proteins is poorly understood. In addition, membrane proteins could alter the membrane dynamics, e.g., the rhomboid intramembrane protease GlpG from E. coli has been proposed to thin its surrounding membrane, possibly explaining the observation that rhomboids diffuse faster than the viscosity limit in thick membranes. To experimentally test the membrane thinning hypothesis, we estimated the thickness of membranes containing differently long deuterated di-saturated phosphatidylcholines (PCs), with and without GlpG using 2H NMR spectroscopy. Surprisingly, GlpG did not influence the membrane thickness of the different PC membranes significantly (C12:0 to C16:0). However, membrane thinning of 1.1Å per leaflet was detected for the E. coli-mimicking membranes containing 16:0/18:1-phosphoethanolamine: 16:0/18:1-phospho-(1'-rac-glycerol) (POPG) at a 3:1 molar ratio. In addition, we tested the transmembrane substrate cleavage kinetics of GlpG in differently thick membranes using a FRET-based substrate cleavage assay. The substrate cleavage velocity of GlpG was dependent on how well the membrane environment hydrophobically matched the thickness of GlpG, with an optimal thickness found for di:14:0-(PC). In conclusion, we suggest specific lipids can enable membrane proteins to thin the surrounding lipid environment, and transmembrane protein function can be drastically altered by the surrounding membrane environment.

P-357
Sequencing-based characterization of site occupancy in functionalized DNA-origami through split-pool barcoding
Enya Engström[1], Elena Ambrosetti[1], Joel Spratt[1], David Fernandez Bonet[2], Ian Torao Hoffecker[2], Ana Teixeira[1]
[1] Karolinska Institutet, Sweden [2] Kungliga Tekniska Högskolan

DNA-origami, the folding for DNA into precise nanoscale structures, is emerging as a platform for controlled and specific drug delivery. Through immobilizing of binders in specific positions or patterns the delivery of the drug can be fine-tuned to precise locations and environments. Therefore, the ability to characterize immobilized binders is vital in using DNA-origami for drug delivery and is currently done using super- resolution imaging. Though these methods yield high resolution images of the structures they only characterize a small population of all structures and additionally have difficulty characterizing more complex and deformable structures. Furthermore, they require extensive training and optimization to yield an accurate characterization. In contrast, our method for characterization of immobilized binders on DNA-origami structures relies on the sequencing of protruding DNA-strands linking the immobilized binders to the structure. By sequentially ligating barcodes through split-pool ligation to the protruding DNA-strands from the DNA-origami we incorporate a structure specific barcode which is used to determine the origin of the strands. The additional incorporation of a position specific barcode in the protruding DNA-strand is then used to trace the exact location of the binder on the structure. Hence, this allows for a fast and simple characterization of entire population of DNA-origami structures, while also enabling characterization of complex and deformable structures. Through this sequencing approach the ability to characterize DNA- origami will also become more readily available and require less training compared to current imaging techniques.

P-358
Identification of Metabolic Traits in Heat Flow Data From Microorganisms of Low Complexity
Karim Fahmy[1], Jana Oertel[1], James Saenz[2]
[1] Helmholtz-Zentrum Dresden-Rossendorf, Bautzner Landstrasse 400, 01328 Dresden, Germany, [2] B CUBE Center for Molecular Bioenegineering, Technische Universität Dresden 01062 Dresden, Germany

Measurements of metabolic heat flow of organisms have reached unprecedented sensitivity. For microbial cultures, heat flow scales directly with the growth rate which is obtained by conventional methods only at very low signal to noise ratio. Isothermal Microcalorimetry allows for the first time the dedicated mathematical modelling of growth metabolism relations. We have demonstrated that „canonical" heat flow patterns are conserved across species.[1] Here, we address the primordial character of the underlying metabolic activities by studying both eukaryotic and prokaryotic parasitic cells with lower complexity of their metabolic pathways than free-living bacteria. The data reveal the unexpected validity of a long-lasting hyperbolic nutrient dependence beyond exponential growth. A novel analysis tool has been developed which quantitates the influence of environmental factors, such as temperature and diet on metabolic parameters. Using a genetically minimal bacterial cell derived from mycoplasma (about 10% of the genome size of E.coli), we examine the lipid diet-dependent metabolic activity.[2] Modelling heat flow data from such a „minimal metabolism" provides a benchmark for the analysis of more complex systems. The evaluation is currently implemented as a web- based tool for researchers to analyze and compare heat flow data across labs.

P-359
Structural insights into opposing actions of neurosteroids on GABAA receptors
Chen Fan[1], Dagimhiwat Legesse[2], Yuxuan Zhuang[1], Colleen Noviello[2], Ryan Hibbs[2], Reba Howard[1], Erik Lindahl[1,3]
[1] Stockholm University, Sweden [2] UT Southwestern Medical Center, [3] KTH Royal Institute of Technology

GABAA receptors mediate most of the fast inhibitory signaling in the brain and are prolific drug targets. Endogenous neurosteroids are derived from cholesterol and some of them potently modulate GABAA receptors activity. Our current understanding of GABAA receptor modulation by neurosteroids comes from structural, electrphysiology and photolabeling studies, these informations paint the picture of where the neurosteriods could bind, but the modulation mechanisms are still poorly understood. As a complement for the conventional structural biology methods, Molecular dynamics (MD) simulations allow the study of the dynamics of protein during the time course. We used MD simulations to study the mechanisms of neurosteriod modulation, based on the recent CryoEM structures solved by our collaborators. The positively modulatory neurosteroid allopregnanolone stably binds at the intracellular end of the transmembrane domain between the β-α interface during the simulation. The presence of allopregnanolone at the intracellular end of the transmembrane domain influences the conformational state and dynamics of the entire receptor including changes from the pore to the outer ECD consistent with increased activity. Pregnenolone sulfate (PS) and dehydroepiandrosterone sulfate (DHEAS) are two well studied negatively modulatory neurosteriods, we performed the simulations of them bound in two different sites, TMD or pore site, and they only stably bind inside the pore during the simulation. Combined with the functional experiments we conclude the negatively modulatory neurosteriods function as the pore blocker. In summary, our MD simulation research provide the insight about the mechanism of the distinct effects of neurosteriods to GABAA receptors.

Poster Presentations
– Session 2 –

P-360

How the Dynamics of an Ultralong Ceramide Challenges our Understanding of the Human Skin Barrier as a Rigid Layer

Ferdinand Fandrei[1], Tomáš Havrišák[2], Lukáš Opálka[2], Oskar Engberg[1], Albert A. Smith[1], Petra Pullmannová[2], Veronika Ondrejčeková[2], Kateřina Vávrová[2], Daniel Huster[1]
[1] Institute of Medical Physics and Biophysics, University of Leipzig, Leipzig, Germany, [2] Skin Barrier Research Group, Faculty of Pharmacy, Charles University, Hradec Králové, Czech Republic

The most important layer for the barrier function of the human skin is the Stratum Corneum (SC) protecting our body from our surroundings. In our study we investigated the intercellular lipid matrix of the SC with special focus on the ultralong ceramide called Cer[EOS]. This molecule is required to form the LPP (long periodicity phase) - a membrane pattern being essential for the barrier function of our skin. As Cer[EOS] and the LPP are reduced in psoriasis patients its molecular behavior and the lipid organization in the LPP are of special interest for us. We investigated a LPP model using 2H/13C solid-state NMR spectroscopy. Cer[EOS] was deuterated at 4 different segments namely the sphingosine chain, the upper and lower part of the ultralong acyl chain as well as the linoleoyl moiety. Each molecule is measured in a separate mixture containing only one deuterated moiety each. The 2H NMR-data shows that while most lipids – as previously suggested - form a highly ordered crystalline state, Cer [EOS] was much more dynamic. The lower end of the ultralong Cer[EOS] acyl chain, the sphingosine moiety and the linoleic acid chain were found to undergo large amplitude motions suggesting them to be much more fluid or even isotropically mobile at skin temperature. From these surprising observations combined with X-Ray and neutron diffraction data we propose an updated model of the LPP with alternating rigid and rather fluid leaflets. This arrangement could be necessary for a fully functional barrier, which is restricted in psoriasis patients.

P-361

α-Synuclein Cooperativity in Lipid Membranes Binding

Noemi Ferrante Carrante[1], Marija Dubackic[1], Katarzyna Makasewicz[1], Stefan Wennmalm[2], Xiaoyan Liu[1], Tova Hermodsson[3], Sara Linse[4], Emma Sparr[1]
[1] Division of Physical Chemistry, Department of Chemistry, Lund University,, Sweden, [2] Department of Applied Physics, Biophysics Group, SciLifeLab, Royal Institute of Technology- KTH, Sweden, [3] Lund University, [4] Division of Biochemistry and Structural Biology, Department of Chemistry, Lund University, Sweden

α-Synuclein is a 140 a.a. long and intrinsically disordered protein localized in vivo in synapses. As far as is currently known, it is implied in still- poorly-understood healthy functions and also found as the main component of aggregates in Lewy bodies and Lewy neurites, hallmarks of the Parkinson's disease. In both cases, the interaction with lipid membranes is crucial. In this study we investigate the interaction between α-Synuclein and model systems made of DOPC:DOPS (7:3 molar ratio) in a wide range of conditions in order to evaluate the cooperativity of α-Synuclein in lipid membranes binding. Cooperativity is a widespread concept in biochemistry since it is related to many metabolic pathways, signaling and transport processes. In a cooperative event the binding of one molecule onto a surface enhances the affinity of the next binding event on the same surface, resulting in a non-random distribution. Cooperative binding of α-Synuclein in lipid membranes has previously been reported for the same model system in one solution condition. Here we systematically changed parameters such as pH, ionic strength, salt composition and α-Synuclein amino acidic sequence (mutant α-Synuclein H50Q) in order to evaluate the cooperativity, by means of Confocal Laser Scanning Microscopy, Circular Dichroism and Fluorescence Cross Correlation Spectroscopy. Both small and giant unilamellar vesicles were used as model systems. The binding was observed under all the conditions investigated and, in addition, for some of them Fluorescence Cross Correlation Spectroscopy and Confocal Microscopy proof a positive cooperative binding in excess of vesicles.

P-362

Fine tuning of A2AAR conformational dynamics upon ternary complex formation with mini-Gs viewed by NMR in solution

Guillaume Ferré[1,2], Kara Anazia[1], Larissa Silva[1], Naveen Thakur[1], Arka Ray[1], Matthew Eddy[1]
[1] University of Florida, [2] University of Toulouse (presently), France

G protein-coupled receptors (GPCRs) play an important role in human cell communication and are prominent drug targets. Deciphering the molecular mechanisms underlying GPCR signal transduction, from ligand binding to G protein interaction, may provide information to design novel therapeutics. Several works highlighted the importance of conformational plasticity in GPCR functions but more data are needed to better understand the allosteric connection between the bound G protein and the drug-binding site. Here, we investigated the conformational dynamics that underly ligand-induced ternary complex formation with the human A2A adenosine receptor (A2AAR) and an engineered Gs protein (the mini- Gs). Leveraging uniform stable isotope-labeling of the receptor and NMR in solution, we monitored A2AAR conformation at multiple sites throughout the receptor in complexes with an agonist and mini-Gs and with an agonist alone. While previous works revealed widespread changes in A2AAR upon agonist binding, we observed that ternary complex formation produces more subtle differences in the receptor conformational state. These data point to a model whereby agonist binding alone populates a receptor conformation resembling the final activation state. However, we observed that an A2AAR "hot spot" connecting the binding sites of the ligand and the G protein is highly dynamic in the ternary complex. Our data thereby indicates a mechanism for allosteric coupling between these two remote sites of the receptor that involves structural plasticity of the "toggle switch" tryptophan located in the A2AAR "hot spot".

P-363

Expression and purification of connexin 26 for studies of cooperative ion channel behavior.

Manuel Fink[1], Lena Reichert[1], Claudia Steinem[1,2]
[1] Georg-August-University Göttingen, Germany [2] Max Planck Institute for Dynamics and Self-Organization

Ion channel proteins are a vital part of life. They enable transport of metabolites and ions across biological membranes. Many of these channels work in a collective manner. One important class of ion channels are connexins. These proteins form cell-cell connections between adjacent cells. The conducting properties of these channels can be investigated by voltage clamping. In this project, Connexin 26 is overexpressed and purified using an insect-cell baculovirus based expression system. For the measurements, the purified protein is reconstituted into black lipid membranes. With these experiments, the number of channels contributing to one measurement is not evident, which leads to issues when recording multiple channels simultaneously. The problems arising from this fact are usually circumvented by performing measurements of a single channel. By determining the properties of a single connexon, they can be compared to those from a measurement of multiple channels in concert. Additionally, it will be attempted to quantify the number of channels in one measurement to gain a more complete set of data from which possible cooperativity can be investigated.

Poster Presentations
– Session 2 –

P-364
Reconstituting ATP synthase and monitoring its activity in photoacid-containing vesicles
Hendrik Flegel[1], Amelie J. Meyer[1], Julia Bock[1], Alexa Heuer[1], Tobias Weege[1], Claudia Steinem[1,2]
[1] Georg-August-Universität, Institute of Organic and Biomolecular Chemistry, 37077 Göttingen, Germany, [2] Max Planck Institute for Dynamics and Self Organization, 37077 Göttingen, Germany

The ATP synthase is one of the most important protein complexes and responsible for synthesizing the energy currency ATP from ADP and phosphate. This transmembrane protein is a molecular motor driven by an electrochemical gradient called proton motive force (pmf). Since the pmf is composed of the pH difference ΔpH and the electric potential Δφ across the respective membrane, ATP synthesis can be induced by a proton gradient. While the proton pathway within the protein during ATP synthesis is rather understood, the question of how the protons reach the protein entrance is still elusive. A new experimental approach enables the control of proton release in time and location. We use the photoacid 8- hydroxypyrene-1,3,6-trisulfonate (HPTS) exhibiting different pKa values in the ground and in the first excited state. Hence, upon light excitation the highly acidic photoacid acts as proton source. Placing the water soluble HPTS in the vesicle lumen or the amphiphilic derivative C12-HPTS within the vesicle membrane should allow differentiation between the transfer of bulk and interfacial protons towards the ATP synthase. The highly sensitive luminescence spectroscopic luciferin-luciferase assay enables the determination of the protein activity as a function of proton localization.

P-365
Measuring lipid order to assess lipid nanoparticle stability, cell membrane permeability and membrane drug interaction
Nicolas Färber[1,2], Sophie Mauritz[2], Jonas Reitler[2], Anna Nolde[2], Julian Schäfer[1], Christoph Westerhausen[1,2,3]
[1] Experimental Physics I, Institute of Physics, University of Augsburg, Universitätsstr. 1, 86159 Augsburg, Germany, [2] Physiology, Institute of Theoretical Medicine, University of Augsburg, Universitätsstr. 2, 86159 Augsburg, Germany, [3] Center for NanoScience (CeNS), Ludwig- Maximilians-Universität Munich, 80799 Munich, Germany

We here report on reversible and irreversible changes of the lipid order in synthetic and biological systems that open up new applications. First, the lipid order within lipid nanoparticles changes as function of time during storage indicating irreversible structural changes. This allows for assessing in-situ the temperature stability in drying processes such as spray drying but also the long-time storage stability of formulations. Second, the permeability of cellular membranes is directly related to the plasma membrane order. This can facilitate permeabilization and transfection protocols. Third, we found that lipid phase transitions of cellular membranes are strongly influenced by short and long-time exposure of the breast cancer drug tamoxifen. These results might yield a different view on working mechanisms and long-time adaptation effects of membrane targeted drugs. All observations were obtained using the same measurement principle: The fluorescent analysis of solvatochromic dyes over wide temperature ranges. We are convinced that these observations will inspire researchers across different working fields in biophysics to include lipid order measurements in their studies. For this we provide detailed insight into the measurement procedure and introduce a custom-made device that facilitates this kind of studies.

P-366
Pore formation by melittin in porous silica-supported lipid membranes
Nicola Garlet[1], Björn Agnarsson[1], Simon Niederkofler[1], Petteri Parkkila[1], Elin Esbjörner Winters[1], Fredrik Höök[1]
[1] Chalmers University of Technology, Sweden

Small amyloid peptide oligomers are considered the primary neurotoxic species responsible for neurodegenerative disorders such as Alzheimer's and Parkinson's diseases. These amyloid oligomers are prone to form pore-like assemblies in the plasma membrane of brain cells, which is likely to contribute to the pathogenesis. To investigate the formation of lipid-membrane pores caused by peptides, we have used supported lipid bilayers (SLBs) formed on porous silica – a novel substrate which in contrast to SLBs on planar glass allows the bordering of the bilayer by two aqueous regions, thereby better resembling the natural cell membrane. Quartz crystal microbalance with dissipation monitoring (QCM-D) revealed that silica having 6-nm wide pores can be efficiently coated by a bilayer. Furthermore, total internal reflection fluorescence (TIRF) microscopy was used to establish a protocol for efficient trapping of a fluorescent dye, calcein, which from fluorescence recovery after photobleaching (FRAP) analysis appears not to diffuse laterally in the porous substrate. Pore formation was investigated using a model pore-forming peptide, melittin. Onset of calcein leakage allows the study of pore formation induced by melittin, which appears to be initiated at a peptide coverage significantly lower than that required to induce microscopically visible structural alteration of the SLB.

P-367
Investigating lysosomal membrane proteins using SSM-based electrophysiology: Improving amplification and accessibility
Cecilia George[1], Andre Bazzone[1], Maria Barthmes[1], Nina Brinkwirth[1], Niels Fertig[1]
[1] Nanion Technologies, GmbH, Germany

Solid supported membrane-based electrophysiology (SSM-E) offers novel approaches for electrophysiological recordings from lysosomal membrane proteins such as transporters, ligand-gated and leak ion channels in their native membrane environment. The method relies on the adsorption of any membrane, native, cell culture-derived or organellar, to a lipid coated electrode, i.e the solid supported membrane, and the direct current read-out caused by the capacitive charging of the membranes. The 3 mm diameter electrode entails a >1000-fold amplification of the currents compared with conventional patch clamp, allowing for the measurements of low-conducting membrane proteins, such as transporters. The fact that intracellular membranes can also be accessed by SSM-E, their accessibility for investigation and characterization drastically improves. Here, we present a study on TMEM175 channels residing in lysosomes using SSM-E. Parameters such as conductivity, ion selectivity, pH- dependence and pharmacology were investigated. Specifically, the dose-dependent effect on TMEM175 exerted by blockers, i.e. Zn2+ and 4-AP, and enhancers, i.e. DCPIB and Arachidonic acid, was investigated.

Poster Presentations
– Session 2 –

P-368
Ligand Flip-Flop on a GPCR Surface
Cristina Gil Herrero[1,2], Sebastian Thallmair[1]
[1] Frankfurt Institute for Advanced Studies, Germany, [2] Goethe University of Frankfurt, Germany

G-protein-coupled receptors (GPCRs) are transmembrane proteins that regulate physiological responses to external stimuli playing a crucial role as therapeutic targets. Thus, they are the most targeted protein family by drugs such as salmeterol and salbutamol, employed in the treatment of pulmonary diseases by activating the GPCR β2-adrenergic receptor (β2AR). In this study, using coarse-grained Martini 3 molecular dynamics simulations we explore the behaviour of the two agonists in the presence of their target protein, β2AR. Our results show that the ligands exploit the protein surface to change leaflets (flip-flop), traversing the membrane more easily. Salmeterol and salbutamol showed 70% and 50% of flip-flops occurring on β2AR surface along unbiased trajectories, respectively. Furthermore, we analyzed the most populated flip-flop path on the β2AR surface and conducted umbrella sampling calculations starting from snapshots of free simulations. The resulting potentials of mean force, despite differences of pathways and between drugs, exhibit a significantly reduced energy barrier (>15 kJ/mol) compared to the pure membrane. Besides, by exchanging the ligands, trends indicate that the path is the most relevant feature influencing the energetics of the flip-flop. Our findings provide insights into ligand permeation mechanisms and could contribute to the design of drugs requiring cell permeation.

P-369
Unravelling Bcl-2 proteins´ functioning at mitochondrial membrane level
Gerhard Gröbner[1], Tamas Milan Nagy[1], Luke Clifton[2], Jörgen Åden[1], Hanna Wacklin-Knecht[3], Ameeq Ul Musthaq[1]
[1] Department of Chemistry, Umeå University, Sweden, [2] ISIS Pulsed Neutron and Muon Source, Rutherford Appleton Laboratory, Harwell Science&Innovation Campus, Didcot, Oxfordshire, OX11 OQX, UK., [3] European Spallation Source ERIC, ESS, P.O. Box 176, SE-22100 Lund, Sweden

Programmed cell death (apoptosis) is essential for human life. Its intrinsic apoptotic pathway is tightly controlled by the B-cell lymphoma 2 (Bcl-2) protein family regulating the permeability of the mitochondrial outer membrane (MOM) and any release of apoptotic factors. Any imbalance can cause disorders with cancer most prominent, where upregulation of cell protecting (anti-apoptotic) Bcl-2 members such as the Bcl-2 membrane protein itself plays a notorious role by blocking cell-killing – via membrane perforation – proteins like Bax. We use neutron reflectometry (NR) on MOM-mimicking lipid bilayers and solid state/liquid state NMR spectroscopy to unravel the molecular basis driving opposing proteins to interact with each other at the MOM; a mechanism which is only scarcely understood yet due to lack of high-resolution structural insight. Based on our central hypothesis that Bcl-2 drives its cell-protecting function at a membrane-embedded location as revealed by NR, we determine the structure of human, fully functional Bcl-2 protein in its membrane environment near physiological conditions by combining solution and solid-state NMR; we use NR to study the kinetics and lipid/protein pore assembled upon binding of Bax to mitochondrial membranes and its membrane destroying activities there; and we unravel the nature of direct interaction between Bcl-2 and Bax to neutralize each other. Knowledge generated here, will be indispensable in understanding the regulative function of the Bcl-2 family at mitochondrial membranes.

P-370
Deciphering CRAC gating through MD simulations
Carlo Guardiani[1], Alberto Giacomello[1]
[1] Sapienza University of Rome Italy

The Calcium Release Activated Calcium channel (CRAC) is a key player of the immune system and its mutations cause severe immuno-deficiencies. The gating mechanism of this channel is highly controversial and the early model based on the rotation of pore helices has been recently questioned. In order to address this issue, we took advantage of the availability of the closed state structure and the structure of a constitutively open mutant. We could thus ran Targeted Molecular Dynamics simulations to characterize the conformational transition from the closed to the open state. The simulations highlighted a hydrophobic gating mechanism whereby the pore becomes functionally occluded by a vapour bubble even in the absence of steric block. Moreover, we highlighted a complex allosteric transition propagating from the outermost ring of helices to the innermost ring delimiting the pore. The final event of the opening transition is a flipping of the side-chain of H206 that creates an empty space behind the hydrophobic region of the pore that can move back expanding its radius. This pattern confirms the "steric brake hypothesis" that was put forward based on mutagenesis experiments.

P-371
Structural basis of Episodic Ataxia type 6
Albert Guskov[1], Emanuela Colucci[1], Zaid Anshari[1], Mijer Patino-Ruiz[1], Mariia Nemchinova[1], Jacob Whittaker[1], Dirk Slotboom[1]
[1] University of Groningen, Netherlands

Episodic ataxias (EA) are debilitating types of rare neurological conditions leading to motor impairment. EA type 6 is associated with the mutation of an essentially conserved proline into an arginine, located in the glutamate transporter hEAAT1. In vitro studies hinted that this mutation causing a reduction in the substrate transport, together with an increase in the anion conductance. It was hypothesised that it might be caused by the removal of a kink formed by Pro residue in the transmembrane helix 5. In here I will present the combination of SSM anion conductance measurements, the cryo-EM structure of the mutant and molecular dynamics simulations which revealed that the Pro to Arg mutation does not abolish the kink, but indeed leads to the increased anion conductance, which is most likely caused by the widening of an anion channel caused by interactions of Arg side chain with the phospholipid of a bilayer.

Poster Presentations
– Session 2 –

P-372
Investigation of surface-active therapeutics on lipid monolayers
Thomas Gutsmann[1,2], Carla Maximiliane Neitzke[1], Priscillia Masalle[1], Susanne Homolka[1], Christian Nehls[1]
[1] Research Center Borstel, Germany [2] Center for Structural Systems Biology

Lipid membranes consist mostly of a bilayers. One of the few exceptions is the membrane lining the lung alveoli. This consists of a monolayer, the pulmonary surfactant. The function of the lipid monolayer is to reduce the surface tension and thus to prevent the alveoli from collapsing on exhalation. Drugs that are administered via the respiratory tract are therefore often examined with lipid monolayers prepared on the film balance. A subphase and a lipid monolayer are used to mimic the air-water interphase in the lung. The lipid monolayer consists of dipalmitoylphosphatidylcholine (DPPC), which is the main component of the pulmonary surfactant. To investigate the interaction between therapeutic drugs, the standard has been that the drug is injected under the surfactant monolayer into the subphase. However, this does not correspond to reality, as the active drug is then incorporated into the lipid monolayer from the water phase. In contrast, asthma medication, for example, is inhaled and the drug is incorporated into the membrane from the air phase. We have developed a set-up to nebulise the drug onto the lipid monolayers. We compared the results from the injection method with those from the nebulisation method for salbutamol and tyloxapol. It turned out that the methods do not lead to the same results. Consequently, the application of drugs to monolayers must be adapted depending on the scientific problem.

P-373
Assembly mechanisms of the plant protein remorin into membrane nanodomains
Zeren Xu[1], Marie-Dominique Jolivet[2], Anthony Legrand[1], Antoine Loquet[1], Véronique Germain[2], Sébastien Mongrand[1], Birgit Habenstein[1]
[1] Institute of Chemistry and Biology of Membranes & Nano-objects, CBMN/IECB, CNRS, University Bordeaux, Bordeaux INP, UMR 5248, Pessac, France, [2] Laboratoire de Biogenèse Membranaire, CNRS, Université Bordeaux, UMR 5200, Villenave d'Ornon, France

Plant-specific REMORINs (REMs) are crucial proteins involved in plant defense against viral propagation by regulating cell-to-cell connectivity. They are tightly associated with the clustering of nanodomains at the plasma membrane, driven by specific protein-protein and protein-lipid interactions. REMs can be classified into 6 groups, containing a membrane-associating C-terminal anchor (REMCA), neighboring a coiled-coil domain that is followed by an intrinsically disordered N-terminal region (IDR). We have recently contributed to understanding the precise underlying mechanisms of nanodomain clustering by REMs, involving interactions of REMCA with specific phosphoinositolphosphates (PIPs). Moreover, our data have revealed that StREM1.3's nanodomain clustering depends on the phosphorylation status in the IDR and REM's oligomerization potential. We then addressed the role of the structural divergence between the different REM groups and found that REMs rely on diverse sequence motif arrangements and REMCA sequences. We investigate REM's structural and dynamic organization based on domain-specific analysis and considering the context of the three-domain protein. Bioinformatics analysis suggests implications of motif distribution in the regulation of nanodomain clustering. Based on 3D structure determination of REMCAs of different REM groups by NMR, we discovered the REMCA structural diversity, further highlighting the role of sequence adaptation and structure modulation to control membrane association. Towards re-contextualization, we have then extended our analysis to investigating the molecular architectures of stable REM multimers, searching for the most energy-favorable coiled-coil structures.

P-374
Engineering Annexin A3 to Mimic Annexin A4: Insights into Plasma Membrane Repair Mechanisms
Ali Asghar Hakami Zanjani[1], Anna Mularski[1], Anne Sofie Busk Heitmann[2], Catarina Dias[2], Michelle Ege Muller[1], Kenji Maeda[2], Jesper Nylandsted[2], Adam Cohen Simonsen[1], Himanshu Khandelia[1]
[1] Department of Physics, Chemistry and Pharmacy, University of Southern Denmark, Odense, Denmark, [2] Danish Cancer Society Research Center, Copenhagen, Denmark

Annexins are a family of calcium-dependent peripheral membrane proteins that play a crucial role in regulating various cellular processes, including plasma membrane repair (PMR). Annexin A4 (ANXA4) is known to form trimeric structures on the membrane surface, which induce high negative curvature and accelerate PMR. However, Annexin A3 (ANXA3), despite being highly homologous to ANXA4, does not form trimers on the membrane surface. We investigated the factors driving annexin-mediated PMR by using molecular dynamics simulations to engineer a mutant form of ANXA3 (ANXA3-MUT) that can trimerize and induce high curvature on the membrane surface, similar to ANXA4. Atomic force microscopy images confirmed the formation of crystalline arrays of the engineered protein on a supported lipid membrane. However, in vivo experiments showed that ANXA3-MUT failed to accumulate near the site of a membrane lesion in laser-punctured cells and was not recruited to repair the lesion, unlike ANXA4. Our investigation indicates that the membrane repairing property of trimer-forming annexins depends not only on trimer formation and curvature induction but also on high membrane binding affinity. These findings provide valuable insights into the factors driving annexin-mediated PMR and may facilitate the development of novel therapeutics targeting PMR in diseases such as cancer.

P-375
Structure determination of the malaria parasite's lactate transporter mutant PfFNT G107S with bound small molecule inhibitor
Christian Hansen[1], Eric Beitz[1]
[1] Christian-Albrechts-University of Kiel, Germany

We discovered the missing plasmodial lactate/H+ symporter PfFNT and validated it as a novel drug target for the potential treatment of malaria. PfFNT is a member of the strictly microbial, homopentameric protein family of formate-nitrite transporters. Screening of the 'malaria box' compound library with unknown antimalarial targets yielded two hits (MMV007839, MMV000972), which block PfFNT and kill Plasmodium falciparum malaria parasites at nanomolar concentrations. Under sub-lethal selection of cultured parasites, MMV007839 lead to the emergence of a PfFNT G107S mutant with severely decreased inhibitor affinity. Further development of our inhibitor class of pentafluoro-3-hydroxy-pent-2-en-1- ones yielded BH267.meta that circumvents the resistance mutation and blocks both, wildtype PfFNT and the G107S mutant, with similar efficiency. Alongside with the establishment of a yeast-based phenotypic assay for rapid high-throughput inhibitor screening, we aim to solve the binding mode of BH267.meta to PfFNT G107S for rational drug development. Therefore, we employ high-yield cell-free protein synthesis and reconstitution of PfFNT G107S into nanodiscs. The procedure avoids the need for solubilization from cellular membranes, and rapidly yields milligram amounts of purified protein. Transmission electron microscopy of cell-free PfFNT G107S showed high homogeneity and particle density. In parallel, we use Pichia pastoris for the cell-based production of PfFNT G107S. Protein solubilization and purification trials indicate the successful extraction of thermostable pentamers. The inhibitor-bound protein structure will visualize the binding pose and mode of action of PfFNT G107S inhibitors, and will facilitate further development of small molecule antimalarials.

Poster Presentations
– Session 2 –

P-376
Monitoring the folding and misfolding of amyloid beta peptides in a lipid membrane environment using SEIRA spectroscopy

Saskia Heermant[1], Jacek Kozuch[1]
[1] Freie Universität Berlin, Germany

Alzheimer's disease affects tens of millions of people worldwide and is tightly associated with amyloid beta (Aβ) peptides that misfold at membrane interfaces to form insoluble fibrils. These fibril aggregates lead to physical and electrostatic changes in the membrane and eventually to dysfunction or cell death, which over time causes symptoms such as cognitive decline. To further increase our knowledge of the misfolding trajectory, it is particularly important to extent experimental studies to techniques that combine various, necessary aspects relevant to peptide-membrane interactions. Towards this goal, we investigate the Aβ (mis)folding process at membrane interfaces using surface-enhanced infrared absorption (SEIRA) spectroscopy, which supports the simultaneous electrochemical and spectroscopic analysis at tethered lipid membrane systems. To evaluate the resistance and capacitance, and thus the quality of the membrane systems, electrochemical impedance spectroscopy (EIS) is performed using the gold surface as an electrode. By focusing on the amide I and amide II bands in the SEIRA spectra, the changes in the secondary structures of Aβ during its interaction with the membranes are followed. We observe β-sheet structures as intermediates towards a potentially misfolded state, but the amount varies depending on the architecture of the membrane, such as the content of negatively charged lipids or the tethering lipids at the gold surface. This demonstrates the usefulness of SEIRA spectroscopy towards understanding how the membrane influences the Aβ folding.

P-377
Mechanisms of lipid membrane perturbations by antimicrobial polymers and a misleading side-effect

Maria Hoernke[1,2], Shuai Shi[1], Katharina Beck[1]
[1] Albert-Ludwigs-Universität , [2] Martin-Luther-Universität, Germany

The interaction of antimicrobial peptides or biomimetic polymers with lipid membranes can cause a variety of membrane perturbations. Systematic and mechanistic investigations are therefore crucial for the rational design of biomimetic compounds in the search for alternatives to classical antibiotics or therapeutic agents enhancing drug delivery. Using various fluorescence methods and microcalorimetry, my group thoroughly characterizes membrane perturbations, most importantly membrane leakage induced by antimicrobial, biomimetic polymers. With a focus on varying lipid compositions, we distinguish different leakage mechanisms and find that changes in leakage mechanisms in different membranes constitute an important contribution to selectivity for different microbial species or cells. Most importantly, we establish membrane fusion in model vesicles to be a common leakage mechanism that is less relevant in microbes. Even worse, vesicles composed of phosphatidylglycerol and phosphatidylethanolamine commonly used to model bacterial membranes are biased for this leaky fusion, potentially leading to misinterpretation in widespread model studies. Various implications of leaky fusion and vesicle aggregation are discussed alongside strategies to prevent them. A positive aspect of our findings is that leaky fusion is probably useful for drug delivery applications.

P-378
Entropic barrier of water permeation through single-file channels

Andreas Horner[1], Johann Wachlmayr[1], Gotthold Flaeschner[2], Kristyna Pluhackova[3], Walter Sandtner[4], Christine Siligan[1]
[1] Institute of Biophysics, Johannes Kepler University Linz, Gruberstr. 40, 4020 Linz, Austria, [2] Department of Biosystems Science and Engineering, Eidgenössiche Technische Hochschule (ETH) Zürich, Mattenstr. 26, 4058 Basel, Switzerland, [3] Stuttgart Center for Simulation Science, Cluster of Excellence EXC 2075, University of Stuttgart, Stuttgart, Germany, [4] Center of Physiology and Pharmacology, Institute of Pharmacology, Medical University of Vienna, Vienna, Austria

Facilitated water permeation through narrow biological channels is fundamental for all forms of life. This process involves dehydration of bulk water entering the single-file region and hydrogen bond formation with channel lining amino acid residues. Despite its significance in health and disease as well as for biotechnological applications (i.e., the design and engineering of artificial water channels), the energetics of water permeation are still elusive. Gibbs free energy of activation is composed of an enthalpic and an entropic component. Whereas the enthalpic contribution is readily accessible via temperature dependent water permeability measurements, estimation of the entropic contribution requires information on the temperature dependence of the rate of water permeation. Here, we estimate, by means of quantitative single channel permeability values and accurate activation energy measurements of water permeation in combination with molecular dynamics simulations of water passage, probing the probability of recrossing the energy barrier, the entropic component of the activation barrier for water permeation through Aquaporin-1. Thereby the calculated value for $\Delta S^{\ddagger} = 2.01 \pm 0.82$ J/(mol·K) links the activation energy of 3.75 ± 0.16 kcal/mol with its efficient water conduction rate of ~10^10 water molecules/second. This analysis can be extended to other biological and artificial water channels to understand the variability of the energetic contributions due to different channel architectures.

P-379
Vesicle Budding relieves Lysolipid-induced Asymmetry Stress: How it starts and why it ends

Lisa Hua[1], Michael Kaiser[1], Heiko Herrklotz[1,2,3]
[1] Institute of Pharmaceutical Sciences, University of Freiburg, Germany, [2] Signalling Research Centers BIOSS and CIBSS, Freiburg, Germany , [3]Leslie Dan Faculty of Pharmacy, University of Toronto, Canada

Asymmetry stress is a regulator in membrane remodeling processes and is discussed as a mode of action of various antimicrobial peptides. It describes the intrinsic area mismatch between the inner and outer leaflet in the membrane, which can result from asymmetric partitioning of molecules that have a low transbilayer diffusion rate, such as lysolipids (LPC). To deal with asymmetry stress, different mechanisms have been reported or hypothesized, such as micellar solubilization, staying out, vesicle budding and many more. The question is, which relaxation mechanisms are actually pursued and why. Vesicle budding induced by LPC has been studied qualitatively with giant unilamellar vesicles, but the studies lack a quantitative perspective which is needed to assign the contribution of the relaxing mechanism to the asymmetry stress. Using asymmetric flow field-flow fractionation (AF4), we were able to establish an assay quantifying the fraction of lipid budding off from large unilamellar vesicles (LUVs). We discovered that it needs only astonishingly little LPC in the outer leaflet (2 mol%) to trigger budding. The extent of budding depends, above a threshold, not on the amount of LPC added. Instead, it is limited by the excess surface stored in the initial LUV. As the vesicle becomes ideally spherical, a hypertonic vesicle interior opposes further budding and further LPC is prevented from entering the membrane. Our studies show how vesicle budding is an effective but limited mechanism to relieve asymmetry stress. Furthermore, it provides insights on the effect of LPCs and other membrane-impermeant amphiphiles on the membrane.

Poster Presentations
– Session 2 –

P-380
Investigating Nanodiscs as a Membrane Protein Environment
Veera Hägg[1], Shreyas Kaptan[1], Tomasz Rog[1], Ilpo Vattulainen[1]
[1] University of Helsinki, Finland

Nanodiscs are a very useful model system for elucidating the structure and properties of membrane proteins. The challenge is the interpretation of these data. Although nanodiscs provide proteins with an operating environment, it is not at all clear what operating environment it corresponds to, or whether it corresponds to any of the conditions in which membrane proteins function in native membrane structures. The first goal of this work is to find out with atomic-level computer simulations, both in terms of structure and dynamic behavior, what operating environment membrane proteins experience when they are in nanodiscs, and how it differs from native GUV-like membrane structures, when the composition of the membranes remains the same. Our results confirm previous observations about the properties of protein-free nanodiscs, but supplement the insight they provide into the effect of proteins in this operating environment. The main goal of this work is to find out how the structure, conformation and dynamic behavior of membrane proteins differ when their operating environment is not the native membrane environment but a nanodisc with the same composition. The results, based on a systematic machine learning analysis of the simulation data, give rise to a fascinating picture, highlighting both the good and the bad sides of nanodiscs.

P-381
TRPM7 ion channel-coupled protein kinase mediates AKT-dependent signaling in human immune cells and leukemia cells Birgit Hoeger[1], Wiebke Nadolni[2], Kilian Hölting[2], Sarah Hampe[2], Marco Fraticelli[2], Nadja Zaborsky[3,4], Roland Immler[5], Vladimir Chubanov[2], Roland Geisberger[3,4], Richard Greil[3,4], Markus Sperandio[5], Inrid Boekhoff[2], Thomas Gudermann[2], Susanna Zierler[1,2]
[1] Institute of Pharmacology, Johannes Kepler University Linz, Austria, [2] Walther Straub Institute of Pharmacology and Toxicology, Ludwig- Maximilians-University Munich, Germany, [3] Department of Internal Medicine III, Oncologic Center, Paracelsus Medical University, Salzburg, Austria, [4] Salzburg Cancer Research Institute, Salzburg, Austria, [5] Walter Brendel Centre of Experimental Medicine, Institute of Cardiovascular Physiology and Pathophysiology, Ludwig-Maximilians-University Munich, Germany

Ion channels of the TRP family are crucial for cellular homeostasis. TRPM7 holds a unique position as ion channel-coupled protein kinase, with a ubiquitous expression pattern. Besides its ability to conduct divalent cations such as Mg2+and Ca2+, it drives intracellular signaling by a constitutively active alpha-kinase which is dependent on a functional ion channel. TRPM7 channel and kinase have been identified to shape immune homeostasis, platelet function, and a number of signaling pathways facilitating cell activation, proliferation, migration and differentiation in various cell types. Previously suggested molecular effectors downstream of TRPM7 signaling include the PI3K/AKT pathway. We study the role of TRPM7 channel and kinase in human immune cells and leukemia cells, by a variety of approaches spanning electrophysiology, CRISPR/Cas-9 genome editing and molecular biology methodologies. To understand TRPM7 ion channel and kinase-dependent signaling, we apply pharmacological inhibitors and genome-editing approaches. We found that genetic knock-out or inactivation of TRPM7 kinase diminishes phosphorylation capacities of AKT, affecting a number of related signaling networks and highlighting the AKT signaling hub as molecular target of TRPM7 kinase function in these cell systems. Downstream of AKT, we could link this to induction of cell proliferation and immune cell activation. Altogether, we confirm the important role of TRPM7 channel-kinase in AKT-mediated signaling, driving pro-inflammatory and pro-activatory responses of human immune cells and leukemia cells.

P-382
Enzymatically Triggered Lipid Conjugation of Membrane Active Peptides
Alexandra Iversen[1], Johanna Utterström[1], Robert Selegård[1], Daniel Aili[1]
[1] Laboratory of Molecular Materials, Division of Biophysics and Bioengineering, Department of Physics, Chemistry and Biology, Linköping University, Sweden

Liposomal drug delivery systems are widely used to improve drug pharmacokinetics, but they often suffer from slow and non-targeted release of the active pharmaceutical ingredient (API). Release kinetics can be modulated using membrane-active peptides, but controlling the interactions between the peptides and lipid membranes is challenging. Here we show a novel enzyme-mediated strategy for the conjugation of a de novo designed membrane-active peptide to vesicles. The peptide is a lysine-rich 42 amino acid helix-loop-helix peptide that triggers lipid membrane destabilization when conjugated to vesicles as a result of peptide folding and partitioning. An N-terminal cysteine enables peptide conjugation to maleimide-functionalized vesicles via Michael addition reaction. Incorporation of a cysteine-protection group, Phacm, on the thiol-moiety prevents undesired thiol-oxidation prior to conjugation. The Phacm group is removed by Penicillin G Acylase (PGA), generating a free thiol that can then react with the maleimide lipids, resulting in a peptide-concentration-dependent release of encapsulated cargo. The possibility to optimize peptide-lipid conjugation provides better means to tune the release process. Additionally, Phacm prevents the issue of thiol-oxidation, allowing for better means of controlling peptide surface concentration. PGA-triggered conjugation of membrane-active peptides to vesicles dramatically facilitates the development of bioresponsive liposome-based drug delivery systems.

P-383
Effect of ERG6 gene deletion on the composition of selected sterols of the Candida glabrata plasma membrane
Juraj Jacko[1], Martina Velísková[1], Nora Tóth-Hervay[2], Yveta Gbelská[2], Libuša Šikurová[1]
[1] Faculty of mathematics, physics and informatics of Comenius university in Bratislava, [2] Faculty of Natural Sciences of Comenius university in Bratislava, Slovakia

Yeasts are eukaryotic, single-cell microorganisms classified as members of the fungus family. Candida yeasts are the most common reason of human fungal infections. One of the essential components of yeast plasma membrane is ergosterol which has the same function as cholesterol in animal cells. In the ergosterol biosynthetic pathway, ERG6 gene plays an important role. It encodes the enzyme Δ(24)-sterol-C-methyltransferase, which converts zymosterol to fecosterol. The ERG6 gene deletion causes changes in plasma membrane architecture and transmembrane potential, leading to disturbances in the barrier function of membrane. It was reported that the ERG6 gene deletion change yeast susceptibility to azole antifungals. This is important because the increase of resistance rate of yeast to antifungal drugs causes a medical problem in the treatment of yeast infections. In our study, we monitored the selected sterols composition of the two groups Candida glabrata (wild-type and Δerg6 mutant) to prove, that the deletion of the ERG6 gene in Δerg6 mutant leads to the inhibition of ergosterol synthesis, ergosterol depletion and accumulation of zymosterol in the plasma membrane. Sterol samples were isolated from both groups of yeasts and analyzed using High-performance liquid chromatography (HPLC) with different settings of absorption detector. Based on our results we found that ERG6 gene deletion causes ergosterol depletion and zymosterol accumulation occur in the plasma membrane of the Δerg6 Candida glabrata yeast.

Poster Presentations
– Session 2 –

P-384
An Allosteric Cholesterol Site in Glycine Receptors Defined Through Molecular Simulations

Farzaneh Jalalypour[1], Rebecca.J. Howard[2], Erik Lindahl[1,2]
[1] Science for Life Laboratory, Department of Applied Physics, KTH Royal Institute of Technology, Stockholm, Sweden, [2] Science for Life Laboratory, Department of Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden

Glycine receptors, belong to pentameric ligand-gated ion channels, facilitate fast inhibitory neurotransmission. Interactions of these channels with their surrounding membrane affect their function. However, the role of lipids in conformational modulation remains unclear. The long timescales of channel gating transitions and lipid diffusion generally preclude statistical sampling by classical methods. To fill this gap, we performed coarse- grained simulations (22 μs each) in a model neuronal membrane for closed, open, and desensitized states of receptor, extracted in identical experimental conditions. We then converted the systems to all-atom models to examine lipid interactions in atomistic detail. Finally, using protein perturbation approach, we identified residues effective in conformational modulation of the receptor. Cholesterol bound to the receptor at the inter-subunit site in a state-dependent manner, indicating that it can bias receptor function. Frequent cholesterol contacts clustered with residues identified by perturbation analysis to be important for allostery and included mutation sites known to influence channel function. Cholesterol binding site appeared to be conserved in human heteromeric glycine receptor, likely representing a more physiologically relevant assembly. Results demonstrate the applicability of multi-scale molecular simulations to pinpoint state-dependent lipid interactions, relevant to allosteric transitions of a membrane protein, and useful for biophysical modeling and pharmaceutical design.

P-385
Modeling Antimicrobial Peptides' Interaction with Bacterial and Mammalian Membranes

Lorant Janosi[1], George Necula[2], Ioan Turcu[1], Mihaela Bacalum[2]
[1] National Institute for Research and Development of Molecular and Biomolecular Technologies, Romania [2] Horia Hulubei National Institute for Research and Development in Physics and Nuclear Engineering

Introduction: Wound healing is considered a dynamic and complex process, which involves a series of events that include cell migration, inflammation, angiogenesis, collagen synthesis and deposition, and re-epithelialization. Thus, an accurate assessment, appropriate diagnosis and optimal wound dressing are essential to ensure proper healing. In recent years, a growing interest towards the use of short (below 50aa) antimicrobial peptides (AMPs) that have a broad-spectrum activity, including multi-drug resistant bacteria was seen. Methods: We used in silico molecular modeling to test the binding of each peptide against both bacterial and mammalian membrane models (DOPC+DOPG 85:15, and DOPC, respectively). The implemented methods use (i) equilibrium molecular dynamics, to assess the AMPs' adhesion times to the membrane models, and (ii) potential of mean force calculations from non-equilibrium simulations, to assess the binding free energy of one specific AMP. Results: Here we present results on (histidine) modulated arginine- and tryptophan-based designed short AMPs (RW-AMP) that will be used in the design of smart wound patches functionalized with these specific AMPs. Conclusion: Our results indicate more stable binding of RW-AMPs to bacterial membrane model versus the mammalian membrane model, yielding RW-AMPs as excellent candidates for future smart wound patching.

P-386
High Deborah numbers in membrane-mimetic bolaamphiphile assemblies

Jelena Jeftic[1,2,3], Mathieu Berchel[4], Cristelle Mériadec[1,5], Loïc Lemiègre[1,2,3], Franck Artzner[1,5], Thierry Benvegnu[1,2,3]
[1] University of Rennes, France, [2] Institut des Sciences Chimiques de Rennes, [3] ENSCR, [4] Université de Bretagne Occidentale, [5] Institut de Physique de Rennes

The remarkable stability of Archaebacteria and their resistance to extreme environmental conditions, such as high temperature (hot springs), high salinity (salt marsh), high acidity (volcanic environment) or low temperature (Arctic or Antarctic) is mostly due to their very stable membranes. Membranes of Archaebacteria are composed of tetraethers from the lipid family of bolaamphiphiles. The concern in our laboratory is to synthesize bolaamphiphiles that could form vectors with function to carry drugs toward target cells in the body. The new family of bolamphiphiles that we have synthesized has one polar head based on a sugar moiety and the other polar head is glycine betaine, both issued from natural sources (sugar beet, wheat). They are interconnected with a hydrocarbon chain of 12, 22 or 32 methylene units. One side hydrocarbon chain, of 8 or 4 methylene units is attached to the anomeric position of the sugar moiety. Bolaamphiphile molecules organise in membrane-mimetic lamellar structures (Lc, L , L ', L ...). They undergo a thermal phase transition upon heating. When cooled back to room temperature, they remain in the undercooled high-temperature phase. In order to follow the relaxation back to the thermodynamically stable room-temperature phase the small-angle and wide- angle X-ray spectra were recorded every hour. The Deborah number (De), defined as De = time of relaxation / time of observation, often used in rheology to characterize the fluidity of materials under specific flow conditions, was evaluated. For two bolaamphiphile structures with the shortest hydrocarbon chain the De(C8C12) = 35 and the De(C8C12X2) = 62.

P-387
An Atomistic Model of the Human Stratum Corneum: Permeation of Solutes Through the Lipid Matrix

Christian Jorgensen[1], Fabien Leonforte[2], Gustavo S. Luengo[2], Sébastien Grégoire[2], Bruno Biatry[2], Peter D. Olmsted[1], Ann Detroyer[2]
[1] Dept. of Physics, Georgetown University, Washington DC 20007, USA, [2] L'Oréal Research and Innovation, Aulnay-sous-Bois, France

The stratum corneum (SC) layer of the human skin is considered to be the outermost and primary barrier against chemical topical exposure. From neutron diffraction data, the SC peaks comprise a short periodicity phase (SPP) with a repeat distance of 6 nm, and a long periodicity phase (LPP) with a repeat distance of 13 nm. The LPP captures long-scale dynamics and can be adequately assigned to a molecular construct termed a sandwich model by Bouwstra, which is composed of two bilayers surrounding a fluid interior slab with lipid headgroups pointing in both directions, i.e. a bilayer-slab-bilayer arrangement. These models of the SC are characterized by an equimolar composition of ceramide, cholesterol and free-fatty acid. A systematic study of transdermal solute penetration through the LPP has not yet been performed. In our model, we find that dipropylene glycol increases the hydrogen-bonding profile and, by consequence, reduces the lipid diffusivity in the lateral direction (DL) by one order of magnitude. Using multimicrosecond constant-velocity steered molecular dynamics of several solutes (hydrophilic and hydrophobic) we expect to be able to construct a chemical profile model of the human stratum corneum of broad applicability in Cosmetics.

Poster Presentations
– Session 2 –

P-388
Shape- based design & purification of bitopic transmembrane peptides
Michael Kaltenegger[1], Louis Percifull[2], Toni Radanovic[2], Jona Causemann[2], Birgit Grill[3], Sandro Keller[1], Gustav Oberdorfer[3], Robert Ernst[2], Georg Pabst[1]
[1] University of Graz, Austria [2] Saarland University, Germany, [3] Technical University Graz, Austria

In the past, bitopic peptides like KALPs and WALPs were used to probe non-specific membrane/protein interactions, in particular hydrophobic mismatch. However, there is significant evidence for asymmetric amino acid distribution of protein transmembrane domains. In an effort to examine the effect of this asymmetric structure, we started designing bitopic peptides to resemble asymmetric shapes. The Rosetta software was used to analyse the sequences we came up with. They consist of stretches of hydrophobic amino acids, which impedes purification during chemical synthesis, leading to protein overexpression as the method of choice for production. We started with an asymmetric sequence of a valine- and an alanine-stretch, including a cysteine as binding site for follow-up experiments and experimented with two different solubility tags, namely a Smt3 and a maltose binding protein tag. The expression in E. Coli cells was successful with both fusion- tags. In both cases we screened different expression, as well as solubilization conditions. I will present the latest results of our protein purification and reconstitution experiments

P-389
Structure and regulation of GSDMD pores at the plasma membrane of pyroptotic cells
Shirin Kappelhoff[1], Michael Holtmannspötter[1], Rainer Kurre[1], John S. Danial[2], Jacob Piehler[1], Katia Cosentino[1]
[1] Center for cellular Nanoanalytics, Osnabrück, Germany [2] Yusuf Hamied Department of Chemistry, University of Cambridge

Pyroptosis is a highly inflammatory form of regulated cell death implicated in pathogen defence and inflammation. Pyroptosis is executed by the Gasdermin (GSDM) family member GSDMD upon cleavage and translocation of the GSDMD-N domain from the cytosol to the plasma membrane (PM), where it oligomerizes and forms pores that allow the release of cytokines and promote cell lysis. Elucidating the structure of GSDMD pores at the PM of cells is key to understanding the role of GSDMD in modulating inflammation by the release of inflammatory molecules. To date, the supposedly small size of GSDMD pores, strong cytosolic background and morphological PM changes during pyroptosis prevented resolving GSDMD pores in their native PM environment. To overcome these limitations, we implemented DNA-PAINT super-resolution microscopy on polymer-supported PMs (PSPMs), a newly developed technique to produce PM sheets generated from cells. This strategy preserves PM topography and integrity while removing any cytosolic fluorescence contribution. Additionally, it makes the inner PM leaflet accessible to techniques such as AFM and SEM. Using this powerful approach, we were able to resolve GSDMD nano-structures directly on the PM of pyroptotic cells. We revealed the presence of different shaped GSDMD structures with a wide variety of size and stoichiometry and identified ring- and arc-like oligomers as pyroptotically-relevant shapes. Furthermore, we started exploring the role of the lipid environment in modulating GSDMD pore formation in cells. Specifically, we observed an increase in PIP3 levels that correlated with faster pyroptosis kinetics and the formation of larger ring-like structures.

P-390
Simplified biomolecule micropatterning for investigation of functional consequences of ERBB2 driver mutations
Tina Karimian[1], Atena Yasari[2], Peter Lanzerstorfer[1], Julian Weghuber[1], Irene Tiemann-Boege[2]
[1] University of applied sciences Wels, Austria [2] Johannes Kepler University Linz

Depositing biomolecule micropatterns on solid substrates via microcontact printing (μCP) usually requires complex chemical substrate modifications to initially create reactive surface groups. Here, we present a simplified activation procedure for untreated solid substrates based on a commercial polymer metal ion coating that allows for direct μCP and the strong attachment of proteins via avidity binding. In proof-of-concept experiments, we identified the optimum working concentrations of the surface coating, characterized the specificity of protein binding and demonstrated the suitability of this approach by subcellular micropatterning experiments in living cells. Furthermore, we used this approach to elaborate on the activation state of novel identified ERBB2 (erb-b2 receptor tyrosine kinase 2) rare mutant variants, as this method allows for the investigation of receptor-mediated protein-interactions at the live cell membrane by total internal reflection fluorescence microscopy (TIRFM). We could unequivocally show that single amino acid changes at the intracellular domains of the ERBB2 are of activating nature, which might lead to constant pathway activity resulting in a growth advantage and clonal expansion. Altogether, this method represents a significant enhancement and simplification of existing μCP procedures and further increases the accessibility of protein micropatterning for cell biological research questions.

P-391
Modulation of a ligand-gated ion channel by an amphetamine derivative
Emelia Karlsson[1], Olivia Andén[1], Chen Fan[1], Yuxuan Zhuang[1], Marc Delarue[2], Rebecca Howard[1], Erik Lindahl[1,3]
[1] Department of Biochemistry and Biophysics, Stockholm University, Solna, Sweden, [2] Unité Dynamique Structurale des Macromolécules, Institut Pasteur, Paris, France, [3] Department of Applied Physics, KTH Royal Institute of Technology, Stockholm, Sweden

Pentameric ligand-gated ion channels (pLGICs) are critical mediators of electrochemical signal transduction in neurons and are important targets of psychoactive drugs. Structural data for these complex proteins are limited, particularly among eukaryotic pLGICs. Interestingly, several homologs of eukaryotic pLGICs are found in prokaryotes. Bacterial channels are often structurally and functionally simpler than eukaryotic subtypes. Nonetheless, in some cases they have been shown to be sensitive to clinically important drugs, including alcohol and anesthetics. Accordingly, bacterial pLGICs offer valuable model systems for structure-function studies of gating and modulation. One bacterial pLGIC, known as sTeLIC, was recently shown to share structural features with its eukaryotic relatives, including a vestibular loop previously identified in serotonin-3 receptors. The reported X-ray structure depicts sTeLIC in an apparent open state with binding pockets in its extracellular domain, compatible with binding a drug with structural similarities to amphetamines. The project aimed to provide the first structure-function evidence for direct modulation of a pLGIC by an amphetamine, by using two-electrode voltage-clamp electrophysiology in Xenopus oocytes. Our modulation data showed that 4-bromoamphetamine is an allosteric potentiator and, at high micromolar concentrations, an allosteric agonist. We also used molecular docking to the X-ray open state and a cryo-EM structure of the closed state to predict key amino acid interactions of the drug. Consistent with these predictions, mutating residues W75 or Y104 to alanine or valine reduced drug potentiation. These results offer new observations of pLGIC potentiation by a psychoactive drug derivative, with potential implications for mechanistic modeling and pharmaceutical development.

Poster Presentations
– Session 2 –

P-392
Membrane Adsorption Enhances Translocation of Antimicrobial Peptide Buforin 2

Mehrnoosh Kh. Hazrati[1], Robert Vácha[1,2,3]

[1] CEITEC – Central European Institute of Technology, Masaryk University, Kamenice 753/5, 625 00 Brno, Czech Republic, [2] National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic, [3] Department of Condensed Matter Physics, Faculty of Science, Masaryk University, Kotlářská 267/2, 611 37 Brno, Czech Republic

Antibiotic resistance has been identified as one of the top three threats to human public health. Promising alternatives to antibiotics are antimicrobial peptides (AMPs) that can selectively kill bacterial cells. Buforin 2 (BF2) is an AMP that kills bacteria via intracellular interactions rather than membrane lysis. BF2 membrane translocation is known to be independent of any cellular receptor because BF2 can readily enter bacterial cells and vesicles that contain only lipids in their membrane. However, the free energy barrier for BF2 translocation across a symmetric membrane suggests a non-spontaneous process, demonstrating our poor understanding of the molecular details of how BF2 crosses biological membranes. Here, we show that inducing membrane asymmetry by BF2 adsorption on one leaflet significantly enhances BF2 translocation across bilayers, indicating that membrane asymmetry may act as a driving force for membrane transport. Our results shed light on the crowding effect of AMP on antibacterial activity and are expected to be helpful in the design of new AMPs.

P-393
Osh6 mediates lipid transport in lipid composition-dependent manner

Alena Koukalova[1], Andrea Eisenreichova[1], Evzen Boura[1], Jana Humpolickova[1]

[1] Institute of Organic Chemistry and Biochemistry AS CR, v.v.i., Czech Republic

Non-vesicular lipid transport is essential for cellular lipid homeostasis and maintaining the identity of individual membranes. Oxysterol-binding protein-related proteins (ORPs) form a large family of proteins that act in this process. We study a member of this family Osh6, which is in yeast involved in the transport of phosphatidylserine (PS) from its site of synthesis in the endoplasmic reticulum to the plasma membrane. The main feature of the transport is the exchange between two cargo molecules, PS and phosphatidylinositol-4-phosphate (PI4P). However, the exact transport mechanism is poorly understood. We have developed assays based on fluorescence cross-correlation spectroscopy to monitor lipid extraction and transfer in model membranes. We demonstrate that occupancy of Osh6 by a particular cargo has an impact on the recognition of a distinct membrane, which determines the transport directionality. Besides, we show that Osh6 tethers membranes in a lipid composition dependent manner confirming the crucial role of membrane characteristics in this process. These findings give insight into the mechanism and regulation of lipid transport in cells.

P-394
Atomic force microscopy of interactions of amyloid precursor protein with lipid rafts

Vladimir Krasnobaev[1,2], Eduard Bocharov[2,3], Oleg Batishchev[1]

[1] Frumkin Institute of Physical Chemistry and Electrochemistry, Russian Academy of Sciences, Moscow, Russia, [2] Moscow Institute of Physics and Technology (National Research University), Dolgoprudny, Russia, [3] Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow Russia

One of the hallmarks of Alzheimer's disease (AD) is the accumulation of amyloid beta (Aβ) peptides in the brain. The processing of amyloid precursor protein (APP) into Aβ is dependent on the location of APP in the membrane, membrane lipid composition and, possibly, presence of lipid rafts. In this study, we used atomic force microscopy (AFM) to investigate the interaction between transmembrane fragment APP 672-726 (corresponding to Aβ 1-55) and its amyloidogenic mutant L723P with membranes combining liquid-ordered and liquid-disordered lipid phases. Our results show that most of the APP 672-726 is located either in the liquid-disordered phase or at the boundary between ordered and disordered phases, and hardly ever in rafts. We did not notice any major changes in the domain structure induced by APP 672-726. We observed the ring-like structures of APP 672-726 only in membranes without cholesterol, and especially for L723P mutants. These findings suggest that APP interacts differently with different phases of the membrane, and that its presence may interfere with the formation of ordered domains. Our study contributes to a better understanding of the complex interactions between APP fragments and model membranes, which is important for developing treatments for neurodegenerative diseases associated with APP.

P-395
Impact of water scarcity conditions on the nanoscale structural arrangement of biomimetic cell membranes

Emilia Krok[1], Madhurima Chattopadhyay[1], Hanna Orlikowska-Rzeznik[1], Henri G. Franquelim[2], Petra Schwille[3], Lukasz Piatkowski[1]

[1] Poznan University of Technology, Poland [2] Interfaculty Centre for Bioactive Matter, [3] Max Planck Institute for Biochemistry

Cell membranes in physiological conditions are surrounded by an abundant amount of water molecules. However, we can distinguish multiple membrane fusion events that require partial or even complete dehydration at the boundary of two lipid membranes such as cell-cell fusion, viral entry endo- and exocytosis. Although phase-separated lipid membranes have been widely characterized in full hydration conditions, still little is known about their lateral organization when the water level in the surrounding them environment is decreased. In the following study, we used the combination of fluorescence and atomic force microscopy to unravel the potential structural changes within biomimetic cell membranes both in macro- and nanoscale occurring under a wide range of hydration conditions. We revealed that upon removal of bulk water and gradual decrease of membrane hydration, lipids from the liquid-disordered (Ld) phase start to migrate into the liquid-ordered (Lo) phase, forming Ld nanodomains embedded within Lo matrix. The analysis of height histograms showed decreasing in height mismatch between phases with a lowering of the membrane hydration. Finally, we addressed the response of the membrane to dehydration conditions in terms of line tension, which is considered to be the main driving force leading to phase separation in biological membranes and which has so far been only measured as a function of fatty acid chain lengths but never through the direct impact of water. Finally, we believe that the presented here pioneering methodology of AFM measurements under controlled hydration conditions can be readily applied to study other model cell systems.

Poster Presentations
– Session 2 –

P-396
Effects of a phase transition in lipid-asymmetric vesicles
Mona Krompers[1], Heiko Heerklotz[1,2]
[1] Institute of Pharmaceutical Sciences, University of Freiburg, Germany, [2] Leslie Dan Faculty of Pharmacy, University of Toronto, Canada

Membrane asymmetry (by means of lipid and area asymmetry) is associated with various physiological processes such as apoptosis or intra- and extracellular transport. Lipid asymmetry occurs in most biological membranes in terms of containing other lipids in the outer than in the inner leaflet. To prepare lipid-asymmetric large unilamellar vesicles (aLUVs), we apply a method based on the exchange of outer leaflet lipids with complexes in an aqueous solution. The protocol allows for controlled lipid exchange of e.g. phosphatidylglycerol (PG) in the outer leaflet of phosphatidylcholine (PC) liposomes via cyclodextrin. We use zeta potential measurements to quantify lipid-asymmetry in aLUVs. Our study aims to generate aLUVs from two components in a gel/fluid coexistence. In the initially relaxed membrane, intrinsic area asymmetry is induced by increasing the temperature as asymmetrically inserted PG melts. This asymmetry stress may be relieved by budding off little daughter vesicles. Using Asymmetric Flow Field-Flow Fractionation (AF4), we observed budding of aLUVs containing 40 mol% PG in the outer leaflet. We compared phase transitions in aLUVs with symmetric vesicles using Differential Scanning Calorimetry (DSC) and Pressure Perturbation Calorimetry (PPC). We have found SOPC melting independently of DPPG/SOPC in the two leaflets of aLUVs. With future research, we will proceed to investigate phase transitions in aLUVs and the effects on membrane structure.

P-397
Reconstitution of the proposed lipid II flippase MurJ in peptidoglycan precursor containing biomimetic membranes
Annika Marie Krüger[1], Lisa Fritz[2], Jan Peter Siebrasse[1], Tanja Schneider[2], Ulrich Kubitscheck[1]
[1] Clausius Institute of Physical and Theoretical Chemistry, University of Bonn, Wegelerstr. 12, 53115 Bonn, Germany, [2] Institute for Pharmaceutical Microbiology, University Hospital Bonn, University of Bonn, Meckenheimer Allee 168, 53115 Bonn, Germany

Lipid II is the key bacterial cell wall precursor. It is synthesized in the cytoplasm and needs to be flipped across the cell membrane by a flippase for cell wall biosynthesis, putatively this is accomplished by MurJ. Our goal is to observe the flipping process in biomimetic artificial membranes directly via microscopy. To this end we employ Giant Unilamellar Vesicles (GUVs), which are not created by electroformation, but by exploiting agarose films. MurJ probably requires a membrane potential to be functional. Therefore, we established a membrane potential of -100 mV across the GUV membrane. We succeeded to observe the lateral diffusion of single lipid II-Atto565 molecules as well as single MurJ-Atto550 with diffusion coefficients of D(Lipid II) = 5.4 ± 0.2 µm2/s and D(MurJ) = 4.9 ± 0.3 µm2/s, respectively. Furthermore, we developed a Flipping Assay based on the binding of labeled monomeric Streptavidin to biotinylated lipid II which is present at the outer leaflet of the GUV membrane.

P-398
Studying biomolecular dynamics and structure with high-speed atomic force microscopy (HS-AFM)
Andre Koernig[1], Andreas Kraus[1], Dimitar Stamov[1], Heiko Haschke[1]
[1] JPK BioAFM, Bruker Nano GmbH, Germany

Molecular dynamics and structural conformations of biological molecules are important for understanding the function and biological significance of samples ranging from single membrane proteins to complex macromolecular systems. Recent atomic force microscopy (AFM) developments have led to unprecedented imaging rates in fluid, enabling temporal resolution on the sub-20-milisecond scale. We will give three examples in which high-speed AFM was applied for studying of structural transitions and biomolecular dynamics in samples, containing annexin V (A5), and thermodynamic DNA rehybridization. A5 serves as an important regulator of membrane repair in eukaryotic cells, where it shows a strong Ca2+ binding affinity to phosphatidylserine. We have used high-speed AFM to study the 2D crystal formation in a model system containing supported lipid bilayers and A5 molecules. We demonstrate the lateral dynamics and preferred structural orientations of the mobile A5 trimers. We previously demonstrated that pUC19 plasmids bind to poly-L-ornithine substrate in supercoiled states that are very high in torsional energy, thereby driving dehybridization of the double-helical DNA strands. Here we have quantified the process kinetics with a temporal resolution of 25 ms per frame and identified stages that include formation of metastable dehybridization bubbles, thermodynamic single strand fluctuations, and ultimately rehybridization to an intact double-stranded state.

P-399
Proton dynamics in the light-driven bacterial chloride pump NmHR
Mariafrancesca La Greca[1], Sabine Oldemeyer[1], Mohamad Yahia Dekmak[1], Przemyslaw Nogly[2], Joachim Heberle[1], Ramona Schlesinger[1]
[1] Freie Universität Berlin, Germany [2] Jagiellonian University Krakow

NmHR, a halorhodopsin from the organism Nonlabens marinus, is an inward chloride pump, discovered less than 10 years ago in a marine bacterium. Although NmHR and the other two known halorhodopsins, (HsHR ans NpHR), transport the same anion, NmHR shows a higher sequence similarity to the sodium pump rhodopsin KR2, and a similar conserved motif of residues involved in the ion transport. Here we report single point mutations on residues involved in the chloride pathway, to understand their functions and involvement in the chloride transport, by carrying out spectroscopic techniques. Using time resolved UV-Vis spectroscopy and steady state FTIR, we are investigating the pathway of release and uptake of the chloride ion from the extracellular side to the intracellular side of the membrane. Furthermore, we report the insertion of the unnatural aminoacid p-cyano phenylalanine, via Amber codon suppression. The C=N stretching vibration of the cyano group can be used as an infrared marker of local environment, due to its sensitivity to hydrogen bonding and local electric field. Via introduction of the p-cyano phenylalanine, exchanging a fundamental residue (W99), part of the binding pocket of the chromophore we are trying to elucidate the environmental changes around the retinal.

Poster Presentations
– Session 2 –

P-400
Insights into the gating mechanisms of channelrhodopsins using FTIR-spectroscopy

Kristin Labudda[1], Mohamed Javad Norahan[1], Philipp Althoff[1], Max-Aylmer Dreier[1], Till Rudack[1], Carsten Kötting[1], Klaus Gerwert[1]

[1] Ruhr-University Bochum, Germany

Optogenetic is a powerful method for studying and manipulating cells and tissues, with applications in neurons and neuron-related diseases. Neurons can be manipulated by using light activated proteins which are often microbial rhodopsins, more specifically channelrhodopsins. In channelrhodopsins retinal is present as a chromophore. Illumination is initiating a photocycle, in which the retinal absorbs a photon, leading to its isomerisation. This triggers further conformation and protonation-changes in the protein leading to the opening and closing of the ion channel. To target cells precisely, high photocurrents and low current inactivation levels are desirable in optogenetic applications. To improve channelrhodopsins for those optogenetic applications, a deep understanding of their functionalities and mechanisms is required. We are investigating channelrhodopsins and their photocycles by the combination of IR-spectroscopy and molecular dynamic simulations. Previously, we found that two parallel photocycles evoked by different retinal conformations are present in cation-conducting channelrhodopsin-2 (CrChR2): one with high conductivity and one with low conductivity. The latter is responsible for the inactivation of the protein, causing a largely reduced photocurrent under continuous illumination. (Kuhne et.al, 2019). In contrast, the highly conducting photocycle was the sole operating cycle in anion-conducting channelrhodopsin 1 (GtACR1). (Dreier et.al, 2021) After comparing residues in the central gate of CrChR2 with residues in the central gate of GtACR1, we carried out mutagenesis towards a more GtACR1 like CrChR2 and vice versa. This comparison provided us more insight in the gating mechanism of channelrhodopsins to advance the development of optimized optogenetic tools.

P-401
MurG membrane-bound proteins do domains

Elodie Lafargue[1], Ignacio Casuso[2]
[1] Aix-Marseille University, [2] INSERM, France

Bacterial membranes have distinct components and are exposed to different environments, such as temperature variations, compared to eukaryotic cells. Microdomains of increased fluidity, known as Regions of Increased Fluidity (RIFs), have been observed in bacterial membranes and may act as platforms for the assembly of proteins. However, the exact mechanisms governing the organization of proteins in bacterial membranes are still not fully understood. It is possible that protein assembly may not always rely on lipid segregation or pre-existing lipid domains, and other factors, such as electrostatic interactions between proteins, protein concentration, and lateral pressure, may be important. This study used High-Speed Atomic Force Microscopy (HS-AFM) to investigate the partitioning of MurG glycosyltransferases from Gram positive and Gram negative bacteria into phospholipid bilayers. Surprisingly, the MurG proteins were found to partition the membrane without requiring any preliminary lipid-induced partitioning. This study reveals important information about the membrane organisation of MurG proteins in phospholipid bilayers, providing insight into how these proteins help structure the bacterial membrane.

P-402
Amphiphilic nanoparticles aggregation on lipid membranes

Enrico Lavagna[1], Davide Bochicchio[1], Giulia Rossi[1]
[1] Department of Physics - University of Genoa, Italy

The aggregation of membrane inclusions is fundamental for many biological processes. For instance, it can favor the activation of membrane receptors; aggregation can stabilize membrane structure by driving curvature or even induce structural transitions, like in the case of vesicle fusion. In this study, we approach the fundamental understanding of aggregation within the bilayer looking at functionalized Au NPs that are embedded in model lipid bilayers. Using Molecular Dynamics and enhanced sampling techniques, we show that Au NPs functionalized by an anionic, amphiphilic shell spontaneously aggregate in fluid zwitterionic lipid bilayers. We show that the aggregation thermodynamics varies depending on the state of penetration of the NPs in the bilayer, and we disentangle the short-range and long-range aggregation driving forces. At short inter- particle distances, ion-mediated, charge-charge interactions (ion bridging) stabilize the formation of large NP aggregates. Long-range interactions, instead, are driven by lipid perturbations, with aggregation induced by lipid depletion or curvature minimization. Ion bridging, lipid depletion, and the effect of NPs on membrane curvature all stem from the configurational flexibility of the NP shell. Our simulations show that the aggregation of same-charge membrane inclusions derives from intrinsic nanoscale effects at the inclusion-inclusion and inclusion-bilayer soft interfaces. Due to the NP size and surface chemical patchiness, the forces that drive NP-NP aggregation are similar to those responsible for other membrane inclusion interactions, like proteins or peptides.

P-403
Exploring Amyloid β self-assembly in native-like membranes

Anthony Legrand[1,2], Chao Song[3], Jan Česnek[1], Martin Kopřiva[1], Antonín Kunka[4], Andrew deMello[3], Jiří Damborský[1,2], Stavros Stavrakis[3], Zbyněk Prokop[1,2]

[1] Loschmidt Laboratories, Department of Experimental Biology and RECETOX, Faculty of Science, Masaryk University, Kamenice 5/C13, 625 00 Brno, [2] International Clinical Research Center, St. Anne's University Hospital Brno, Pekařská 53, 656 91 Brno, [3] Institute for Chemical and Bioengineering, Department of Chemistry and Applied Biosciences, ETH Zürich, Vladimir-Prelog-Weg 1 8093 Zürich, Switzerland, [4] Department of Biotechnology and Biomedicine, Technical University of Denmark, Kongens Lyngby, Denmark

As the global population is ageing, dementia becomes a cause of worldwide epidemiological concerns. Alzheimer's disease (AD) accounts for 60% to 70% of all cases of dementia. AD is characterised by the presence of senile plaques of Amyloid β (Aβ) peptides and neurofibrillary tangles of hyperphosphorylated Tau protein. Aβ is cut-out from the plasma membrane-spanning Amyloid Precursor Protein (APP), through the action of secretases in the brain, yielding Aβ peptides of varying length, with the most up-regulated in AD being Aβ42. The latter may either aggregate into amyloid fibrils or pore-forming oligomers in the plasma membrane. Controlling this process is a promising therapeutic concept, but it requires a thorough understanding of Aβ42's aggregation pathway in its native membrane environment. We developed a microfluidic platform to generate highly homogeneous giant unilamellar vesicles (GUVs), whose lipid composition mimics neuron plasma membranes, and we used these to monitor Aβ42 aggregation and liposome destruction by fluorometry. Tweaking GUV lipid composition, we reveal interactions between specific lipids and Aβ42, and assess structural changes using infrared spectroscopy, and NMR. Finally, we build a kinetic model of Aβ42 aggregation and membrane toxicity in a native-like environment, which could guide future drug discovery in the field of AD.

Poster Presentations
– Session 2 –

P-404
Asymmetrical Calcium Ions Induced Stress and Remodeling in Lipid Bilayer Membranes
Chang Liu[1]
[1] Peking University, China

Ca2+ ions play crucial roles in regulating many chemical and biological processes, but their impact on lipid bilayer membranes remains elusive, especially when the impacts on the two leaflets are asymmetrical. Using a recently developed multisite Ca2+ model, we performed molecular dynamics simulations to study the impact of Ca2+ on the properties of membranes composed of POPC and POPS and observed that both the structure and fluidity of the membranes were significantly affected. In particular, we examined the influence of asymmetrically distributed Ca2+ on asymmetric lipid bilayers and found that imbalanced stress in the two leaflets was generated, with the negatively charged leaflet on the Ca2+- rich side becoming more condensed, which in turn induced membrane curvature that bent the membrane away from the Ca2+-rich side. We employed continuum mechanics to study the large-scale deformations of the membrane and found that membranes can develop into locally pearl-shaped or globally oblate, depending on the specific Ca2+ distributions. These results provide new insights into the underlying mechanisms of many biological phenomena involving Ca2+-membrane interactions and may lead to new methods for manipulating the membrane curvature of vesicles in biological, chemical, and nanosystems.

P-405
Structure and Mechanism of Prokaryotic Dissolved Inorganic Carbon-Concentrating Transporter
Yat Kei Lo[1], Adel Beghiah[2], Ville Kaila[2], Jan Schuller[1]
[1] CryoEM of Molecular Machines, SYNMIKRO Research Center and Department of Chemistry, Philipps-University, Marburg, Germany, [2]Department of Biochemistry and Biophysics, Stockholm University, Sweden

The dissolved inorganic carbon-concentrating transporter (DIC-CT) is a recently discovered membrane potential dependent carbon-concentrating mechanism (CCM) which enable autotrophic bacteria to maintain a high level of intracellular DIC ($CO_2 + HCO_3^- + CO_3^{2-}$) for sustaining CO_2 fixation. It is shown to be essential in several chemoautotrophs, and its homologs can be found in more than 12 classes of bacteria. Despite its physiological significance, the molecular mechanism remains largely enigmatic. Here we present the first cryo-EM structure of a DIC-CT reconstituted in lipid nanodiscs. The system comprises of i) a transmembrane subunit which cognates well with the Complex I (NADH:ubiquinone oxidoreductase) proton-pumping subunits, and ii) a cytoplasmic subunit that partially resembles β-carbonic anhydrases. We performed structure guided mutagenesis and complementation assay to identify key amino acids essential for the transporter to elevate intracellular DIC. Furthermore, we demonstrated that it is capable of conducting proton in proteoliposomes. In contrast to cytoplasmic carbonic anhydrases, DIC-CT might represent a novel class of proton motive force coupled vectorial carbonic anhydrase catalysing CO_2 hydration.

P-406
Alterations in model membranes during lipid oxidation
Sara Lotfipour Nasudivar[1], Ana J. Garcia-Saéz [1], Lohans Pedrera Puentes[1]
[1] Institute for Genetics, CECAD Research Center, University of Cologne, Germany

Ferroptosis is a form of regulated necrosis characterized by the accumulation of iron-dependent lipid peroxides in cell membrane. By mimicking the cell membrane through minimalist artificial lipid bilayer model systems, we investigate changes based on the underlying mechanical properties of phospholipid interactions during the oxidation processes, triggered with ascorbic acid and iron. Lipid compositions with phase coexistence of ordered (saturated lipid species enriched with cholesterol) and disordered (unsaturated lipid species) liquid phases allow observation of temporal changes in the characteristic round shape of the ordered phase domains that origins from a line tension due to the height difference. A loss of circularity during the oxidation process indicates reduced line tension, possibly due to reduced repulsive forces of the hydrophobic lipid tails. Force measurements using atomic force microscopy of bilayers in the oxidized and non-oxidized states have shown a significant decrease in the force required to pierce through the membrane, indicating weaker molecular interactions and less elasticity. This leads us to believe that mechanical changes due to a continuous oxidation process of the phospholipids could have a sufficiently large influence to explain the destabilization of the plasma membrane and possibly the formation of pores.

P-407
Effect of proton collecting antenna residues at the K-channel entrance and enzyme activity modulation in cytochrome c oxidase
Victor Manuel Loyo Cruz[1], Ulrike Alexiev[1], Jens Balke[1]
[1] Freie Universität Berlin- Physics department, Germany

Cytochrome c oxidase (CcO) is part of the electron transport chain. As a redox-coupled proton pump, CcO establishes a proton motive force across the membrane. This mechanism employs two proton uptake channels, the D- and the K-channel. We aim at investigating the contribution of potential proton collecting antennae residues (Asp, Glu, His) for proton uptake through the K-channel by measuring enzymatic activity, H-bond network and protein dynamics changes by site-directed mutagenesis and a fluorescent sensor group covalently bound at the K-channel entrance of P. denitrificans CcO in position 301. H73II, H526, and E78II were mutated to alanine; individually and in a combination. Our results show how a long-range H-bonding network along the protein surface affects enzyme activity and protonation changes. Modulation of enzymatic activity by detergent/ligand binding, and by specific conformational changes of loop structures at the K-channel entrance attribute to the effects.

Poster Presentations
– Session 2 –

P-408
Calcium-dependent and membrane-specific binding of the C2 domain protein CaLB studied by ATR-FTIR spectroscopy

Shane Maguire[1], Christian Scheibe[1], Terese Eisgruber[1], Niccolò Mosesso[2], Erika Isono[2], Karin Hauser[1]

[1] Department of Chemistry, University of Konstanz, [2] Department of Biology, University of Konstanz, Germany

Membrane trafficking events are controlled by various C2 domain-containing proteins that are targeted to specific membrane compositions. Although these membrane interacting proteins have been reported, the molecular mechanisms of these interactions and conformational dynamics are poorly understood. Here, we used attenuated total reflection (ATR)-Fourier-transform infrared (FTIR) spectroscopy and biomimetic membrane systems to analyse CalB, a yet uncharacterized C2 domain protein. Membrane binding, lipid specificity, as well as calcium dependency, were studied with solid-supported lipid membranes (SSLB) and small unilamellar lipid vesicles (SUVs). Membranes were composed of both pure POPC lipids or POPC/PI(3)P lipid compositions. A significant increase in protein binding affinity was observed when the membrane contained 1% PI(3)P, indicating a high binding specificity of CaLB for PI(3)P. Furthermore, membrane binding was observed in a calcium-dependent manner, with a higher calcium concentration increasing the CaLB binding to membrane compositions containing PI(3)P. Secondary structure analysis of IR- spectra indicates that only small conformational changes take place upon binding with a slight increase in the helical and disordered region of CaLB.

P-409
Dependence of cell's membrane potential on extracellular voltage observed in algae

Manohara Mahadeva[1], Sebastian Niestępski [1], Magdalena Kowacz [1]

[1] Institute of Animal Reproduction and Food Research of Polish Academy of Sciences, Department of Reproductive Immunology and Pathology, Olsztyn, Poland.

Cytoplasmic or extracellular modifications affect cell's membrane potential (Vm). It has been recently recognized that adjacent to red blood cells there is a nanometer-wide zone of extracellular voltage (Vz) intimately related to Vm. Here, we are showing the presence of Vz within a large zone of a few microns next to cell walls of algae (C. globularis), its dynamic response to external CO_2, and its relation to Vm. At resting conditions, Vm = (-184.12 ± 21.41) mV, Vz = (-17.33 ± 2.50) mV, and the zone width is 4-6 µm. Electrostatic interactions between ions and perm-selective cell walls instigate Vz. External CO_2 increases negativity of Vz by (-15.90 ± 3.05) mV while depolarizing Vm by (16.84 ± 3.91) mV. Changes in Vz and Vm in response to external CO_2 correspond to each other, what suggests capacitive coupling between cell interior and exterior rather than ion transport. External CO_2 affects both Vz and Vm, while internal CO_2 (respiration) affects only Vm. This implies that Vz is affected by interaction of cell wall and extracellular ions and there is no immediate ion transport across the cell wall. Vz can be expressed as a particle-free zone which we observed next to damaged and newly growing cells, where the uptake of ions in enhanced. Therefore, Vm can result not only from ion exchange across the cell membrane but also from electrostatic interactions between the cell and its surrounding environment, which opens the door to exploring Vm beyond ion channel activities.

P-410
Testing the adhesive ability of cardiolipin on a mammalian ATP synthase dimer through molecular dynamics

Marcin Makowski[1,2,3], Victor G. Almendro-Vedia[2,4,5,6], Manuel N. Melo[1], Iván López-Montero[2,4,6]

[1] Instituto de Tecnologia Quimica e Biologica, Universidade Nova de Lisboa, Av. da República 2780-180 Oeiras, Portugal, [2] Departamento Química Física, Universidad Complutense de Madrid, Avda. Complutense s/n 28040 Madrid, Spain, [3] Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa, Av. Egas Moniz, 1649-028 Lisbon, Portugal, [4] Instituto de Investigación Hospital Doce de Octubre (Imas12), Avda. de Córdoba s/n, 28041 Madrid, Spain, [5] Sección Departamental Farmacia Galénica y Tecnología Alimentaria, Universidad Complutense de Madrid, Avda. Complutense s/n 28040 Madrid, Spain, [6] Instituto Pluridisciplinar, Universidad Complutense de Madrid, Paseo Juan XXIII 1 28040 Madrid, Spain

The membrane protein F1Fo ATP synthase is responsible for the production of most of the ATP requirements of mammalian cells. ATP synthases organize in dimers at the curved rims of mitochondrial cristae. Its function depends on the rotation of its membrane-spanning motor, hinting on a foreseeable prominent role of the lipid environment on this protein's function and biogenesis. Cardiolipin (CL), an exclusively mitochondrial, four-tailed lipid, reportedly could aid in the maintenance of the u-shaped curvature of mitochondrial cristae. Furthermore, it has been proposed that CL might act as a molecular glue of mitochondrial supercomplexes. We explore using molecular dynamics simulations the lipid environment of an ATP synthase dimer, finding that it is absolutely crowded in CLs. We identify key CL binding regions and characterize their affinity for ATP synthase through Alchemical Free Energy Perturbation. We also study CL's adhesive ability of the dimer through Potential of Mean Force calculations.

P-411
Precise description of the GPCRs activation

Antoni Marciniak[1], Darko Mitrovic

[1] KTH Royal Institute of Technology, Sweden

G-Protein Coupled Receptors (GPCRs) are transmembrane proteins made of seven transmembrane helices responsible for transducing chemical signals through the membrane. These receptors are usually grouped into a few classes based on their functionality, with the biggest one (class A) spanning multiple commercial drug targets. Despite significant effort and funding put into understanding those receptors – with the goal of designing new drugs - the precise description of GPCR activation remains elusive. It is known that helix-6 outward movement is necessary for the receptors to activate and commonly accepted that various microswitches constitute a good indication of the process, at least in class A. However, the sequential resolution of the structural changes is not understood, and the energetics behind it are not studied well. In this work, we present the development of a unique approach combining evolutionary information, data science, and molecular dynamics that allows us to accurately describe the activation of GPCRs. By obtaining the free energy surfaces of activation of receptors bound with different drugs, we are able to describe the drivers of the structural change in an energy-based manner and pinpoint the residues that are essential for the process.

Poster Presentations
– Session 2 –

P-412
Molecular mechanisms of Gasdermin D pore assembly by single-molecule microscopy
Eleonora Margheritis[1], Shirin Kappelhoff[1], John Danial[2,3], Katia Cosentino[1]
[1] Department of Biology/Chemistry and Center for Cellular Nanoanalytics (CellNanOs), University of Osnabrück, Germany, [2] Yusuf Hamied Department of Chemistry, University of Cambridge, Cambridge, United Kingdom, [3] UK Dementia Research Institute, University of Cambridge, Cambridge, United Kingdom

Pore-forming proteins of the Gasdermin (GSDM) family are distinctly involved in immunity, inflammatory diseases, cancer, and neurodegeneration and represent promising therapeutic targets. The Gasdermin D (GSDMD) family member is synthesized as a cytosolic and inactive protein characterized by a C-terminal auto-inhibitory domain (CTD) and an N-terminal functional domain (NTD). The NTD is released, upon processing at the NTD-CTD linker by inflammatory caspases, and self-assembles into oligomeric complexes at the plasma membrane creating pores that allow membrane permeabilization, a crucial step in the execution of pyroptosis. The exact mechanism of GSDMD membrane recruitment, assembly, and perforation is still poorly understood. Here, we investigate the molecular mechanisms of GSDMD pore assembly by stoichiometric brightness analysis of GSDMD oligomers using single-molecule total internal reflection fluorescence (TIRF) microscopy. To this end, we reconstituted recombinant mouse GSDMD-mGFP oligomers in mimetic membrane systems with specific lipid composition. Initial characterization of the full- length protein state in solution, supported by NATIVE PAGE analysis, reveals that GSDMD assembles as stable dimers and trimers in an aqueous environment. Dimeric and trimeric oligomers are also the minimal GSDMD unit that can insert into the membrane but are not sufficient for permeabilization. Further higher-order oligomerization proceeds by sequential addition of these di-trimeric assembly units in a protein- concentration-dependent manner. Furthermore, our analysis discloses a cooperative contribution of cysteine residues in the formation of trimeric units and in the further oligomerization of GSDMD. Overall, our study provides unprecedented structural details on the initial steps of GSDMD pore assembly during pyroptosis.

P-413
Calmodulin interacts with membranes in a Ca2+ and lipid specific manner
Martin hof[1], Federica Scollo[1], Carmello Tempra[2], Hector Martinez-Seara[2], Marek Cebecauer[1], Pavel Jungwirth[2], Piotr Jurkiewicz[1]
[1] J. Heyrovsky Institute of Physical Chemistry, Czech Academy of Science, Czech Republic [2] Institute of Organic Chemistry and Biochemistry, Czech Academy of Science

Calmodulin (CaM) is a multifunctional calcium-modulated protein expressed in all eukaryotic cells. It binds to a wide variety of target proteins, by converting changes in the intracellular calcium concentration to signaling events. Up to date, there are evidences in the literature showing that CaM, upon calcium binding, interacts with certain membrane proteins. Therefore, it is tempting to speculate that the membrane plays a role in the regulation of CaM functions. However, if at all and how CaM interacts with lipid membranes is unclear. In this work we investigate the behavior of CaM with different model membranes by Confocal Microscopy, Fluorescence Correlation Spectroscopy, Laurdan General Polarization and Surface Plasmon Resonance. Our data clearly show adsorption of CaM to lipid membranes in a lipid and calcium-dependent manner. We complement these experiments with Confocal Microscopy on living cells and MD simulations. These experiments indicate the physiological relevance and molecular mechanism of the CaM membrane interaction, respectively.

P-414
Mechanistic insights into the interactions between S. aureus amyloids and cell membranes
Marion Mathelié-Guinlet[1], Laura Bonnecaze[1], Katlyn Jumel[1], Lucie Khemtemourian[1], Cécile Feuillie[1], Michael Molinari[1], Sophie Lecomte[1]
[1] CBMN, France

The virulence of S. aureus, a multi-drug resistant pathogen, notably depends on the expression of PSMα3, secreted as either formylated (f-) or deformylated (df-) peptides. PSMα3 is able to self-assemble into amyloid-like fibrils, and, depending on its capping and conformation, can participate in diverse physiological functions, e.g. cytotoxicity and immune stimulation. The specific interactions with cell membranes underlying such activities remain so far elusive. We thus aim at unveiling the structure-function relationship of PSMα3, in light of its dynamic interactions with biomimetic membranes. We first showed that f- and df-PSMα3 behaves similarly in terms of fibrillation kinetics and structural content. Atomic force microscopy additionally demonstrated that while df-PSMα3 tends to accumulate and elongate at any membrane interface, f-PSMα3 has a much higher affinity for eukaryotic membranes, especially for fluidic phases, on which it fibrillates while partially inducing morphological and mechanical disruption. Our data support the idea that f-PSMα3 oligomers might be the membrane-active entities and that local lipid disorganization could, in turn, triggers further aggregation. Besides, unlike df-PSMα3, f-PSMα3 does not deposit on bacterial membranes but has gained a bactericidal activity. Such discrepancies in the behavior of f- and df-PSMα3 might be explained by the change in hydrophobic and electrostatic interactions imposed by the presence of a formyl group at the N-terminus or alternatively by differences in their intrinsic structures, that would require further work e.g. by solid state NMR and cryo-EM.

P-415
Structural and Functional Characterization of a Novel Aminosugar-specific Porin from the Marine bacterium Vibrio harveyi
Piyaporn Meesrikaew[1], Robert C. Robinson[2], Wipa Suginta[1]
[1] Vidyasirimedhi Institute of Science and Technology (VISTEC) 555 Moo 1 PayupNai, Wangchan, Rayong 21210 Thailand, [2] Research Institute for Interdisciplinary Science (RIIS), Okayama University, Okayama 700-8530, Japan

Poster Presentations
– Session 2 –

P-416
Water permeation through the sodium glucose cotransporter SGLT1
Franci Merzel[1], Marko Sever
[1] National Institute of Chemistry, Slovenia

The underlying transport mechanism of SGLT1 is based on switching between inward- and outward-facing conformations, known as the alternating access model, which is crucial for the sugar transport and has also been postulated for water permeation. However, the nature of water transport remains unclear and is disputed along the passive and active transport, the latter postulating the presence of pumping effect. To better examine the water transport in SGLT1 we performed a set of equilibrium all-atom molecular dynamics totaling over 6 microseconds of sampling on different SGLT1 systems including representative conformational states and complexes with sugars, ions, and inhibitor. We elucidate factors of water transport including energetics of the channel-water and dynamical features of protein using principal component analysis. We have identified dependence of instantaneous water flux on the channel opening and local water diffusion in the channel supporting the hypothesis of passive water transport in hSGLT. However, a strong correlation between the local water diffusion and protein domain motion resembling the "rocking-bundle" vibrational modes, reveals its facilitating role in water transport.

P-417
Investigating the Fusion Efficiency of Respiratory Virus-Like Particles with Model Cell Membranes
Mahsa Mohammadian[1], Ralf Seemann[1], Jean-Baptiste Fleury[1]
[1] Department of Experimental Physics and Center for Biophysics, Saarland University, Germany

Viral infections are initiated when a virus attaches to a host cell membrane, and the virus then penetrates the cell through a process called membrane fusion. The fusion process depends on specific fusion proteins located on the viral particle surface, which contain a short, hydrophobic segment called "fusion peptide". While these peptides typically bind to their corresponding receptors on host cells to initiate the fusion process, fusion may also occur through direct cell-to-cell contact. To investigate the fusion efficiency of various fusion peptides, we create non-infectious virus-like particles (VLPs) decorated with different fusion peptides and fuse them with an artificial cell membrane with or without virus receptors. For this purpose, 3D microfluidic devices are used to create either supported or free-standing lipid bilayers and the fusion process is then studied using fluorescence microscopy. Our research aims to gain a better understanding of the interactions between virus particles and cell membranes, which can facilitate the development of new therapeutic strategies and more effective viral vectors for therapeutic applications.

P-418
FRET studies of the Pulmonary Surfactant protein C (SP-C): oligomer stoichiometry and protein-protein interactions.
Mishelle Morán-Lalangui[1], Ana Coutinho[2,3,4], Manuel Prieto[2,3], Jesús Pérez-Gil[1], Luís M.S. Loura[5,6,7], Begoña García-Álvarez[1,8]
[1] Complutense University, Spain, [2] Univ Lisbon, Inst Super Tecn, Portugal., [3] Univ Lisbon, Portugal., [4] Univ Lisbon, Portugal. , [5] Univ Coimbra, Portugal., [6] Univ Coimbra, Portugal., [7] Univ Coimbra, Portugal., [8]Complutense University, Spain.

Pulmonary surfactant (PS) is a lipid-protein complex that covers the alveolar lining film and reduces surface tension. Its main functions depend on its composition which is mainly lipids (90% of PS mass) and surfactant specific proteins (SPs). Surfactant Protein C (SP-C) is essential to the biophysical function of PS due to its ability to rearrange lipids of PS membrane during compression-expansion cycles of breathing. SP-C is the smallest protein of PS (4.2kDa) and encompasses about 1% of PS mass. The N-terminal segment of SP-C contains cysteines stoichiometrically palmitoylated, which affects the stability and structure of the molecule, and it is not embedded in the membrane, adopting an undefined structure. The C-terminal region comprises transmembrane α-helix that contains a ALLMG motif analogous to GxxxG in glycophorin A, which is well-studied for its homodimerization state. Recently, Barriga et al (2021) have shown that SP-C molecules could interact with each other using Bimolecular Fluorescence Complementation assays. In this work, the oligomerization state of SP-C in membrane systems was studied using fluorescence spectroscopy techniques. We have performed self-quenching and Förster resonance energy transfer (FRET) assays to analyze SP-C dimerization and the effect of palmitoylation state on it. We have used fluorescently labeled proteins in different lipid systems. Our results show that double palmitoylated native SP-C is mainly monomeric while recombinant protein of SP-C (rSP-C) could dimerize at high concentration and oligomerization is enhanced when there is lipid phase separation. The palmitoylation of this protein appears to inhibit the interaction between monomers of SP-C.

P-419
Interaction of a homologous series of amphiphiles with P-glycoprotein in a membrane environment – Contributions of polar and nonpolar interactions
Maria João Moreno[1,2], Hugo Filipe[1,3], Susana Cunha[1], Cristiana Ramos[1,2], Patrícia Martins[1], Biebele Abel[4], Luís Loura[1], Suresh Ambudkar[5]
[1] University of Coimbra, Portugal, [2]University of Coimbra, Portugal, [3]Center of Potential and Innovation of Natural Resources, Polytechnic of Guarda, Guarda, Portugal, [4] University of the Health Science, USA, [5] National Cancer Institute, USA

Efflux transporters limit drug bioavailability and are a major determinant of drug resistance development by cancer cells and pathogens. Although extensively studied, the molecular determinants of their specificity are still poorly understood. In this work we explore the role of polar and nonpolar interactions in the specificity of P-glycoprotein (Pgp). The interaction of a series of amphiphiles containing the polar NBD fluorescent group and different alkyl chain lengths was evaluated through effects on Pgp's ATPase activity, inhibition of [125I]IAAP binding, and partition to the whole native membranes containing Pgp. The results were complemented with partition to representative lipid bilayers, and details on the interactions established were obtained from MD simulations. We show that the commonly used formalism (ignoring ligand sequestration by the lipid bilayer) leads to apparent binding parameters that are assay dependent and do not reflect the affinity for Pgp. A new formalism is proposed that includes partition of the amphiphiles to the lipid bilayer and the binding of several molecules in Pgp's binding pocket. This allows obtaining the intrinsic binding affinities which were essentially independent on ligand hydrophobicity and assay (KM from 3 to 10 μM). An increase in the ligand's lipophilicity and amphiphilicity leads to a more efficient association with the lipid bilayer but not with Pgp. Amphiphiles with long hydrophobic tails maintain part of their tails in the lipid bilayer while the polar groups interact with Pgp's binding pocket. The presence of several amphiphiles in this orientation is proposed as a mechanism for Pgp inhibition.

Poster Presentations
– Session 2 –

P-420
Unlocking the secrets of membrane protein dynamics with innovative optical trapping-assisted fusion
Guillermo Moreno-Pescador[1], Mohammad Reza Arastoo[1], Victoria Thusgaard Ruhoff[1], Salvatore Chiantia[2], Robert Daniels[3], Poul Martin Bendix[1] [1] Niels Bohr Institute, University of Copenhagen, 2100 København Ø, Denmark, [2] Institute of Biochemistry and Biology, University of Potsdam, 14476 Potsdam, Germany, [3] Division of Viral Products, Center for Biologics Evaluation and Research, Food and Drug Administration, Silver Spring, Maryland 20993, United States

The cellular plasma membrane is a complex and dynamic structure composed of a vast landscape of different lipids and proteins. The interaction between these components regulates many vital cellular processes, making their study under control conditions extremely challenging. To overcome these difficulties, we have developed a novel approach that allows for the delivery of native plasma membranes into model systems using a combination of optical trapping, thermoplasmonic-mediated membrane fusion, and confocal imaging. By labeling proteins with fluorescent markers, we are able to monitor the lateral segregation within native plasma membranes in real time. We demonstrate the versatility and potential of this approach by investigating the phase affinity of influenza virus spike proteins at physiological relevant temperatures without requiring clustering chemicals previously used. Our generic platform can be used to investigate any plasma membrane constituent labeled with a fluorescent marker. In addition, it allows triggering of chemical reactions or ligand interactions with native membrane proteins, offering new avenues for studying dynamics of soft matter systems.

P-421
F[−] ion transport mechanism in CLC^F analyzed by molecular dynamics simulation
Akihiro Nakamura[1], Takashi Tokumasu[2], Takuya Mabuchi[2,3] [1] Graduate School of Engineering, Tohoku university, Japan [2] Institute of Fluid Science, Tohoku University, [3] Frontier Research Institute for Interdisciplinary Sciences

Many bacterial strains have evolved to be resistant to F[−] ions that inhibit bacterial growth by using exporters located in the bacterial cell membranes that rapidly reduce the intracellular F[−] ion concentration. CLC^F is a transmembrane protein as exporter that exchanges F[−] ions and protons. It has been hypothesized that a glutamate E118 (Gluex) in CLC^F rotates, transporting proton from the extracellular to the intracellular solution and driving F[−] ion through the pore. However, the transport mechanism is difficult to understand based on the experimental structure without dynamics. Therefore, we investigate the mechanism of F[−] ion export in CLC^F using molecular dynamics (MD) simulation and analyze the potential of mean force (PMF) of F[−] and Cl[−] ions to understand the transport mechanism and selectivity of F[−] ions. The results suggest that upon protonation of Gluex, a state of CLC^F is suitable for F[−] ion export.

P-422
Developments in Single-Molecule Study of Transporters with Optical tweezers
Kaori Nakao[1,2], Kasia Tych[1,2] [1] University of Groningen, Netherlands [2] Groningen Biomolecular Sciences and Biotechnology Institute

Living cells have a number of ways to regulate which solutes can pass through their membranes. One of the means is by transmembrane transport proteins. They are embedded in the plasma membrane and play important roles in recognising substrates before mediating their transport. There are various mechanisms which enable them to transport solutes, along or against the electrochemical gradient across the lipid bilayer. One group of transporters work by a common 'elevator' mechanism despite the structural diversity within the group. Here, one domain, as a rigid body, slides across the membrane against the other, together with the substrate. We are developing tools to investigate this dynamic structural change at the single-molecule level, by combining biochemical techniques with optical tweezers. These enable the direct observation of the order and kinetics of changes between the intermediate states, providing additional information that obtained from structural biology. We anticipate that the optical tweezers will become a powerful tool to describe the kinetics of transmembrane transport processes.

P-423
The effective dynamic elastic modulus of cancer cells as function of temperature and membrane order
Simon Neidinger[1,2], Isabella Kroiß[1], Anna Jötten[1,3], Christoph Westerhausen[1,2,3] [1] University of Augsburg, Institute of Physics, Experimental Physics I, Germany [2] University of Augsburg, Institute of Theoretical Medicine, Physiology, [3]Ludwig-Maximilians-Universität Munich, Center for NanoScience (CeNS)

The precise characterization of the mechanical properties of cells, e.g., by employing atomic force spectroscopy (AFS), is important for both fundamental research, and numerous applications. AFS delivers information about the topography, viscoelasticity of cells and adhesive forces. Here, we systematically compare differences in the results depending on the measurement concept. For A375 melanoma cells we find local differences in the Young's modulus E covering about one order of magnitude and a logarithmic dependence of E on the loading rate, as known for binding forces between single proteins. Moreover, we compare different tip geometries and fit models resulting in considerable differences, stressing the limitations of comparability of the data between publications. Comparing AFS data using tipless cantilevers with the results of deformation analysis of cells in microchannels we find good agreement of the obtained effective Young´s moduli. Finally, we employ rheological measurements on Hela cells to correlate membrane order and mechanical properties as function of the temperature.

Poster Presentations
– Session 2 –

P-424
Toward overcoming pyrethroid resistance in mosquito control with sodium channel blocker insecticides
Beata Niklas[1], Jakub Rydzewski[1], Bruno Lapied[2], Wieslaw Nowak[1]
[1] Nicolaus Copernicus University in Torun, Poland, [2] University Agners, France

Diseases spread by mosquitoes lead to death of 700,000 people each year. The main way to reduce transmission is vector control by biting prevention with chemicals. However, the most commonly used insecticides lose efficacy due to the growing resistance. Voltage-gated sodium channels (VGSCs), membrane proteins responsible for the depolarizing phase of an action potential, are targeted by pyrethroids and sodium channel blocker insecticides (SCBIs). Although SCBIs – indoxacarb (a pre-insecticide bioactivated to DCJW in insects) and metaflumizone – are used in agriculture only, they emerge as promising candidates in mosquito control. Therefore, a thorough understanding of molecular mechanisms of SCBIs action is urgently needed to break the resistance and stop disease transmission. In this study, by performing an extensive combination of equilibrium and enhanced sampling molecular dynamics simulations (3.2 μs in total), we found the DIII-DIV fenestration to be the most probable entry route of DCJW to the central cavity of mosquito VGSC. Results explain the role of the F1852T mutation found in resistant insects and the increased toxicity of DCJW compared to its bulkier parent compound, indoxacarb. We also delineated residues that contribute to both SCBIs and non-ester pyrethroid etofenprox binding and thus could be involved in the target site cross-resistance.

P-425
Ball-and-chain inactivation in potassium channels and its modulation by bilayer properties
Crina Nimigean[1]
[1] Weill Cornell Medical College, United States,

Inactivation is how ion channels terminate ion flux through pores while opening stimulus is still present. Inactivation of Na and K channels is crucial for action potential generation and regulation of firing frequency. It was proposed that a cytoplasmic domain of the channel plugs the open pore to inactivate it via a "ball-and-chain" mechanism, but no structural evidence existed. We used cryo-EM to determine the gating mechanism of MthK channel in lipid nanodiscs. In the presence of Ca2+, we obtained open and inactivated channels, with the N-terminus plugging the pore via a ball-and-chain inactivation mechanism. In the absence of Ca2+, we obtained a closed channel with a sterically shut bundle-crossing and with fenestrations connecting the channel pore with the membrane. We showed that these fenestrations are used by quaternary amine compounds to access the channel in the closed state, highlighting a novel state-dependent access for these compounds. Finally, we show that ball-and-chain inactivation in MthK channels is lipid bilayer-thickness dependent. We showed that this was not due to a change in the pore dimensions in the different thickness bilayers, but to a stronger interaction between inactivation ball domain and thicker bilayers. These results highlight the ease of modulating channel activity by simply changing bilayer thickness.

P-426
Influence of different membrane mimetic systems on intra-membrane protein hydration and function
Lisa Nucke[1], Yun-Hsuan Huang[1], Jana Oertel[1], Karim Fahmy[1]
[1] Helmholtz-Zentrum Dresden-Rossendorf, Germany

Active transport across biological membranes is crucial for the homeostasis of the cell. An often neglected but important molecular constituent in the transport of ions is water. However, it is challenging to monitor, quantitate and site-specifically resolve the functional role of water within membrane proteins. We have adressed these problems and studied specifically the influence of the lipid environment on ion transport and intra- membrane protein hydration in the copper-transporting ATPase CopA, a system amenable for spectroscopic observations of intra-membrane protein hydration by fluorescence solvatochromism. The recombinant protein was reconstituted into different membrane mimetic systems. Functional assay revealed differences in the activity of CopA in mixed lipid-detergent micelles as compared to protein-based (MSP1E3D1), whereas no activity was detected in polymer-based (DIBMA) nanodisc. These differences correlated with intra-membrane protein hydration as revealed by spectroscopic response to osmotic pressure changes induced by PEG. The polarity-sensitive fluorophore BADAN was used as a probe for the dielectric environment of one of two conserved cysteine residues at the protein's active (Cu-binding) site. The results show that lipid lateral pressure in MSP1E3D1 nanodiscs reduces the degree of hydration relative to detergent micelles by five to ten internal water molecules. Interestingly, the unexpected lack of functionality of CopA in DIBMA nanodiscs correlated with high lateral pressure on the transmembrane region which may constrain functionally important hydration changes.

P-427
NMRlipids Databank makes data-driven analysis of biomembrane properties accessible for all
Samuli Ollila[1], Anne Kiirikki[1], Hanne Antila[2], Lara Bort[3], Pavel Buslaev[4], Fernando Favela[5], Tiago Ferreira[6], Patrick Fuchs[7], Rebeca Garcia- Fandino[8], Ivan Gushchin, Batuhan Kav[9], Patrik Kula[10], Milla Kurki[11], Alexander Kuzmin, Jesper Madsen[12], Markus Miettinen[13], Ricky Nencini[1], Thomas Piggot[14], Angel Pineiro[15], Suman Samantray, Fabian Suarez-Leston[8]
[1] University of Helsinki, Finland [2] Max Planck Institute of Colloids and Interfaces, [3] University of Potsdam, [4] University of Jyväskylä, [5] Tecnologico Nacional de Mexico, [6] Martin Luther University Halle-Wittenberg, [7] Sorbonne Universite, [8] Universidade de Santiago de Compostela, [9] Forschungszentrum Julich, [10] Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, [11] University of Eastern Finland, [12] University of South Florida, [13] University of Bergen, [14] University of Southampton, [15] University of Santiago de Compostela

Cellular membrane lipid composition is implicated in diseases and major biological functions in cells, but membranes are difficult to study experimentally due to their intrinsic disorder and complex phase behaviour. Molecular dynamics (MD) simulations have been useful in understanding membrane systems, but they require significant computational resources and often suffer from inaccuracies in model parameters. Applications of data-driven and machine learning methods, currently revolutionizing many fields, are limited to membrane systems due to the lack of suitable training sets. Here we present the NMRlipids Databank, a community-driven, open-for-all database featuring programmatic access to quality evaluated atom-resolution MD simulations of lipid bilayers. The NMRlipids databank will benefit scientists in different disciplines by providing automatic ranking of simulations based on their quality against experiments, flexible implementations of data-driven and machine learning applications, and rapid access to simulation data via graphical user interface. To demonstrate the unlocked possibilities beyond current MD simulation studies, we analyzed how anisotropic diffusion of water and cholesterol flip–flop rates depend on membrane properties.

Poster Presentations
– Session 2 –

P-428
STUDYING THE IMPACT OF VAPE LIQUID ADDITIVE, α-TOCOPHERYL ACETATE, ON PULMONARY SURFACTANT

Agnieszka Olżyńska[1], Wojciech Pajerski[1], Hanna Korolainen[2], Ilpo Vattulainen[2], Waldemar Kulig[2], Lukasz Cwiklik[1]
[1] J. Heyrovský Institute of Physical Chemistry, Czech Academy of Sciences, Prague, Czech Republic, [2] Department of Physics, University of Helsinki, Helsinki, Finland

Pulmonary surfactant (PS) is a lipid-protein film present at the aqueous-air interface in the alveoli of the lungs that undergoes significant periodic restructuring during breathing. Functionally, PS serves as a semi-permeable barrier between the inhaled air and the lungs, lowers the interface surface tension, and protects from the alveoli collapse. Consequently, any alteration of the structural and mechanical properties of PS can lead to the destabilization of the alveoli interface and worsen its functions. One potential agent that could trigger such unfavorable modifications in PS is α-Tocopheryl acetate and its topical application in the form of inhalation is suspected to be harmful. Specifically, the presence of α-Tocopheryl acetate in the vape liquids could be related to the condition of vaping-associated pulmonary injury (VAPI). We developed and used a novel in vitro system consisting of an environmental chamber with a model pulmonary surfactant, allowing us to investigate directly the influence of vape smoke enriched with α-Tocopheryl acetate on PS film. Furthermore, we studied model PS supplemented with α-Tocopheryl acetate performing epifluorescence microscopy measurements with implemented Langmuir technique parallel with molecular dynamics simulations. Our results clearly show that α-Tocopheryl acetate influences the structural and mechanical properties of both model PS and poractant alfa. It incorporates into PS film at low surface pressure and restructures it at higher pressures. It could explain the changes observed in the lungs of patients suffering the VAPI.

P-429
Cholesterol's role in cell membranes is even more multifaceted

Hanna Orlikowska-Rzeznik[1], Jan Versluis[2], Huib J. Bakker[2], Lukasz Piatkowski[1]
[1] Poznan University of Technology, Poland [2] AMOLF, Ultrafast Spectroscopy

Cholesterol is a key component of mammalian cell membranes and biomimetic drug-delivery vesicles. It is well known for regulating the dynamics and structure of lipid bilayers as well as mediating membrane trafficking, signal transduction, and host-pathogen interactions. Yet, there is an ongoing debate regarding its effect on the hydration properties of membranes. The orientation of interfacial water molecules can affect the electrical potential of the membranes as well as the strength and specificity of their interactions with peripheral proteins and other molecules, which in turn can affect their function. To address this, we employed surface-specific heterodyne-detected vibrational sum-frequency generation (HD-VSFG) spectroscopy to provide molecular-level insights into the effect of cholesterol on the hydration properties of zwitterionic lipid monolayers at the air/water interface. Results indicate that cholesterol reduces the anisotropic, non-uniform orientational distribution of interfacial water molecules, which for pure neutral zwitterionic lipids, interestingly, is prominent. Our intriguing finding adds to the multiple actions of cholesterol toward lipid membranes and has important implications for our understanding of cell membrane biophysics and designing biomimetic drug-delivery systems. This work has been supported by the Polish National Agency for Academic Exchange (NAWA) under the STER programme, Towards Internationalization of Poznan University of Technology Doctoral School (2022-2024). This work was co-financed from the budget funds allocated for science in the years 2019–2023 as a research project under the Diamond Grant program 0042/DIA/2019/48 and by National Science Centre, Poland 2020/37/B/ST4/01785.

P-430
The molecular identity of TNF receptor-ligand complexes in cells revealed by quantitative single-molecule localization microscopy

Tanja Ott[1], Isabell Lang[2], Marius Glogger[1], Mathilda Glaesmann[1], Christos Karathanasis[1], Claudia Catapano[1], Marina S. Dietz[1], Sjoerd van Wijk[3], Harald Wajant[2], Mike Heilemann[1]
[1] Institute of Physical and Theoretical Chemistry, Goethe-University Frankfurt, Max-von-Laue Strasse 7, 60438 Frankfurt, Germany, [2] Division of Molecular Internal Medicine, Department of Internal Medicine II, University Hospital Würzburg, Auverahaus, Grombühlstrasse 12, 97080 Würzburg, Germany, [3] Institute for Experimental Cancer Research in Paediatrics, Goethe-University Frankfurt, Komturstrasse 3a, 60528 Frankfurt, Germany

The tumor necrosis factor receptor (TNFR) superfamily constitutes cell membrane receptors that orchestrate cell signaling and immune responses in health and disease. The diverse cellular responses initiated by different TNFRs are determined by the molecular identity of receptor clusters that initially form in the cell membrane. Understanding this "molecular code" demands for experimental methods that can probe the composition of such protein assemblies in intact cells. Here, we use single-molecule localization microscopy (SMLM), a super-resolution microscopy method that achieves near-molecular spatial resolution, enables multi-target imaging and molecular quantification. We developed a strategy for stoichiometric labeling of TNFR1/2 and its ligand TNFα, colocalized receptor-ligand complexes in intact cells, and extracted the oligomeric state of TNFR1/2 prior and post ligand stimulation. Our imaging and analysis approach is transferable to other membrane-protein signaling hubs.

P-431
Biophysical study on Small extracellular vesicles (sEVs) interaction with model plasma membrane

Carolina Paba[1,2], Beatrice Senigagliesi[3], Nicolò Tormena[4], Pietro Parisse[2,5], Kislon Voitchovsky[4], Loredana Casalis[2]
[1] University of Trieste, Italy [2] Elettra Sincrotrone Trieste, [3] IINS Bordeaux, [4] Durham University, [5] CNR-IOM

Small extracellular vesicles (sEVs) represent nowadays the most promising communication route between distant cells for delivering biological information throughout the body, strongly impacting the fate of recipient cells. Despite their extreme biological relevance, sEVs small size (30–500 nm) and heterogeneity still pose a great challenge to the characterization of their function. Moreover, a high spatial and temporal resolution is required for the detection of the molecular players involved in EVs' uptake and internalization pathways, e.g. cell membrane subdomains, called lipid rafts, involved in sEVs-mediated endocytosis. Here we propose a time-resolved Atomic Force Microscopy (AFM) study on supported lipid bilayer. (LBL) systems displaying a phase separation that mimics the presence of lipid rafts, to highlight the biophysical/biochemical characteristics that regulate triple-negative breast cancer sEVs fusogenic pathway and membrane remodelling. We focused on a mixed-phase LBL, formed by a liquid-disordered phase given by DOPC and a solid-ordered (So)/liquidorder phase given by SM or DPPC enriched with 0-17% cholesterol concentration, respectively. We found a preferential interaction of sEVs with the ordered phase, regardless of the chemical composition of such domains, as well as an increased fusion with such domains with increasing cholesterol, i.e. fluidity of the ordered phase. Our findings suggest that monitoring/tuning the biophysical characteristics of cell membranes might be instrumental to detect/regulating EVs uptake for biosensing/EVs based drug delivery applications.

Poster Presentations
– Session 2 –

P-432
Vascular lipid droplets and endothelial Na+ signalling: brothers in arms or partners in crime?
Marta Z. Pacia[1], Natalia Chorazy[1,2], Kamila Wojnar-Lason[1,3], Stefan Chlopicki[1,3]
[1] Jagiellonian University, Jagiellonian Centre for Experimental Therapeutics, Bobrzynskiego 14, Krakow, Poland, [2] Jagiellonian University, Doctoral School of Exact and Natural Sciences, Lojasiewicza 11, Krakow, Poland, [3] Jagiellonian University, Chair of Pharmacology, Grzegorzecka 16, Krakow, Poland

The formation of vascular lipid droplets (LDs) is thought to be a protective mechanism against cellular stress. Hypoxia results in the formation of LDs that could be linked to altered cellular homeostasis, including increased sodium influx into endothelial cells. Here, we hypothesized that the formation of endothelial LDs is mediated by the Na+ level, and inhibitors of endothelial sodium influx inhibit the formation of LDs. In the present work, we have characterized the formation of LDs in endothelium in isolated murine blood vessels under hypoxic conditions by manipulating pathways that lead to the formation and degradation of LDs such as diacylglycerol transferase (DGAT-1) and adipose triglyceride lipase (ATGL). We have suggested that HIF-α-dependent hypoxia-inducible lipid droplet-associated protein (HILPDA) was responsible for the impairment of LDs breakdown by inhibiting ATGL and promoting DGAT-1. The enhanced formation of vascular LDs under hypoxic conditions was inhibited by sodium transport inhibitors, including empagliflozin (inhibitor of sodium-glucose cotransporters, SGLT2-I) and cariporide (inhibitor of the sodium-hydrogen exchanger, NHE-I). Furthermore, in the presence of cariporide that profoundly inhibited LDs formation, empagliflozin did not further affect hypoxia-induced LDs formation, pointing out that empagliflozin might have attenuated LDs formation through inhibition of NHE. In summary, our results suggest that the formation of vascular LDs in hypoxia is a Na+-dependent process, revealing hitherto unknown aspects of the pathobiology of vascular LDs.

P-433
Solid-State NMR footprints of the Growth Hormone Secretagogue Receptor in different conformational states
Emelyne Pacull[1], Sabrina Stecher[1], Peter Schmidt[1], Holger A. Scheidt[1], Daniel Huster[1]
[1] Leipzig University, Germany

The well-described physiological A204E mutant of the Growth Hormone Secretagogue Receptor (GHSR) leads to obesity and short stature in patients. This mutant GHSR is lacking its characteristic constitutive activity of 50%, a seldom feature among G protein-coupled receptors (GPCRs). Using 13C-13C DARR NMR, we aim to investigate the conformational changes of wild-type and A204E GHSR upon ligand binding by monitoring the changes in the local chemical environment of the receptor's native histidines as a variation of their 13C NMR chemical shift. These three histidines, conveniently located in sensitive receptor sites (helix 6 and extracellular loop 2), were 13C labelled through an established cell-free expression system where the labeled GHSR is expressed in the precipitated form with a yield of up to 1.5 mg per 1 mL reaction volume and subsequently functionally reconstituted into DMPC bilayer membranes. Upon ghrelin binding, the two helical histidine residues showed characteristic downfield shifts indicative of the structural alterations in the molecule upon the outward movement of helix 6. In contrast, the shifts for the constitutive activity mutant highlighted the existence of an alternative conformation that can be rescued by ghrelin binding.

P-434
Molecular rotors as tools to explore the mechanical behaviour of lipid membranes under stress
Miguel Paez Perez[1], Nicholas Brooks[1], Marin Kuimova[1]
[1] Imperial College London, United Kingdom

The cell's lipid membrane plays a central role in cellular adaptation, homeostasis, and disease. This is achieved thanks to the intricate molecular interactions between the membrane components, which are ultimately reflected in the membrane's mechanical behaviour. As such, there has been a growing interest in understanding how the biophysical properties of the cell's lipid membrane are affected by pathogenic stress. Here, we explore the relationship between the membrane's molecular structure and its mechanical behaviour by combining the unique capabilities of molecular rotors (MRs), fluorescent molecules capable of reporting on their microviscosity, with fluorescence lifetime imaging microscopy (FLIM), together with X-Ray diffraction (XRD) characterization. Using this approach, we examined how the mechanical behaviour of lipid membranes changes during drug treatment, under mechanical load, under oxidative stress, or in the presence of atherogenic trans-fatty acids. Our results show how the membrane's composition influences the rheological properties and the lateral organization of lipid membranes. We monitor how these changes lead to the uncoupling of the membrane's viscoelastic properties, i.e. a higher viscosity corresponds to a lower bending rigidity. The emergence of such non-classical behaviour can be linked to an increased resilience of the cell membrane to pathogenic stress, emphasizing the importance of the membrane's mechanics in disease development.

P-435
Amphipathic helices can sense negative membrane curvature
Peter Pajtinka[1,2], Robert Vácha[1,2,3]
[1] CEITEC - Central European Institute of Technology, Masaryk University, Kamenice 753/5, 625 00 Brno, Czech Republic, [2] National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic, [3] Department of Condensed Matter Physics, Faculty of Science, Masaryk University, Kotlářská 267/2, 611 37 Brno, Czech Republic

Proteins that sense and respond to membrane curvature are essential for biological processes such as vesicle trafficking, endocytosis, and cell migration. These proteins are able to localize to membranes with specific curvatures, allowing them to perform their functions with high precision. The most prominent class of curvature sensors - BAR domains - can sense either positive or negative membrane curvature, depending on the shape of their membrane-binding surface. Many short amphipathic helices (AHs) have also been shown to sense positive membrane curvature, but no AH with a preference for negative membrane curvature has been discovered yet. Using a systematic computational approach, we derived AHs that preferentially localize to negatively curved membrane regions in both coarse-grained and atomistic simulations. In addition, we identified properties of AHs that are necessary for sensing positive or negative membrane curvature. Therefore, our results reveal a novel mechanism by which the amphipathic helices can contribute to the membrane localization of peripheral and transmembrane proteins.

Poster Presentations
– Session 2 –

P-436
Gamma-Hemolysin Components: Computational Strategies for LukF-Hlg2 Dimer Reconstruction on a Model Membrane

Costanza Paternoster[1,2], Thomas Tarenzi[1,2], Raffaello Potestio[1,2], Gianluca Lattanzi[1,2]
[1] University of Trento, Italy [2] INFN-TIFPA-Trento Institute for Fundamental Physics and Applications

Gamma-hemolysin protein represents one of the most common pore-forming toxins expressed by the pathogenic bacterium Staphylococcus aureus. The toxin is used by the pathogen to escape the immune system of the host organism, by assembling into octameric transmembrane pores on the surface of the target immune cell and leading to its death by leakage or apoptosis. Despite the high potential risk associated with Staphylococcus aureus infections and the urgent need for new treatments, several aspects of the pore formation process from gamma-hemolysin are still unclear. These include the identification of the interactions between the individual monomers that lead to the formation of a dimer on the cell membrane, which represents the unit for further oligomerization. Here, we employed a combination of all-atoms explicit solvent molecular dynamics simulations and protein-protein docking to determine the stabilizing contacts that guide the formation of a functional dimer. The simulations and the molecular modeling concur to reveal the importance of the flexibility of specific protein domains, in particular the N-terminus, to drive the formation of the correct dimerization interface through functional contacts between the monomers. The results obtained are compared with the experimental data available in the literature.

P-437
Substrate and membrane interactions of the glycosyltransferase WaaG investigated by site-specific 19F labeling and NMR

Pontus Pettersson[1], Joan Patrick[1], Lena Mäler[1]
[1] Department of Biochemistry and Biophysics, Stockholm University, Sweden

WaaG from E. coli is a glycosyltransferase (GT) participating in the synthesis of the lipopolysaccharide (LPS), a major component of the Gram- negative outer membrane. WaaG is a type B GT, consisting of two Rossman fold domains separated by a cleft containing the active site. To perform its function WaaG brings a bulky, membrane-bound, nascent LPS and the sugar donor UDP-glucose together which requires conformational flexibility between the domains and membrane interaction. The large GT-B enzymes and their complex dynamics make them inaccessible to investigation by standard multidimensional NMR. Instead WaaG's native and inserted Trps were fluorinated at a single position, allowing collection of 19F NMR spectra from this highly sensitive nucleus also displaying a large chemical shift range. Spectra revealed dynamics stabilization throughout WaaG in presence of UDP-glucose or membrane mimetics relative to in solution, and a conformational change together with UDP-glucose likely due to transition into a 'closed' inter-domain conformation. A paramagnetic probe was furthermore used to confirm WaaG's N-terminal domain positioning relative the membrane.

P-438
Allosteric Modulation of Integral Protein Activity by Differential Stress in Asymmetric Membranes

Paulina Piller[1], Enrico F. Semeraro[1], Gerald N. Rechberger[1], Sandro Keller[1], Georg Pabst[1]
[1] University of Graz, Austria

The activity of integral membrane proteins is tightly coupled to the properties of the surrounding lipid matrix. In particular, transbilayer asymmetry, a hallmark of all plasma membranes, might be exploited to control membrane-protein activity. Here, we hypothesized that the membrane-embedded enzyme outer membrane phospholipase A (OmpLA) is susceptible to the lateral pressure differences that build up between such asymmetric membrane leaflets. Upon reconstituting OmpLA into synthetic, chemically well-defined phospholipid bilayers exhibiting different lateral pressure profiles, we indeed observed a substantial decrease in the enzyme's hydrolytic activity with increasing membrane asymmetry. No such effects were observed in symmetric mixtures of the same lipids. To quantitatively rationalize how the differential stress in asymmetric lipid bilayers inhibits OmpLA, we developed a simple allosteric model within the lateral-pressure framework. Thus, we find that membrane asymmetry can serve as the dominant factor in controlling membrane-protein activity, even in the absence of specific, chemical cues or other physical membrane determinants such as hydrophobic mismatch.

P-439
Biophysical assessment of aquaporin-3 inhibition by the natural compound rottlerin

Catarina Pimpão[1,2], Inês Paccetti-Alves[1,2], Marta S. P. Batista[3], Bruno L. Victor[3], Graça Soveral[1,2]
[1] Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, 1649-003 Lisbon, Portugal, [2] Department of Pharmaceutical Sciences and Medicines, Faculty of Pharmacy, Universidade de Lisboa, 1649-003 Lisbon, Portugal, [3] Biosystems and Integrative Sciences Institute, Faculty of Sciences, Universidade de Lisboa, 1649-003 Lisbon, Portugal

The polyphenolic compound Rottlerin (RoT) showed anticancer properties in a variety of human tumors by inhibiting several proteins that are implicated in tumorigenesis. However, the effect of RoT in aquaporins (AQPs), membrane channels that are implicated in cancer progression and metastasis, has never been studied. AQPs are specialized transmembrane protein channels that facilitate the passive transport of water, glycerol and other small solutes through cell membranes in response to osmotic gradients. AQPs are found overexpressed in different types of cancers being promising anticancer pharmacological targets. Increasing evidence has suggested that the water and glycerol channel aquaporin-3 (AQP3) plays a key role in cancer cell migration, proliferation and invasion. Here, we investigated the inhibitory effect of RoT on AQP3 activity by permeability assays using stopped-flow spectroscopy, and by computational approaches. RoT was found to inhibit human AQP3 activity on red blood cells, with an IC50 in the micromolar range (22.8 ± 5.82 μM for water and 6.7 ± 2.97 μM for glycerol permeability inhibition). These results were confirmed by inhibition assays in yeast cells transformed to overexpress human AQP3. Moreover, through molecular docking and molecular dynamics simulations we observed that RoT blocks AQP3-glycerol permeation by establishing strong and stable interactions at the extracellular region of AQP3 pores interacting with residues essential for glycerol permeation. Altogether, our results disclose RoT as a potent AQP3 inhibitor and with potential use as an anticancer drug against tumors where AQP3 is highly expressed.

Poster Presentations
– Session 2 –

P-440
PIP2 binding at the voltage sensor domain modulates KCNQ1 VSD activation
Tanadet Pipatpolkai[1], Lu Zhao[2], Darko Mitrovic[1], Jianmin Cui[2], Lucie Delemotte[1]
[1] KTH Royal Institute of Technology, Sweden [2] Washington University in St. Louis

The KCNQ1 channel is a voltage-gated potassium channel involved in cell repolarisation, and the mutations on the channel are associated with cardiac arrhythmia, which can cause sudden cardiac arrest. The voltage sensor domain (VSD) of the KCNQ1 channel senses membrane depolarisation, resulting in a conformational change which then couples to the pore opening. The recently solved structure of KCNQ1 has revealed a phosphatidylinositol-4,5-bisphosphate (PIP2) binding to VSD, leading to our proposing the role that PIP2 may stabilise a certain VSD conformation. Here, we used fluctuation application of specific traits (FAST) to capture VSD deactivation at hyperpolarising voltages. Our simulation proposes that PIP2 binding at the VSD changes the pattern of salt-bridge formation at the bottom of the S4 helix and may act as a counter-charge pausing point during the voltage-dependent activation process. This may suggest how PIP2 stabilises a certain VSD conformation on the KCNQ1 channel, leading to a different open state. Together, our work highlights the importance of PIP2 in regulating VSD activation, in addition to our previous knowledge as the key coupler between the VSD and the pore.

P-441
Modulating membrane tension of giant vesicles using Atlastin
Shreya Pramanik[1], Amelie S. Benk[2], Miroslaw Tarnawski[2], Joachim Spatz[2], Rumiana Dimova[1], Reinhard Lipowsky[1]
[1] Max Planck Institute of Colloids and Interfaces, Science Park West, Potsdam, Germany, [2] Max Planck Institute for Medical Research, Jahnstrasse 29, Heidelberg, Germany

The bottom-up approach to synthetic biology aims at making functional cells or organelles with minimal components. Most of these cell organelles have a lipid bilayer surrounding them that separates the inner aqueous medium from the outer aqueous medium. Many protocols can be employed to make closed lipid bilayer systems from commercially available lipids. Generally, these systems are spherical with size ranges of diameters from nanometers to micrometers. However, the endoplasmic reticulum (ER) is made of a continuous membrane that extends throughout the cell interior. It forms a complex network of nanotubes connected by three-way junctions. These nanotubes form irregular polygons with contact angles of approximately 120°. Atlastin, a dynamin-like GTPase, is known to form and maintain networks mimicking the ER. Dimerization of the Atlastin on two membranes facilitates the fusion of the membrane segment but the reason for the dimerization of Atlastin on the same membrane remains unknown. We hypothesize that this process of homo-dimerization could generate membrane tension necessary to maintain the irregular polygonal geometry of ER and prevent it from breaking apart. In this work, we optimize the protocol for reconstituting the Atlastin into a giant unilamellar vesicle (GUV) using detergent-mediated incorporation in the membrane. The proteo-GUVs would make it possible to systematically study the ER network morphology and the effective membrane tension using optical microscopy.

P-442
Phase separation and dynamics on "hybrid polymer/lipid vesicles", from time-resolved fluorescence and microscopy.
Manuel Prieto[1], Fábio Fernandes[1], T. Tuyen Dao[1]
[1] iBB—Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, Lisboa, Portugal

Mixing up lipids and polymers, a new entity is obtained the "hybrid polymer/lipid vesicle", aiming to reach an optimization of the characteristics of both partners in a single vesicle, such as the biocompatibility of lipids as observed in liposomes, and the mechanical properties of pure polymer vesicles, the "polymersomes". This work deals with its biophysical characterization, and a detailed study of phase separation (lipid and polymer domains) was carried out by microscopy using Giant Hybrid Vesicles, using polymer and lipid fluorescent probes. Several types of polymers with different hydrophobic thicknesses were studied, which allowed to rationalize its influence on vesicles morphology (e.g., budding alterations vs. increasing of line tension), and different types of polymer architecture were considered (triblock vs. grafted). For the situation where no macroscopic phase separation is observed, the so-called "homogeneous distribution" under the confocal microscope, the detection of nano-domains was carried out by FRET-FLIM (donor in the polymer and acceptor in the lipid). The same nanodomains were also detected in Large Unilamellar Hybrid vesicles (LUHVs), in this case using quantitative approaches for FRET in two dimensions, in order to obtain the theoretical expectations both for "homogeneous distribution" and "infinite phase separation". The lateral diffusion coefficients were determined by FRAP, and it was concluded that the presence of lipid domains hinders polymer diffusion, at variance with the diffusion of lipids which is not slowed down.

P-443
Elucidating the molecular basis of voltage- and lysophospholipid-induced TRPC5 channel gating
Alexandra Ptakova[1,2], Michal Mitro[1,3], Lucie Zimova[1], Ivan Barvik[4], Viktorie Vlachova[1]
[1] Department of Cellular Neurophysiology, Institute of Physiology, Czech Academy of Sciences, Prague, CZ, [2] Department of Physiology, Faculty of Science, Charles University, Prague, CZ, [3] Department of Genetics and Microbiology, Faculty of Science, Charles University, Prague, CZ, [4] Division of Biomolecular Physics, Institute of Physics, Faculty of Mathematics and Physics, Charles University, Prague, CZ

TRPC5 channel has recently emerged as a novel potential target for the treatment of peripheral and visceral pain, and its abnormal functioning has been linked to pathophysiologically increased levels of lysophosphatidylcholine (LPC). TRPC5 can be also tightly regulated by membrane voltage. The underlying molecular mechanisms of the LPC- and voltage-induced TRPC5 regulation are poorly understood. We measured whole-cell membrane currents from recombinant human TRPC5 channels expressed in HEK293T cells in response to LPC 18:1 at various membrane potentials (-100 to +200 mV). To study the structural bases of these modes of regulation, we used molecular dynamics simulations. Replacing the highly conserved tryptophan residue 577 by alanine rendered TRPC5 completely insensitive to depolarizing voltage, but LPC activated this mutant to a similar extent as the WT channel. Substitution of conserved glycine 606, located directly opposite W577, with tryptophan rescued the channel sensitivity to the voltage and LPC. Given the considerable degree of homology between TRP proteins and the similar role of lipids in their regulation, these results may provide insights in the search for a general mechanism of TRP voltage and lipid activation and may help identify sites of effective pharmacological targeting of TRPC5.

Poster Presentations
– Session 2 –

P-444
Electrical excitability-enhancing action of Inositol 1,4,5 trisphosphate (IP3) on macroalgae Nitellopsis obtuse
Vilmantas Pupkis[1], Judita Janužaitė[1], Indrė Lapeikaitė[1], Vilma Kisnierienė[1]
[1] Life Sciences Center, Vilnius University, Lithuania

In response to various environmental stimuli plants generate electrical signals that transfer information about the stressor and induce diverse physiological responses. The involvement of inositol 1,4,5 trisphosphate (IP3) in the initiation of plant action potentials via the activation of Ca2+ channels has been reported but remains contested since no IP3 receptors have been documented in plants. In this study, intracellular microelectrode two-pair current clamp and voltage clamp techniques were employed to analyze the effect of externally applied IP3 on the parameters of action potentials and excitation transients of the macroalgae Nitellopsis obtusa internodal cells. The excitability-enhancing effect of IP3 was manifested via the hyperpolarization of the action potential excitation threshold. In several cases, IP3 induced trains of spontaneous action potentials. However, the amplitudes of the electrically-induced depolarizing currents during excitation were not affected. These effects imply that endogenous IP3 modulates the voltage dependence of Ca2+ channels that release Ca2+ from internal stores into the cytoplasm and not the cytoplasmic calcium level during excitation. Presented data support the role of IP3 as a second messenger activating the Ca2+ channels responsible for the initiation of action potential generation in plants. However, the molecular mechanism of IP3 action remains to be elucidated.

P-445
Multiple Intermediate States in the Detergent-Induced Fusion of Single Lipid Vesicles
Steven Quinn[1,2], Lara Dresser[1], Casper Kunstmann[1], Donato Conteduca[1], Steven Johnson[1,2], Mark Leake[1,2,3]
[1] School of Physics, Engineering and Technology, University of York, Heslington, York, UK [2] York Biomedical Research Institute, University of York, Heslington, York, UK [3] Department of Biology, University of York, Heslington, York, UK,

The structure, dynamics and function of lipid vesicles are heavily influenced by a range of physical forces, local microenvironmental effects and interactions with perturbative molecules, including detergents. Detergent-induced membrane solubilization – critical for biotechnological applications including protein extraction and virus inactivation – varies in magnitude according to the detergent type and membrane composition, but the underlying mechanistic details remain largely under explored. By using a lipid mixing assay based on single-molecule Förster resonance energy transfer (smFRET), and single-vesicle characterization approaches, we find that highly-curved sub-micron sized vesicles are induced to fuse by the widely-used non-ionic detergent Triton-X 100. We show that the fusion process is a dynamic and controllable multi-step process characterized by discrete values of smFRET efficiency between membrane-embedded donor and acceptor fluorophores and broadly involves vesicle docking, structural remodelling, hemi-fusion and full lipid mixing, even at sub-solubilizing detergent concentrations. We present evidence that the fusion process is regulated by factors including cholesterol content, membrane composition and phase, and we dissect the kinetics of vesicle fusion via quantitative analysis of the conformation of immobilized vesicles using a label free quartz-crystal microbalance with dissipation monitoring approach. Our strategies are applicable beyond the vesicle sizes and compositions studied here, and not only provide insight into the multifaceted dynamics of vesicle fusion but may have important implications for vesicle trafficking mechanisms, surfactant sensing and drug delivery processes.

P-446
Investigating contact formation and biophysical properties at the reconstituted immune synapse
Franziska Ragaller[1], Luca Andronico[1], Adnane Achour[2], Erdinc Sezgin[1]
[1] Science for Life Laboratory, Department of Women's and Children's Health, Karolinska Institutet, Solna, Sweden, [2] Science for Life Laboratory, Department of Medicine, Karolinska Institutet, Solna, Sweden

The immune synapse is a spatiotemporally highly organised cell-cell contact between immune cells and their target cells, in which signaling events are tightly controlled. At this contact, the plasma membrane biophysical properties strongly influence the nature and dynamics of protein-protein interactions it harbours. This highlights the need for detailed investigation of biophysical properties alongside protein-protein interactions, to gain a comprehensive understanding of molecular mechanisms at the immune synapse. Here, the immune synapse was reconstituted using model membrane systems alone or together with live cells. The use of synthetic membranes permitted investigation of defined lipid environments and specific ligand receptor pairs, which influence biophysical properties and contact formation at the immune synapse. The formation of and diffusion dynamics within the contact were quantified by confocal imaging and scanning FCS, respectively. Utilising environment-sensitive fluorescent probes and subsequent spectral imaging, the biophysical properties at the immune synapse were examined. In summary, we could create a straightforward and widely applicable cell-cell contact reconstitution model, which allows the investigation of contact formation, diffusion dynamics of specific ligand-receptor pairs as well as biophysical properties at the immune synapse.

P-447
A closer look at the dynamics of single T-Cell Receptors within TCR microclusters.
Neetu Rajendran[1], Christian Wald[2], Gerhard J Schütz[1]
[1] Technische Universität Wien, Austria, [2] Technische Universität Berlin, Germany

T cells are part of our adaptive immune system and T cell activation is the process by which T cells recognize and respond to antigens presented via Antigen Presenting Cells (APC). Within seconds of antigen recognition by T cells, T-cell receptors (TCRs), which are important transmembrane proteins of T cells, form microclusters. These microclusters are considered important as they are believed to be the structures where signaling for T cell activation is initiated. But the dynamics of single TCRs within these structures is still enigmatic. The aim of the project is to utilize super- resolution fluorescence microscopy to investigate the dynamics of single TCRs between and within microclusters. TCRs are fluorescently labelled using two different dyes. The density of labelling of one of the dyes is kept significantly higher than the other, which allows to simultaneously track TCR microclusters and single TCRs in microclusters. By studying the dynamics of single TCRs within microclusters and investigating the possibility of TCR movement between microclusters, the project hopes to gain more insights into the role of TCR microclusters in T cell activation.

Poster Presentations
– Session 2 –

P-448
Studying GPI-anchored proteins complexes on model membranes
Shahneda Ramadan[1,2], Paula Guerrero[2], Nikolai Klishin[1], Ruslan Nedielkov[1], Rumiana Dimova[2], Daniel Varón Silva[3], Heiko M. Möller[1]
[1] University of Potsdam, department of Analytical Chemistry, Germany [2] Max Planck Institute of Colloids and Interfaces (MPIKG), [3] Fachhochschule Nordwestschweiz (FHNW), School of Life Science

Glycosylphosphatidylinositol (GPIs) are glycolipid anchors uniquely present on the surface of eukaryotic cells. Proteins docked by GPI participate in the protection, regulation, and activation of cells via protein-protein interactions. The effect of GPI anchors on the functionality of anchored proteins remains vaguely understood due to the difficult isolation and synthesis of homogenous GPI-protein complexes. Herein, we combine recombinant protein expression, ligation reactions, and chemical synthesis in a multi-disciplinary approach to obtain well-defined GPI-protein complexes. We present the expression of the 19 kDa fragment of the Plasmodium berghei merozoite surface protein 1 (MSP1-19) fused to a selectively reactive domain. We characterize the expressed protein and show its cleavage from the reactive domain via nuclear magnetic resonance and mass spectrometry. As a next step, we will ligate the protein to a GPI model anchor. This protein-GPI complex will be incorporated into giant unilamellar vesicles (GUVs) as cell-sized model membranes. Using fluorescence microscopy-based techniques, we will evaluate the behavior of the conjugated MSP1-19 GPI complex in the membrane, and study characteristics such as partitioning between membrane phases (liquid-ordered and liquid-disordered), clustering and interaction with other proteins.

P-449
Exploring the Proton Transport Properties along Lipid Membranes
Ambili Ramanthrikkovil Variyam[1], Nadav Amdursky[1]
[1] Technion-Israel Institute of Technology, Israel

The process of proton diffusion has an important role in critical processes as diverse as cellular respiration in mitochondria and bacteria, the light- driven production of ATP, and acid secretion in epithelia. How exactly the protons migrate along lipid membranes and reach at specific membrane proteins still remains unresolved. In addition, the role of various lipids which possess different types of head groups or aliphatic tails, in membranes of distinct compositions of lipids, and the influence of physical properties of lipids like melting point & membrane fluidity on the regulation proton translocation need to be explored in depth. In this context, we have synthesized a fluorescent and photoacid probe, C12-HPTS (alkyl chains attached pyranine probe), which can be nicely tethered inside membranes owing to the lipophilic nature of the molecule to explore the proton transport properties of membranes made of two distinct lipids. The probe acts as a proton donor at the excited state and the time resolved fluorescence techniques can be adopted to monitor the released protons. The introduction of the probe into lipid vesicles made of binary mixtures of POPA (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphate) and POPC (1-palmitoyl-2-oleoyl-glycero-3-phosphocholine) results a nonlinear trend for proton transfer rates from C12-HPTS to the membrane concerning the amount of POPA/POPC inside vesicles. This observation points out the significance of lipid composition on regulating proton diffusion properties along membranes.

P-450
Exploring the conformational states of experimental and predicted respiratory complex I structures
Markus Rauhalahti[1]
[1] University of Helsinki, Finland

Recent advances in experimental methods, particularly in high-resolution cryo-EM, and Alphafold2's breakhtrough in structure prediction, have led to a revolution in structural biology. The advances are particularly evident in the study of large biomolecular complexes. Respiratory complex I (CI) is the first enzyme in the respiratory chain responsible for energy production in mitochondria and bacteria. It is a large protein complex consisting of ca. 45 individual subunits, which efficiently catalyze proton translocation across the membrane in strong coupling to the reduction of ubiquinone. Even though the proton pumping mechanism of CI remains to be understood, the advent of high-resolution Cryo-EM structures and molecular simulations have brought understanding of several catalytic states that may form during enzyme turnover. In this study, we conducted a comprehensive analysis of all published experimental CI structures alongside their Alphafold2 predictions, examining their conformational landscape and the structural features associated with the catalytically relevant states. To enable this analysis, we developed automated and general pipelines for processing the heterogeneous structural data. Our findings contribute to a deeper understanding of the structural determinants associated with CI's various states, offering valuable insights into the enzyme's function and mechanism. The automated data processing methods have a potential for broad applicability within the structural biology community, enabling researchers to harness the power of experimental and predicted structural data in tandem.

P-451
Molecular dynamics simulations highlight structural features of lipid scramblases activity.
Cristian Rocha-Roa[1], Stefano Vanni[1]
[1] Department of Biology, University of Fribourg Chemin du Musée 10, 1700 Fribourg, Switzerland.

Nearly a quarter of the proteins encoded in most organisms are membrane proteins, and about 50% of the membrane mass are proteins. For this reason, lipid-protein interactions are crucial for many cellular processes, and lipid transport between membrane organelle is crucial for cellular homeostasis. Recently, it has been discovered that lipid transport proteins functionally interact with lipid scramblases. These interactions have been proposed to be required for membrane expansion, for example during autophagosome biogenesis. Using experimentally-solved and AlphaFold structures, we carried out molecular dynamics simulations (MD) at coarse grained (CG) resolution, in order to assess the ability of CG- MD simulations to reproduce scramblase activity of a series of known scramblase proteins. Our results suggest that our protocol is able to reproduce the experimentally-characterized activity of bona-fide lipid scramblases, including VDAC1, VDAC2, VDAC3, TMEM41B, VMP1, MCP1, ATG9, TMEM16F and GPCR-rhodopsin. Additionally, our results indicate that oligomerization is crucial for scramblase activity. Interestingly, our MD-CG simulations allowed us to further distinguish between flippases and scramblases proteins. Our protocol will allow us to address the possibility of describing this type of biological function in other membrane proteins.

Poster Presentations
– Session 2 –

P-452
Small-Angle X-Ray Scattering of protein-membrane interactions: the case of hHep1 with negatively charged liposomes

Luiz Fernando Rodrigues[1,2], Lauren Matthews[3], Amanda Laís Coto[4], Júlio César Borges[4], Theyencheri Narayanan[3], Leandro Barbosa[1,2]

[1] Institute of Physics, University of São Paulo, São Paulo, Brazil, [2] Laboratório Nacional de Luz Síncrotron, Centro Nacional de Pesquisa em Energia e Materiais, Campinas, Brazil, [3] ESRF – The European Synchrotron, 38043 Grenoble, France, [4] São Carlos Institute of Chemistry, University of São Paulo, São Carlos, Brazil

Molecular chaperones are proteins with functions related to folding processes in the cell, such as correcting misfolding of client proteins, aggregation prevention and solubilization of aggregates. hHep1 is a co-chaperone protein necessary for the activity of an essential chaperone in the cell, the mitochondrial Hsp70 (mtHsp70), for it to remain stable, and needed for protein import through the membrane. Recent works have shown evidence of the interaction of these proteins with liposomes, but little information is known regarding how this process occurs. In this work, we have employed Small-Angle X-Ray Scattering (SAXS) for studying the interaction of hHep1 with negatively charged liposomes of different compositions. Our results indicate that hHep1 docks onto the liposomes but does not penetrate considerably into the lipid membrane. This suggests that hHep1 has a more cooperative interaction with mtHsp70 for protein import through the membrane.

P-453
Identification of metastable states of a large-conductance mechanosensitive channel (MscL) using enhanced sampling methods.

Olga Rogacheva[1], Tiago Costa[2], Andreas Hartel[3], Carsten Kutzner[1], Wojciech Kopec[1]

[1] Max Planck Institute for Multidisciplinary Science, Germany [2] Delft University of Technology, [3] Columbia University

The Large Conductance Mechanosensitive Ion Channel (MscL) is a bacterial channel that senses membrane tension upon osmotic shock. Specifically, the application of membrane tension activates the channel, leading to a major conformational change that results in a channel opening. It is also known that focused ultrasound and some other factors can induce MscL activation. However, the study of this process is hampered by the fact that the mechanism of the MscL gating is poorly understood, and even the structure of the open state has not yet been characterized. Here, we used umbrella sampling and OPES approaches to identify MscL metastable states under high and low membrane tension. We described a “partially open” state with an extremely low conductance (3±8 pS), intermediate states, and an “expanded” state with a conductance almost equal to that of the open state (> 3.2 nS). Despite the high conductance, the “expanded” state is likely not the “true” open state, since it does not satisfy all the distance restraints known from experiments. Therefore, we proposed an alternative model of the open state. Based on our calculations we confirm that membrane tension significantly increases stability of the open states. We also argue that the transition between the “closed” and “expanded” states must break the five-fold symmetry typical for the MscL channel.

P-454
MOLECULAR BASIS FOR INACTIVITY OF MAMMALIAN DELTA IGLURS

Giulio Rosano[1], Timothy Lynagh

[1] University of Bergen, Norway

Ionotropic glutamate receptors (iGluRs) mediate excitatory signals between neurons by conducting transmembrane cation current in response to neurotransmitter binding. In mammals, AMPA- and NMDA-type iGluRs mediate most signals in the mammalian brain. Mammalian delta-type iGluRs form channels that express at the cell surface and bind neurotransmitters, but in stark contrast to other iGluRs, their channel is not activated by neurotransmitter binding, raising interesting biophysical and evolutionary questions about the divergence of delta-type iGluRs from other iGluRs. We therefore investigated the delta iGluR family in depth using phylogenetics, electrophysiology, and site-directed mutagenesis. We find that delta iGluRs are found in numerous bilaterian animals (e.g., worms, starfish, vertebrates) and that they are closely related to AMPA-type iGluRs in amino acid sequence. Secondly, we discovered that delta iGluRs from various invertebrates are indeed functional neurotransmitter- activated channels. Remarkably, they are potently activated by the inhibitory neurotransmitter GABA. Robust transmitter-activated currents in invertebrate delta iGluRs enabled a unique characterization of delta iGluR pharmacology, and we show, for instance, sensitivity to AMPA-type iGluR competitive antagonists such as NBQX and DNQX, reflecting the shared ancestry of delta iGluRs with their AMPA receptor cousins. Finally, we identified five amino acid residues whose substitution in delta receptors of early vertebrates likely gave rise to the mysterious inactivity of mammalian delta iGluRs. Detailed experimental analysis suggests that these residues control receptors desensitization, offering a biophysical mechanism by which inactivity evolved. These results offer mechanistic insight into iGluR activation and highlight the use of comparative approaches in understanding protein function.

P-455
Which moiety drives gangliosides to form nanodomains?

Radek Sachl[1], David Davidovic[1], Mercedes Kukulka[2], Maria Sarmento[3], Ilya Mikhalyov[4], Natalia Gretskaya[4], Barbora Chmelova[1], Martin Hof[1], Lukasz Cwiklik[1]

[1] J. Heyrovsky Institute of Physical Chemistry of the Czech Academy of Sciences, Dolejskova 2155/3, 182 00 Prague, Czech Republic , [2] Faculty of Chemistry, Jagiellonian University, Gronostajowa 2, 30-387 Krakow, Poland, [3] Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, 1649-028 Lisbon, Portugal. , [4] Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Science, Miklukho-Maklaya 16/10, 117997 Moscow, Russia

Gangliosides are important glycosphingolipids involved in a multitude of physiological functions. From a physicochemical standpoint, this is related to their ability to self-organize into nanoscopic domains, even at molar concentrations of one per thousand lipid molecules. Despite recent experimental and theoretical efforts suggesting that a hydrogen bonding network is crucial for nanodomain stability, the specific ganglioside moiety decisive for the development of these nanodomains has not yet been identified. Here, we combine an experimental technique achieving nanometer resolution (MC-FRET) with atomistic molecular dynamic simulations to give the physical chemical explanation for a series of publications describing the formation of ganglioside nanodomains and discussing the impact on the nanodomain formation on the in vivo function of gangliosides.

Poster Presentations
– Session 2 –

P-456
Understanding ESCRT-membrane interaction in plants with ATR-FTIR spectroscopy
Oliva Saldanha[1], Marie-Kristin Nagel[2], Erika Isono[2], Karin Hauser[1]
[1] Biophysical Chemistry, Department of Chemistry, University of Konstanz, 78457 Konstanz, Germany., [2] Chair of Plant Physiology and Biochemistry, Department of Biology, University of Konstanz, 78457 Konstanz, Germany.

Cell membrane repair is an evolutionary tactic for survival and the repair mechanisms have been studied for decades in diverse model systems. It is highly interesting to understand membrane repair in plants, which perform these tasks simply and more effectively than mammals. The machinery controlling the membrane remodeling is made up of a multi-protein group called the endosomal sorting complex required for transport (ESCRTs). Of the several protein complexes, ESCRT-III can polymerize and form filaments crucial for membrane degradation. One of the protein coding gene CHMP7 is important in repairing nuclear membrane in humans and yeast but its role in plant cells is yet to be ascertained. We have explored the role of CHMP7 in membrane interaction in a synthetically generated artificial cell, made up of a phospholipid bilayer. Molecular interplay between lipid and protein in our model is globally tracked with a sensitive analytical technique of attenuated total reflection Fourier-transform infrared (ATR-FTIR) spectroscopy. Additionally, macroscopic fluctuations are tracked with confocal microscopy. This helps in understanding ESCRT mediated sealing and repair of damaged plant membranes in vivo, bringing us one step closer to explaining how plants adapt in the wake of climate change.

P-457
Using collisional mixing in lipid-polymer nanoparticles to probe the native environment of intra-membrane rhomboid protease GlpG and its associated activity
Henry Sawczyc[1], Takashi Tatsuta[2], Sascha Lange[1], Carl Oester[1], Claudia Bohg[1], Thomas Langer[2], Adam Lange[1]
[1] Leibniz-Forschungsinstitut für Molekulare Pharmakologie, Germany [2] Max-Planck-Institute for Biology of Ageing

Lipid polymer nanoparticles can extract integral membrane proteins without the use of detergents, forming discoidal nanoparticles containing the membrane protein, as well as local native membrane lipids. These discs have been shown to be dynamic systems, transferring lipids from one nanoparticle to another through 'collisional lipid mixing'. Rhomboid proteases are intra-membrane proteases, cleaving substrates only within the membrane bilayer. Some rhomboid proteases, such as E. coli's GlpG, have been shown to cause membrane thinning, with recent work showing that this thinning is due to the interaction between GlpG and phosphoethanolamine head group lipids. This work shows the extraction of both the full- length and N-terminal truncated GlpG (GlpG N) with native lipids into DIBMA nanodiscs, where differences in the total lipid composition between the full-length and GlpG N nanodiscs were observed. Introduction of DMPC and POPC into the GlpG-DIBMA nanodiscs through simple incubation with DIBMA nanodiscs generated solely from DMPC or POPC was then achieved. Finally, we compare the activity of GlpG in both native and PC-washed nanodiscs, utilizing this mixing to show cleavage of fluorescently labeled substrate TatA. The method presented here shows it is possible to replace the native lipid environment of a membrane protein without the risk of destabilization through the use of detergent.

P-458
Atomistic molecular dynamics simulations of gasdermin pores
Stefan Schäfer[1], Stefan Schäfer[1,2]
[1] Max Planck Institute of Biophysics, Frankfurt am Main, Germany, [2] Department of Theoretical Biophysics, Max Planck Institute of Biophysics, Germany

Pyroptosis is a recently discovered type of regulated cell death associated with cell lysis and inflammation. After proteolytic activation, gasdermins, the key executing proteins of pyroptosis, bind the plasma membrane, oligomerize, and insert β-pores into the membrane. The exact mechanisms by which binding, assembly and formation of the pores occur remain unclear. We performed multi-microsecond atomistic molecular dynamics simulations of different-sized gasdermin-D (GSDMD) oligomers in pore and pre-pore conformations and studied their lipid interactions, dynamics and structural stability. Using a complex asymmetric plasma membrane mimetic, we identified specific interactions with acidic lipid species that recruit GSDMD to the membrane and may drive oligomerization and β-sheet insertion. Simulations of small membrane inserted GSDMD assemblies explain the sublytic, nonselective ion flow observed during early pyroptosis. For larger oligomeric arcs, we identified the high tension of an emerging membrane edge as the driving force behind the formation of slit and ring-shaped pores. Lastly, our simulations indicate that fully assembled pre-pore rings cause membrane buckling that may prime the membrane for concerted insertion of the β-barrel. Our findings suggest competing, lipid and GSDMD concentration dependent assembly pathways by gradual expansion of sublytic pores or a concerted "cookie-cutter" membrane insertion from pre-pore rings.

P-459
Large throughput single-molecule FRET for screening the effects of correctors on patient-derived CFTR transmembrane hairpins
Mathias Schenkel[1], Andreas Hartmann[1], Philipp Schake[1], Kanish Siddarth Ravi Chandran[1], Dominik Horák[1], Michael Schlierf[1,2]
[1] B CUBE, TU Dresden, Tatzberg 41, 01307 Dresden, Germany, [2] Physics of Life, DFG Cluster of Excellence, TU Dresden, 01062 Dresden, Germany

The advent of small-molecule modulator drugs for cystic fibrosis (CF) made causal treatment available for most people with CF, although the mechanisms of action of these novel drugs remain partly unknown. Furthermore, many rare CF mutations lack causal treatment, while testing approved small-molecule drugs on these mutations could lead to drug repurposing. We focus on the study of structural effects of rare missense mutations in the transmembrane (TM) domains of the cystic fibrosis transmembrane conductance regulator (CFTR). By using a newly developed large throughput single-molecule Förster resonance energy transfer (smFRET) screening platform, we aim to identify rare mutants with local misfolding and investigate their interaction with CFTR correctors. We created a mutant library of CFTR's second TM helix pair (TM3/4) for in vitro folding studies in phospholipid vesicles. After labeling and reconstitution, smFRET was employed to characterize the folding behavior of TM3/4 hairpins and the effect of different CFTR correctors on them. We have identified mutants with varying misfolding severity within this CFTR segment, while corrector addition promoted fold recovery of misfolded TM3/4 mutants with different effective concentrations. Our results could be used as a pre-selection for in cellulo experiments and ultimately lead to drug repurposing for rare CF mutations.

Poster Presentations
– Session 2 –

P-460
Effect of Transmembrane Domains on the Free Energy of Stalk Nucleation during Membrane Fusion
Katharina C. Scherer[1], Jochen S. Hub[1]
[1] Saarland University, Department of Theoretical Physics, Computational Biophysics Group, Saarbrücken, Germany

The nucleation of the stalk is the first step in membrane fusion. The overall fusion process including the stalk formation is facilitated by fusion proteins anchored in the membrane by transmembrane domains (TMDs). Although TMDs of fusion proteins were found to play an active role during fusion, little quantitative or mechanistic understanding of putative TMD effects has evolved. We used molecular dynamics simulations to analyze the influence of TMDs from both the SNARE complex and viral fusion proteins on the free energy of stalk formation. The stalk free energy was computed via potential of mean force calculations along a newly designed reaction coordinate together with the Martini coarse-grained force field. We saw that the presence of TMDs in the fusing membranes decrease the stalk free energy. This free energy decrease scales linearly with the concentration of TMDs in the membrane and strongly depends on the hydrophobic mismatch between TMD and membrane core. Further, we correlated the free energy decrease with an increased disorder in the lipid packing caused by the insertion of TMDs. Additionally, we observed that kinked TMDs promote stalk formation more effectively than straight TMDs. This observation is compatible with previous experimental studies suggesting that the flexibility of the TMDs is a key factor in facilitating fusion. However, our simulations indicate that the kinked structure is likely more responsible for the mechanism than the flexibility per se. Overall, we quantified the effect of TMDs and gained mechanistic insights of how TMDs facilitate stalk formation.

P-461
Quantitative determination of fluorescence labelling efficiency
Chiara Schirripa Spagnolo[1], Aldo Moscardini[1], Rosy Amodeo[1], Fabio Beltram[1,2], Stefano Luin[1,2]
[1] NEST Laboratory Scuola Normale Superiore, [2] NEST Laboratory Istituto Nanoscienze CNR, Italy

Fluorescent labelling of cellular components is paramount in cellular and molecular Biophysics. Quantitative studies of processes like biomolecule interactions require high and known labelling yields. In particular, in our laboratory we optimize and exploit single-molecule techniques by TIRF microscopy in living cells, which need both high labelling efficiency and low background. I discuss how existing approaches for estimating labelling yields are usually inaccurate or even non-quantitative; then, I present a method for robust measurements of fluorescent labelling efficiencies. This exploits a ratiometric analysis of two sequential reactions using two distinct probes; its principle is general and can be employed in living cells or in other systems (e.g. purified proteins or tissues). Introduced advantages include: accuracy of a measurement performed under the same experimental conditions of interest; ease of application; minimization of costs and labour, needing no additional expertise or equipment compared to the final experiment but an auxiliary dye. I show an application on membrane TrkA receptors labelled via Sfp phosphopantetheinyl transferase with organic dyes; I demonstrate the broad potential of our method by studying reaction efficiencies varying reactants concentrations, reaction times, fluorescent probes. I also present a procedure for simultaneously inspecting specific and nonspecific probe interactions until finding the best balance between high efficiency and low background, as needed for critical applications down to the single-molecule level, in single and multicolour experiments. The method can be applied to a variety of labelling strategies to find conditions for quantitative studies of molecular processes via fluorescence-based techniques in different biological contexts.

P-462
Active vs inactive - Using Alchemical Free Energy Simulations to Probe Stabilization Effects within the Human Dopamine 2 Receptor
Lisa Schmidt[1,2], Bert de Groot[1]
[1] Max Planck Institute for Multidisciplinary Sciences, Department of Theoretical and Computational Biophysics, Am Fassberg 11, 37077 Göttingen, Germany, [2] Heidelberg University, Department of Biosciences, Im Neuenheimer Feld 234, 69120 Heidelberg, Germany

G protein coupled receptors (GPCRs) are an important class of signal-transducing membrane proteins, able to bind various types of ligands and activate different cellular signalling pathways. Among the different types of GPCRs aminergic GPCRs are of particular interest as drug targets since they are important for neurological function and signal transduction in nerve cells. To be able to develop highly receptor and pathway specific drugs it is crucial to understand the different modes of action of the target GPCR. Although much is already known about the activation mechanism of these receptors there is still much more to uncover. Here we focus on the human dopamine receptor 2 (DRD2) and apply Alchemical Free Energy simulations with PMX on large mutational scans over the whole receptor domain to calculate mutation free energy differences differentially affecting the active and inactive state. Combining the relative free energy differences of different mutations and their inter residue contact profiles we are able to map residues and interactions that are important for the stabilization of the different functional states of DRD2. Compared to long equilibrium simulations often used to investigate interaction networks, this method is faster and can be applied for large scale screening of interaction networks thereby helping to uncover alternative activation mechanisms

P-463
Dynamic assembly of recombinant human septin complexes
Kita Schmidt[1], Eric D. B. Foley[2], Philipp Kukura[2], Rumiana Dimova[3], Helge Ewers[1]
[1] Freie Universität Berlin, Deparment of Chemistry and Biochemistry, Germany, [2] University of Oxford, Department of Chemistry, United Kingdom, [3] Max Planck Institute of Colloids and Interfaces, Department of Sustainable and Bio-inspired Materials, Germany

Septins are filament forming cytoskeletal proteins encoded by 13 genes in human. In solution, septins are found as heterohexa- or octameric linear palindromic complexes composed of three or four different septin monomers, respectively. Upon membrane binding these septin complexes assemble into filaments that function in a variety of cellular processes, e.g. in cytokinesis. However, the mechanism and dynamics of human septin assembly have not yet been elucidated. To investigate septin filament formation in vitro, we use standard and dynamic mass photometry (MP) on supported lipid bilayers (SLBs) containing negatively charged lipids. By single particle tracking (SPT), we analyse the diffusion of single septin complexes and the mechanism of their polymerization. We show that recombinant septin complexes readily assemble into higher oligomeric species on SLBs without the need for additional factors such as regulatory proteins. Furthermore, we found that the diffusion behaviour of nascent septin filaments on SLBs is directly dependent on the number of septin complexes within oligomeric species. Interestingly, we found that septin assembly and fragmentation into single complexes occur in almost equal proportion and presumably independent of the protein concentration, indicating a distinct polymerization mechanism.

Poster Presentations
– Session 2 –

P-464
Bacterial outer membrane vesicles (OMVs): Dissecting the delivery process to host cells
Andra Schromm[1], Leonard Christian[1]
[1] Research Center Borstel - Leibniz Lung Center, Germany

Outer membrane vesicles (OMV) of Gram-negative bacteria are membrane-derived nanoparticles of 20 – 200 nm size carrying bacterial molecules, lipids, toxins, and genetic material between microbes and to host cells. Interaction of OMVs with host cells is involved in bacterial invasion, inflammation, tissue pathology, and modulation of host cell metabolism. Our study is aimed to provide fundamental information on OMV membrane interaction, fusion and possible subsequent internalization of membrane compartments to provide a better understanding of the mechanisms underlying OMV-mediated delivery of pathogen-factors to the host. We performed liposome studies to define the lipid specificity and obtain time-resolved information on OMV interaction with the eukaryotic membrane. Using a panel of phospholipids and lipid mixtures without and with cholesterol, we systematically analyzed the role of lipids in small-unilamellar liposomes and phase-separated giant unilamellar vesicles. OMV effects on membrane fluidity were analyzed in liposomes and in the cytoplasmic membrane of living cells. Based on our data, we propose a 2- step model for the interaction of bacterial OMV with eukaryotic membranes, which is orchestrated by anionic phospholipids and cholesterol. In addition, employing complex macrophage membrane mimicry systems we have gained indications for self-promoted uptake of membrane areas after OMV-interaction, presumably a post-fusion effect.

P-465
«Specific or not specific, that is the question»: an overview on gal-1 - GM1 interaction
Federica Scollo[1], Gabriele Nicita[2], Waldemar Kulig[3], Anna-Kristin Ludwig[4], Peter Kapusta[1], Marek Cebecauer[1], Hans-Joachim Gabius[4], Herbert Kaltner[4], Ilpo Vattulainen[3], Giuseppe Maccarrone[2], Martin Hof[1]
[1] Biophysical Chemistry, J.H. Institute of Physical Chemistry, Prague, Czech Republic, [2] Department of Chemical Sciences, University of Catania, Catania, Italy, [3] Department of Physics, University of Helsinki, P.O. Box 64, FI-00014 Helsinki, Finland, [4] Faculty of Veterinary Medicine, Institute of Physiological Chemistry, Ludwig-Maximilians-University, Munich, Germany

Galectins are a ubiquitous family of galactose-binding proteins involved in a wide variety of functions, spanning from RNA splicing to cell growth regulation, including cell adhesion, embryogenesis, inflammation and immune function, apoptosis, angiogenesis and tumor metastasis. Fourteen human galectins are known, commonly classified into three different types, according to their diverse architecture. However, they all have a common feature: they possess one or more β-sandwich carbohydrate recognition domain/s (CRD/s) by which they interact with molecules with a galactoside moiety. Among all of them, galectin-1 is one of the most important, being implicated in T-cells communication. Gal-1 is believed to express its function by binding specifically the monosialotetrahexosylganglioside (GM1). The above-mentioned binding has been hypothesized and indirectly shown in previous works, employing too sophisticated or non-physiologically relevant systems, such as neuroblastoma cells or glycodendrimers. Whether this binding is specific and what is the dissociation constant (Kd) are still open questions. In this work, we investigate the interaction of galectin-1 with different types of physiologically relevant model membranes containing GM1 and on living cells, by using a combined experimental and computational approach. Specifically, we employed Molecular Dynamic Simulations and both fluorescence-based, i.e. Förster Resonance Energy Transfer and confocal Microscopy, combined with Quartz Crystal Microbalance with Dissipation Monitoring and Isothermal Titration Calorimetry, with the latter being label-free techniques. To the best of our knowledge, our data show for the first time that galectin-1 is interacting with model membranes and, more importantly, the data suggest a specific interaction with GM1.

P-466
High-throughput and correlative membrane biophysical mapping of health and disease
Erdinc Sezgin
[1] Karolinska Institutet, Sweden

Malfunctions in processes that tightly control cellular physiology are likely causes of diseases and there is extensive search on protein and nucleic acid markers to find these underlying aberrant processes. However, diseases cannot be fully comprehended without a thorough understanding of collective biophysical remodeling such as changes in cellular membrane viscosity, fluidity, mobility, tension and deformability. In our lab, we fill this gap by defining the biophysical principles underlying health and disease. To enable this, we develop (i) robust chemical probes that accurately report on the physical and chemical state of cells, (ii) easy to use, accessible, open, fast, cheap and high-throughput methodologies that allow measuring multiple biophysical properties simultaneously for millions of cells, and (iii) automated software for advanced analysis of biophysical data and its correlation with other multi-omics and physiology data. Applying these biophysical single-cell screening methods to reveal how physical and chemical properties of cells change in different states, diseases and upon different stimuli allow us to create a biophysical atlas of health and disease. Importantly, data from millions of single cells enable us to develop machine learning algorithms to predict the disease phenotypes based on biophysical properties of cells and potential treatments restoring healthy biophysical profile. Our high-content, multi- parametric and data-driven biophysical analysis will transform our understanding of physiology and disease by adding a new physical perspective.

P-467
A biophysical model of phagocytosis
Peyman Shadmani[1], David. M Richards[1]
[1] University of Exeter, United Kingdom

Phagocytosis is a key part of the immune system in which cells identify, ingest, and break down foreign particles such as bacteria. Understanding the underlying biophysics of this process is relevant to improving the immune response, tackling various medical conditions, and designing micron- sized drug-delivery systems. The details of phagocytosis are still poorly understood, particularly how cells capture and internalise particles. Cell shape change, membrane remodelling, and the actin cytoskeleton are fundamental parts of the process, and yet their facilitators and coordinators are still to be elucidated. Further, important questions such as how long internalisation takes, and the effect of target size and shape are at best only partially understood. In this talk, I will describe my recent work to develop a computer simulation of the internalisation stage of phagocytosis. This is a type of vertex model, where biophysical forces act on each vertex. After discussing previous models in this area, I will cover the underlying biophysics of my model, and explain how it was developed and implemented. I will then introduce some new results, including the dependence on target size, the effect of target stiffness and the role of the actin cytoskeleton. Finally, I will discuss possible model extensions and what these will allow us to learn in the future.

Poster Presentations
– Session 2 –

P-468
Molecular rotors as tools to study downstream processes in mitochondria upon inhibition of protein-protein interactions
Petr S. Sherin[1], Roshni Malde[1], Thomas Mills[1], Phoebe Seltzer[1], Anna Barnard[1], Marina K. Kuimova[1]
[1] Imperial College London, United Kingdom

Protein-protein interactions (PPIs) play an important role in numerous biological processes and the correct functioning of live cells. Their mis- regulation can often result in the development of disease. Therefore, their inhibition has been the subject of numerous studies and drug discovery campaigns but the quantitative study of this inhibition in relevant biological environments (in cellulo) is still limited. We demonstrate that the inhibition of a PPI between proteins of Bcl-2 family, regulating cell apoptosis, could be monitored by Fluorescence Lifetime Imaging microscopy (FLIM) combined with environmentally sensitive fluorophores termed molecular rotors. Molecular rotors are synthetic fluorescent molecules, in which fluorescence intensity and lifetime strongly depend on the viscosity of their microenvironment. In this work, we used mitochondria-localised molecular rotors to study the changes in the organisation of the mitochondrial outer membrane (MOM) under different conditions, in different cell lines. Our results have shown that the viscosity within MOM significantly increases with Type-II photodamage and upon starvation. Opposite to these observations, the presence of ABT-737, a well-known inhibitor of the above-mentioned PPI, drastically decreases the viscosity, indicating a large perturbation within the MOM structure. These results open up opportunities for direct visualisation of the consequences of PPI inhibition in live cells.

P-469
Urea and Ammonia Yeast Complementation Assays Challenge the View of Highly Selective Human Aquaporins
Sahar Shojaei[1], Anna Stoib[1], Christine Siligan[1], Nikolaus Goessweiner- Mohr[1], Andreas Horner[1]
[1] Institute of Biophysics, Johannes Kepler University Linz, Austria

Aquaporins (AQP) are small tetrameric membrane channels critically involved in osmotic cell volume regulation and the transport of metabolites. The narrow AQP pores are thought to combine enormous permeability, conducting water in a single-file manner close to the diffusion limit of water in bulk, with exceptional selectivity. The thirteen human variants (hAQP0–12) are expressed in a cell/tissue-dependent manner and are subdivided according to their selectivity and sequence homology. A subset of AQPs, the aquaglyceroporins, are also able to conduct glycerol and other small neutral solutes. Additionally, the characterization of single AQPs already revealed neutral solute permeabilities for some of the other un/orthodox AQPs. Yet, a systematic study of all hAQPs is still missing. We tested urea and ammonia permeability at a pH of 4.5 and 7.0 for all hAQPs utilizing yeast complementation assays. Our results contradict the view of highly selective AQP pores, as all hAQPs facilitated urea permeability except hAQP1. Furthermore, our results suggested pH gating of hAQP5, with enhanced urea permeability at acidic pH. Ammonia seems to be facilitated by all hAQPs except hAQP0,1,10 with greater variability than with urea. Hence, our results indicate that highly selective AQPs seem to be the exception and not the norm.

P-470
EFFECT OF ΔF508 AND G551D CYSTIC FIBROSIS MUTATIONS ON CFTR-PKA INTERACTION
Márton András Simon[1,2], László Csanády[1,2]
[1] HCEMM-SE Molecular Channelopathies Research Group, [2] Department of Biochemistry, Semmelweis University, Hungary

The CFTR anion channel consists of two pore-forming transmembrane domains, two cytosolic nucleotide-binding domains, and a cytosolic regulatory (R) domain. The catalytic subunit of PKA binds to the R domain and phosphorylates it, causing reversible and irreversible channel activation. CFTR mutations can cause cystic fibrosis (CF), a devastating disease. ΔF508, the most common CF mutation impairs maturation and gating of the channel, while G551D disrupts ATP-dependent channel gating. PKA-dependent activation of these mutants is reportedly also defective. Since the stimulatory effect of the FDA-approved potentiator ivacaftor depends on phosphorylation of CFTR, understanding the impairment of the CFTR-PKA interaction in mutants is of great importance. In inside-out patch-clamp recordings, we studied the effects of PKA- binding and phosphorylation on channel activation by using an ATP analog that supports CFTR gating but does not support phosphorylation by PKA, and assessed the rates and fractional amplitudes of reversible/irreversible activation in mutant and wild-type (WT) CFTR. The phosphorylation rate of both mutants was comparable to that of WT channels. Importantly, the relative effect of binding is significantly greater for both mutants compared to WT channels, suggesting that the effect of phosphorylation on channel activation is impaired in ΔF508 and G551D CFTR. In the presence of ivacaftor plus elexacaftor reversible activation is still substantial for both unphosphorylated and phosphorylated G551D and for unphosphorylated ΔF508, but small for phosphorylated ΔF508 CFTR channels.

P-471
Annexin mediated plasma membrane repair
Adam Cohen Simonsen[1], Anna Mularski[1], Anne Sofie Busk Heitmann[2], Stine Lauritzen Sønder[2], Theresa Louise Boye[2], Martin Berg Klenow[1], Weria Pezeshkian[1,3], Jesper Nylandsted[2,4]
[1] University of Southern Denmark, PhyLife - Physical LifeScience, Odense, Denmark, [2] Danish Cancer Society Research Center, Membrane Integrity Group, Copenhagen, Denmark, [3] University of Copenhagen, The Niels Bohr Institute, Copenhagen, Denmark, [4] University of Southern Denmark, Department of Molecular Medicine, Odense, Denmark

The plasma membrane of cells can suffer injury due to external perturbations. Rupture of the plasma membrane must be sealed rapidly to maintain homeostasis. To this, end efficient plasma membrane repair (PMR) mechanisms have been developed. The repair involves recruitment of multiple proteins to the damage region as triggered by the influx of calcium ions from the extracellular fluid. Members of the Annexin protein family are involved in repair although their mechanistic role is incompletely understood.Biophysical membrane experiments and modeling can give additional insight into Annexin mediated repair mechanisms. Using a unique platform of planar, free-edged membrane patches, the edge region of a membrane hole can be experimentally mimicked. The platform allows membrane shape changes and bending near free edges to be observed. We find that many Annexins induce spontaneous membrane curvature leading to distinct, curved morphologies including roll-up of membranes by Annexin A4 and A5. The curvature effects were theoretically modelled and linked to the formation of a membrane neck around holes during repair. The molecular scale formation of 2D Annexin lattices was studied in parallel using AFM and results correlated with curvature effects. Finally, from experiments on MCF7 cells upon laser-induced rupture, we visualize the influx of calcium and from a quantitative model estimate the time of hole closure. In summary, our studies provide insight into the mechanistic role of Annexins during PMR and emphasize the role of induced curvature and shape changes as possible key steps in the repair.

Poster Presentations
– Session 2 –

P-472
Specific cradle loop residues regulate the pore-formation mechanism of Vibrio cholerae cytolysin, a β-barrel pore-forming toxin
MAHENDRA SINGH[1], KAUSIK CHATTOPADHYAY[1]
[1] INDIAN INSTITUTE OF SCIENCE EDUCATION AND RESEARCH MOHALI, India

Vibrio cholerae cytolysin (VCC) is a pore-forming toxin with potent membrane-damaging cell-killing activity. VCC monomers bind to the target cell membrane and forms transmembrane heptameric β-barrel pores. This conversion involves critical reorganization within the protein structure, regulation of which is poorly understood. One crucial transition is opening of the pre-stem loop from each protomer that extends and inserts into target membrane forming the β-barrel pore. Here, we explore the role of the so-called cradle loop that supports the pre-stem in the monomeric form, and moves away from it in the pore-structure of VCC, in the pore-forming mode of action of VCC. We show that two specific residues within the cradle loop play critical roles in regulating the pore-forming function of VCC. We find that the mutations of the cradle loop-residues compromise the pore-forming function of VCC and trap the toxin molecules in a membrane-bound oligomeric state with compromised membrane- insertion ability. Further, we show that these mutations merely delay the kinetics of pore-formation that can be rescued by elevated temperature condition in case of mutation of one of the residues but not for the other one. Our study reveals distinct regulatory role of the cradle loop in the pore-formation mechanism of VCC that has not been documented earlier in the structurally related β-barrel pore-forming toxins.

P-473
The role of flow and membrane state for transport across endothelial cell membranes
Manuel Sirch[1], Lara Clemen[1], Serap Demir[1], Christop Westerhausen[1]
[1] University Augsburg, Germany

In this research, we investigate the role of the endothelial glycocalyx in nanoparticle uptake and molecule transport into cells, as well as its impact on the phase state of the cells' plasma membrane in flow-based cell culture. Our results demonstrate that the presence or absence of the glycocalyx can significantly impact the uptake kinetics of nanoparticles and affect the molecular transport across the endothelial cell membrane. We further show that the glycocalyx can play a crucial role in maintaining the integrity of the cell membrane under flow conditions, which has important implications for the phase state of the cells during culture, in turn affecting the membrane phase state. These findings shed light on the interplay between the glycocalyx and the cell membrane in cellular function under physiological flow conditions and for the development of new therapeutics targeting the endothelium.

P-474
A guideline for quantitative characterization and application of environment-sensitive probes
Yagmur Balim Urem[1], Ellen Sjule[1], Franziska Ragaller[1], Jan Schlegel[1], Luca Andronico[1], Erdinc Sezgin[1]
[1] Science for Life Laboratory, Department of Women's and Children's Health, Karolinska Institutet, Sweden

Investigation of plasma membrane biophysical properties is crucial for a detailed understanding of the biological processes occurring thereof. To study these properties, environment-sensitive fluorescent probes have emerged as important tools. Detailed characterization is needed to gain a comprehensive understanding of these probes. Here, the probes NR12S, NR12A, Pro12A, and Flipper-TR were characterized in-depth using model membrane systems. Different sensitivities to changes in lipid saturation index, double bond position/configuration, phospholipid headgroup and cholesterol content were unraveled by spectral imaging and fluorescence lifetime imaging microscopy (FLIM). Furthermore, FLIM revealed that the probes exhibit different lifetimes depending on the selected emission window. By applying these probes in live cells, biophysical remodeling of the plasma membrane upon viral protein expression (SARS-CoV-2 Delta spike and Ebola glycoprotein) was observed. Together, these results illustrate the potential of environment sensitive fluorescent probes as tools to gain a deeper insight in the biophysical properties of the plasma membrane, while providing a guideline to select a suitable probe depending on application.

P-475
Novel Mechanism for the Transport of Fatty Acid Anions Assisted by ANT1
Sanja Škulj[1], Mario Vazdar[2], Jürgen Kreiter[1,3], Elena E. Pohl[1]
[1] Department of Biomedical Sciences, University of Veterinary Medicine, Vienna, Austria, [2] Department of Mathematics, University of Chemistry and Technology, Prague, Czech Republic, [3] Institute of Molecular and Cellular Physiology, Stanford Medical School, CA, USA

Adenine nucleotide translocase (ANT1) is an inner mitochondrial membrane (IMM) protein with the main function of exchanging ADP and ATP for cellular energy supply. An important but less acknowledged function of ANT1 is the transport of protons in the presence of long chain fatty acids (FA). The molecular mechanism of this transport is not understood.1 FAs act as weak uncouplers, independently of protein. The transport of FA anionic form (FA-) across the membrane is a rate-limiting step. We hypothesized that ANT1 will accelerate this transport of FA- across the IMM. Our recent MD simulations, together with experimental results, suggest that anions of arachidonic acid (AA-) are attracted by the positively charged amino acids cloud at the matrix side and slide between transmembrane helices 2 and 3 to the cytosolic side. The suggested mechanism is energetically favourable because the AA- "head" interactions with the positively charged amino acids complemented by hydrophobic tail stabilization both within the hydrophobic surface of ANT1 and in the surrounding lipids. The translocation path terminates at R79, where AA- is protonated with the help of D134. The protonated AA spontaneously exits the protein, flips across the IMM and releases the proton at the matrix side, completing the catalytic cycle. Knowledge of the precise proton transport mechanism will enable the development of effective drugs for the treatment of diseases involving ANT1, such as progressive external ophthalmoplegia, myopathy, and hypertrophic cardiomyopathy.

Poster Presentations
– Session 2 –

P-476
Significance of hydrogen bond in the mechanism of action of bedaquiline

Joanna Słabońska[1], Jacek Czub[1,2], Subrahmanyam Sappati[1,2]
[1] Gdansk University of Technology, Faculty of Chemistry, Department of Physical Chemistry, Gabriela Narutowicza St 11/12, 80-233, Gdańsk, Poland, [2] BioTechMed Center, Gdansk University of Techmology, Gabriela Narutowicza St 11/12, 80-233, Gdańsk, Poland

The binding of bedaquiline (Bq), a multi- and extensively drug-resistant tuberculosis drug to the surface of the membrane-embedded Fo subcomplex of the ATP synthase protein is a special case of the binding of an inhibitor to the protein. This is due to the hydrophobic environment of the membrane, providing an additional challenge to create sufficiently strong protein-ligand interactions. Therefore, finding the basis responsible for the binding of Bq to the c-ring – the rotary part of the protein is crucial part of understanding the mechanism of action. Despite good shape complementarity with a relatively shallow binding pocket, basic non-polar interactions cannot be responsible for the mechanism of drug recognition and binding to the protein. The X-ray and cryo-EM structures show the importance of the hydrogen bond (HB) between carboxyl group glutamic acid (E56) and the amino group of Bq with an extremely short distance between donor and acceptor (2.4 Å). Using hybrid QM/MM ab initio MD approach based on DFT, we determined the effective HB potential, which allowed us to characterize the unique behavior of an exceptionally short, strong HB. Finding the low-barrier potential of HB for protein-ligand binding is an interesting area of research based on this binding mechanism.

P-477
Optical control of photo-Gb3 enriched membranes: impact of the position of the azobenzene on Gb3-Shiga toxin interaction

Larissa Socrier[1], Somayeh Ahadi[2], Robin Gering[3], Ann-Sophie Schmidt[3], Daniel B. Werz[4], Claudia Steinem[5]
[1] Max Planck Institute for Dynamics and Self-Organization. Am Faßberg 17, 37077 Göttingen, Germany, [2] Technische Universität Braunschweig. Hagenring 30, D-38106 Braunschweig, Germany, [3] Institute for Medical Physics and Biophysics, Leipzig University, Härtelstraße 16–18, D-04107 Leipzig, Germany, [4] Albert-Ludwigs-Universität. Albertstraße 21 D-79104 Freiburg, Germany, [5] Georg-August-Universität. Tammannstraße 2, 37077 Göttingen, Germany

Cellular membrane organization has widely been discussed since the introduction of the fluid mosaic model 50 years ago. Initially it was proposed that proteins are randomly distributed in a lipid bilayer matrix. The model has been complemented with the raft-theory which remains controversial to this day. Over the past decade, lipids bearing a light-sensitive azobenzene group have gained in popularity to investigate membrane properties and lateral organization in combination with coexisting lo/ld artificial membranes. We attached the photo-sensitive azobenzene to the fatty acid of globotriaosylceramide glycolipids (Gb3) which constitute natural receptors for the bacterial protein Shiga Toxin (STx). In vitro studies performed with phase-separated lipid systems have shown that, dependent on the position of the azo group on the fatty acid, the compounds can greatly alter the size of membrane domains and impact protein density on the surface of the membrane. We next investigated the influence of the position of the azobenzene group on lipid-lipid and lipid-protein interactions. By means of surface plasmon resonance and surface tension, we found that when the azobenzene is deeply embedded in the hydrophobic core of the membrane, the area occupied by photo-Gb3s diminishes while the miscibility with sphingomyelin and binding affinity (KD) for STx increase, suggesting a preferential partitioning of the compounds in lo domains. These findings can bring further understanding on the lipid raft concept given that a control of membrane lateral organization, protein-lipid interactions and cluster formation is accessible with this approach.

P-478
Investigating early events of hiIRE1αactivation: insights from molecular dynamics simulations

Elena Spinetti[1,2], Jan Stuke[3], G. Elif Karagöz[4], Roberto Covino[1,5]
[1] Frankfurt Institute for Advanced Studies, Frankfurt am Main, Germany , [2] Goethe Universität, Frankfurt am Main, Germany, [3] Max Planck Institute of Biophysics, Frankfurt am Main, Germany, [4] Max Perutz Labs, Vienna Biocenter (VBC), Vienna, Austria, [5] International Max Planck Research school for cellular Biophysics, Frankfurt am Main, Germany

The Unfolded Protein Response (UPR) is a cellular process that helps eukaryotic cells respond to stress conditions in the endoplasmic reticulum (ER) and is essential for maintaining health and preventing disease. When stress sensors on the ER membrane are activated, the UPR is initiated. The most evolutionarily conserved sensor is the membrane protein IRE1, which triggers the UPR by forming dimers and larger assemblies.
Although IRE1 has been shown to exist as a dimer in non-stress conditions and interact with unfolded polypeptides via its luminal domain (LD), the mechanism by which the LD dimer directly binds to unfolded proteins in humans remains unknown. Our study aimed to shed light on the early events of human IRE1α(hIRE1α) activation, specifically how the LD dimer detects unfolded proteins and propagates the signal. We used molecular dynamic (MD) simulations to investigate the stability of the LD dimer and its interactions with unfolded polypeptides at atomistic resolution. Our findings demonstrated that hIRE1α LD dimer could constitutively form a dimer, even in the absence of ER stress. Our results also showed that specific unfolded polypeptides could stably bind to the surface of the LD dimer, even if the central groove was unable to accommodate them inside. These new findings support a model in which the direct interaction between IRE1 and unfolded proteins is a critical initial step towards the formation of clusters. Overall, these new insights into the molecular mechanisms of IRE1 activation may have implications for the development of therapies targeting the UPR pathway.

P-479
Label-free nanopore approach to distinguish histone protein variants

Marija Srnko[1], Gašper Šolinc[1], Ana Crnković[1], Gregor Anderluh[1]
[1] Department of Molecular Biology and Nanobiotechnology, National Institute of Chemistry, Hajdrihova 19, 1000 Ljubljana, Slovenia

The varying cavity sizes and ease of genetic modification accelerated the use of pore-forming proteins in biotechnological applications. A biosensor based on pore-forming proteins consists of biological pores inserted into planar membranes that separate chambers with an electrically conducting solution. The applied electrical voltage generates nanopore- and analyte-specific electrical electric current. By modifying an actinoporin from Orbicella faveolata (Fav), we developed octameric pores (confirmed by Cryo-EM) that stably insert into MinION membranes and exhibit improved electrophysical properties compared to wild-type pores. The successful insertion of Fav nanopores into MinION membranes enables rapid, accurate, and high-throughput detection of medically relevant positively charged histone proteins that are susceptible to various post-translational modifications. Blockades of histone H3.1 were longer and less discrete compared to the blockages of less positively charged histone variant H4, whereas the addition of H3.1 citrullinated at multiple arginine residues (reduced positive net charge) caused discrete blockades with smaller amplitudes. The observed blockades, which differ in amplitude, current noise, and dwell times, are the result of different electrostatic interactions between negatively charged amino acid residues of the pore entrance/lumen and histone proteins.

Poster Presentations
– Session 2 –

P-480
Impact of native-like lipid membranes on the architecture and contractility of actomyosin networks
Claudia Steinem[1], Nils Liebe[1], Andreas Janshoff[1]
[1] University of Göttingen, Germany

At the plasma membrane of mammalian cells, the linkage of the filamentous (F)-actin network is of pivotal importance leading to cell-specific and dynamic F-actin architectures, which are essential for the cell's shape, mechanical resilience, and biological function. These networks are established through the coordinated action of diverse actin-binding proteins and the presence of the plasma membrane. We established phosphatidylinositol-4,5-bisphosphate-doped supported planar lipid bilayers to which contractile actomyosin networks were bound via the membrane–actin linker ezrin. This membrane system, amenable to high-resolution fluorescence microscopy, enabled us to analyze the connectivity and contractility of the actomyosin network. We found that the concentration of phosphatidylserine, a characteristic lipid of the inner leaflet of mammalian plasma membranes, plays a pivotal role in the binding of the membrane-cytoskeleton linker protein ezrin and the resulting architecture and contractile behavior of the adjacent F-actin network. Phosphatidylserine drives the attached network into a regime, where low but physiologically relevant connectivity to the membrane results in strong contractility of the actomyosin network, emphasizing the importance of the lipid composition of the membrane interface.

P-481
Investigating the pH dependent solute permeabilities of Helicobacter pyloris urea channel UreI
Anna Stoib[1], Christine Siligan[1], Andreas Horner[1]
[1] Institute of Biophysics, Johannes Kepler University, Linz, Austria

Over 50% of the world population suffer from chronic gastric infection with Helicobacter pylori which is linked to peptic ulcer disease and stomach cancer. Yet, the efficacy of the common therapy including antibiotics is decreasing. An alternative drug target could be H.pyloris pH gated inner- membrane urea channel UreI, which is pivotal for the survival of the pathogen in the acidic environment of the human stomach. Besides regulating urea permeability, with its hydrolysis products stabilizing the pathogens inner pH, HpUreI was shown to facilitate water in its open state. In order to investigate the pH dependent selectivity mechanism of HpUreI we tested variants with modified N- and C-termini as well as periplasmic loop 1 (PL1) with in-vivo yeast freeze survival and complementation growth assays. Thereby, the functionality and pH gating behaviour of these constructs are compared to the wild type protein in the physiological relevant pH range from 4.0 – 7.0. Our results question the expected pH dependent solute selectivity, with HpUreI being closed at neutral pH and being open at acidic pH. Contrary to urea, our findings suggest that additional amino acids at the termini and in PL1 have no significant effect on the water permeability and HpUreI exhibits its maximal water permeability at neutral pH. This highlights a complex pH dependent solute selectivity mechanism of HpUreI. Overall, yeast freeze survival and complementation assays are a cost-effective tool for testing qualitative differences in protein variant activity and functionality for a broad range of channels.

P-482
Structural model of the chitin uptake channel of the marine Vibrio bacteria: the important role of the N-terminal plug
Surapoj Sanram[1], Anuwat Aunkham[1], Robert C Robinson[1]
[1] Vidyasirimedhi Institute of Science and Technology, Thailand

VhChiP is an outer membrane porin identified from the marine bacterium Vibrio campbellii type strain ATCC® BAA 1116. This protein channel is responsible for the uptake of chitooligosaccharides. The structure of VhChiP contained three identical subunits, to which each subunit has the 19-aa N-terminal segment that serves as a molecular plug (the 'N-plug') that controls the closed/open states of the neighboring pores. In this study, the crystal structures of VhChiP lacking the N-plug were determined in the absence and presence of chitohexaose. Binding studies by single channel recordings and ITC experiments suggested that the deletion of the N-plug peptide significantly weakened the sugar binding affinity due to the loss of four hydrogen bonds around the central affinity sites. However, the addition of the N-plug peptide on the trans side restored the binding affinity. Molecular dynamic simulations revealed that the movement of the sugar chain along the sugar passage triggered the ejection of the N-plug, at the same time the hydrogen bonds formed between the reducing-end GlcNAc units of the sugar chain with the N-plug peptide provide weak pulling force that facilitated sugar translocation. The findings enable us to propose a structural model for chitooligosaccharide uptake by the marine Vibrio bacteria

P-483
Ion Permeation, Voltage Gating and Pharmacological Modulation in K2P TREK Channels Studied by Atomistic Molecular Dynamics Simulations
Han Sun[1,2], Berke Türkaydin[1,2], Yessenbek Aldakul[1,2]
[1] Leibniz Forschungsinstitut für Molekulare Pharmakologie, [2] Technical University of Berlin, Germany

Many questions still remain regarding ion permeation, gating, and pharmacological regulation of two-pore domain potassium (K2P) channels. Our simulations revealed two dynamic hotspots in the selectivity filter (SF) of TREK channels, which are concentrated at the S1 and S3 binding sites. Notably, we found that the residues around the S1 binding site in TREK channels were more dynamic than those in the reference MthK potassium channel, which allowed water to enter the SF. This may be considered as the first step in the inactivation of TREK channel at negative transmembrane voltages. In addition, we conducted simulations of the TREK-2 channel with an extended C-terminal (pCt) tail. We found that the non-conductive state of the channel was only present in the down-state simulations, where a conformational change at the S1 K+ binding site in the SF was responsible for ion non-conductivity. Using an extensive interaction network analysis, we identified two different pathways of that connect the pCt and SF dynamics. Together, our finding not only shed light on how different gating regions work in concert to regulate TREK channel function, but also offer atomistic insights into the molecular mechanism of pharmacological modulation in TREK K2P channels.

Poster Presentations
– Session 2 –

P-484
COLLAGEN TYPE I BIOSYNTHESIS IN THE PRESENCE OF DIFFERENT CONCENTRATIONS OF β-HYDROXY β-METHYLBUTYRIC ACID

Izabela Świetlicka[1], Eliza Czarnecka[1], Krzysztof Gołacki[2], Michał Świetlicki[3], Marta Arczewska[1]

[1] Department of Biophysics, Faculty of Environmental Biology, University of Life Sciences in Lublin, Akademicka 13 St, 20-950 Lublin, Poland, [2]Department of Mechanical Engineering and Automatics, Faculty of Production Engineering, University of Life Sciences in Lublin, 28 Głęboka St., 20-612 Lublin, Poland, [3] Department of Applied Physics, Faculty of Mechanical Engineering, Lublin University of Technology, Nadbystrzycka 38D St., 20-618 Lublin, Poland

Collagen type I is a major structural protein in the extracellular matrix of various tissues and organs. Its biosynthesis is a complex process leading to the creation of fibrils. β-Hydroxy β-methyl-butyric acid (HMB) is a metabolite of the branched-chain amino acid leucine, which has been reported to stimulate collagen synthesis. However, the mechanism of HMB-induced collagen synthesis is not well understood. This study investigated the effect of different concentrations of HMB on the molecular structure and morphology of collagen type I using FTIR spectroscopy and atomic force microscopy. Our results showed that HMB treatment at the lower concentration increased collagen type I biosynthesis and improved the quality of collagen fibrils, while in contrast, the highest concentrations of HMB disturbed collagen polymerisation, leading to various changes. Specifically, a decrease in the intensity of the amide I band and alterations in the amide III spectra were observed, suggesting disruptions in the secondary structure. AFM images also revealed a significant decrease in the thickness of collagen fibrils, indicating a disorder in collagen organisation. In conclusion, our results suggest that optimal concentrations of HMB are necessary for enhancing collagen type I biosynthesis and improving collagen fibril quality.

P-485
Novel loss-of-function mutations in KV7.2, and their possible involvement in epilepsy.

Tibor Szanto[1], Istvan Balogh[2], Gyorgy Panyi[1]

[1] University of Debrecen, Faculty of Medicine, Department of Biophysics and Cell Biology, Debrecen, Hungary, [2] University of Debrecen, Faculty of Medicine, Division of Clinical Genetics, Department of Human Genetics, Debrecen, Hungary

KV7.2 potassium channels play a key role in controlling excitability of neurons in the central nervous system by contributing to shaping of the action potential. Therefore, mutations of the KCNQ2 gene encoding the KV7.2 channel may lead to hyperexcitability syndromes, such as benign familial neonatal seizures (BFNS). Exploring the molecular identity and altered function of novel KCNQ2 mutants may guide us to a deeper understanding of the pathogenesis of BFNS. Accordingly, we aimed at fully characterizing the biophysical properties of two potentially pathogenic KV7.2 mutations identified in patients, namely Ser113Phe localized in the extracellular S1-S2 loop and Ala306Val located in the water filled cavity of the pore. The mutations were introduced by site-directed mutagenesis and the mutant channels were transiently expressed in CHO cells. Potassium currents (IK) were measured using whole-cell patch-clamp technique and compared the gating parameters of the mutants to the wild-type KV7.2. We found that the peak current density is substantially reduced in the Ser113Phe mutant together with a slower activation kinetics of IK and a significant rightward shift on the steady-state activation curve. The Ala306Val mutation resulted in non-functional channels. Pharmacological studies revealed that Retigabine restores the normal function of Ser113Phe. In addition, the expression of functional KV7.2/7.3 heteromers partially rescues the Ser113Phe mutant phenotype. Our conclusion is that the analyzed mutations in the KCNQ2 gene result in biophysical changes in the Kv7.2 currents and thus, may be responsible for BFNS.

P-486
Nascent Collagen-III Strongly Misfolds: A Single-Molecule Characterisation

Jack Tait[1], Jack Tait[1], Sander Tans[1]

[1] AMOLF, Amsterdam, Netherlands

Collagen is the most abundant protein in the human body, comprising over one third of the total protein content. Consequently, a number of diseases result from defects in the collagen quality control system, including osteoporosis and Ehlers-Danlos syndrome. A deeper understanding of collagen assembly is required to develop more effective treatments for these conditions. The native folding pathway of type-III collagen is known to be complex, requiring both chaperones and an array of co- and post-translational functionalisation in vivo. However, this assembly pathway has only ever been studied in bulk experiments. Here, the behaviour of nascent COL3A1 monomers at the ribosome is studied using optical tweezers, providing the first investigation of collagen folding at the single-molecule level. In this study the first direct observation of the misfolding of COL3A1 monomers is demonstrated, as well as the link between this misfolding propensity and the stage of protein translation. Collectively, these data present a new challenge for which molecule chaperones are required, and give new detail to the picture of how the collagen synthesis machinery interacts with newly-synthesised monomers to successfully produce native collagen trimers in humans.

P-487
The Effects of Calcium on Lipid-S100 Proteins Interactions

Rimgailė Tamulytė[1], Evelina Jankaitytė[1], Darius Šulskis[2], Marija Jankunec[1]

[1] Institute of Biochemistry, Life Sciences Center, Vilnius University, Vilnius, Lithuania, [2] Institute of Biotechnology, Life Sciences Center, Vilnius University, Vilnius, Lithuania

Pro-inflammatory, calcium-binding proteins S100A9 and S100A8 are localized in the cytoplasm of a wide range of cells and regulates several intracellular and extracellular processes. One of them is the participation in the inflammation associated with the pathogenesis of Alzheimer's disease (AD). The number of studies on the impact of S100 proteins in co-aggregation processes with amyloid-like proteins is increasing. However, the interest in the interaction mechanism of proteins S100A9 and S100A8 with biological membranes is still limited. In this work various biomimetic membrane models as lipid vesicles in solution and tethered bilayer lipid membranes were used to examine the interaction between the S100 proteins and the membrane surface in the absence or presence of Ca^{2+}-free environment. For this purpose we employed atomic force microscopy and fluorescence spectroscopy techniques. Our results indicate that the damage induced by S100A9 in a calcium-free environment is mainly determined by membrane fluidity rather than by negatively charged lipids. The most significant loss of integrity was observed in lipid bilayers composed of lipid mixture (brain total lipid extract). Furthermore, we demonstrate that the presence of Ca^{2+} ions promotes the membrane disruption induced by S100A9. However, our latest data indicates that the S100A8 protein induce membrane leakage and promotes even a greater effect than S100A9 which might correspond to a higher cytotoxicity. These results might broader the understanding of S100 family proteins interactions with lipid membrane and potentially affect the development of new diagnostic and therapeutic approaches for AD or other related diseases.

Poster Presentations
– Session 2 –

P-488
Molecular details of a Gram-positive Type 4 Secretion System

Josy ter Beek[1], Alexandra Berg[1], Annika Breidenstein[1], Krishna Chaitanya Bhattiprolu[1], Kai Ehrenbolger[1], Anaïs Lamy[1], Wei-Sheng Sun[1], Dennis Svedberg[1], Ronnie P-A Berntsson[1]
[1] Department of Medical Biochemistry and Biophysics and Wallenberg Centre for Molecular Medicine, Umeå University, Sweden

Type 4 Secretion Systems (T4SSs) are a main driver for the spread of antibiotic resistance genes and virulence factors in bacteria. T4SSs are versatile and megadalton sized complexes that facilitate the transfer of proteins and DNA from a donor bacterium to a recipient cell, which can be from a different species. Over the past decades, studies have advanced our understanding of how these systems work in Gram-negative bacteria. However, so far little is known about their structure and function in Gram-positive bacteria, while these bacteria account for most hospital acquired infections. Our lab studies the T4SS that is encoded on the conjugative pCF10 plasmid from the Gram-positive Enterococcus faecalis. By combining methods from molecular biology, biochemistry, and structural biology we are making progress in our aims to: Structurally and functionally characterize the DNA transfer channel Determine how the initial cell adhesion, DNA processing and substrate recruitment to the T4SS channel works.

P-489
Probing the influence of transmembrane peptide charge on its interaction with lipid membrane

Garima Thakur, Arunima Uday[1], Wouter Roos[2], Lukasz Cwiklik[1], Marek Cebecauer[1], Martin Hof[1], Piotr Jurkiewicz[1], Adela Melcrova[2]
[1] J. Heyrovsky Institute of Physical Chemistry, [2] Zernike Institute for Advanced Materials, Rijksuniversiteit Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands

Transmembrane proteins perform vital functions such as channelling ions, transporting molecules across the membrane, and transducing signals. In addition to several factors, the rough surface of their transmembrane domains (TMDs) was shown to directly affect the neighboring phospholipids, which in turn can modulate the vital functions during cellular transport. To this end, we constructed model membranes and studied their interactions with two peptides i.e., zwitterionic WALP-KD and positively charged WALP-KK^{4+} using fluorescence of Laurdan. Lipid mobility probed by Laurdan generalized polarization (GP) and time-resolved fluorescence revealed that both peptides rigidified the membrane. Moreover, energy transfer from tryptophan residues of the peptides to Laurdan molecules in its surrounding (FRET-GP) revealed that this rigidification is driven by the local interactions with the peptide and lead to inhomogeneity in lipid bilayer fluidity. Comparison of GP and FRET-GP results for the two peptides showed that the cationic charge of the native TMDs can additionally hinder lipid mobility, and that this interaction is of longer range than that caused by the rough surface of the TMD. These results are complemented by AFM investigation of membrane elasticity as well as molecular characterization by MD simulations.

P-490
'Take 1' on TAOK2: structural and biophysical insights into TAOK2 and its complexes with TRIM4 and viral dsRNA

Lisse Thierens[1,2], Savvas N. Savvides[1,2]
[1] Unit for Structural Biology, VIB-UGent Center for Inflammation Research, [2] Department of Biochemistry and Microbiology, Ghent University, Ghent, Belgium

Thousand-and-one-amino-acid kinase 2 (TAOK2) is a member of the MAP3K family that plays critical roles in various physiological and pathophysiological cellular responses, such as regulation of microtubule dynamics and antiviral immunity. The literature links sensing of double-stranded RNA (dsRNA) and activation in innate immunity by TAOK2 to its interaction with the ubiquitin ligase protein TRIM4. However, the structural and mechanistic basis of these pleiotropic functions remains poorly understood, thereby presenting the field with a progress bottleneck. TAOK2 is the largest of three TAOK family members and features a conserved kinase domain (KD) coupled to a much larger segment predicted to adopt a coiled-coil (CC) region and an additional leucine-rich (Leu) region. These scaffolding parts of TAOK2 are thought to mediate interactions with TRIM4, dsRNA, and cellular membranes. At the same time, the most readily studied function of TAOK2 as a kinase via its conserved KD has not yet been linked to its much more substantial non-kinase segment. Here, at the forefront of molecular and structural biology, we propose to elucidate the structure-function landscape of TAOK2 and its complexes with TRIM4 and viral dsRNA. Therefore, the interaction network of candidate interactors of TAOK2 will be studied through the use of biophysical tools, such as bio-layer interferometry, mass photometry, isothermal titration calorimetry and multi-angle laser light scattering. Such knowledge will provide insights into the modular function and mechanistic synergies comprised in the enigmatic structure of TAOK2, and will facilitate further interrogation of TAOK proteins in physiology and disease.

P-491
Impact of the substrate on the phase transitions properties in supported lipid bilayers

Nicolo Tormena[1], Kislon Voitchovsky, Teuta Pilizota
[1] University of Durham, United Kingdom

Membranes play a crucial role in the survival of the organism, from providing a physical barrier with the surroundings, to controlling membrane trafficking. Membranes are self-assembled structures, comprising a phospholipid bilayer where proteins and sugars are embedded. The activity of the proteins and their response to external stimuli depends on the biophysical properties of the lipid bilayer such as thickness, and molecular mobility. Additionally, membranes are always in close contact with various structural filaments such as cytoskeleton tubules which can all influence the movement of macromolecules in the bilayer. E. coli is routinely used as a platform to investigate fundamental biological questions about the function of membrane proteins, making it a key bacterial system. We create a minimal model system for E. coli's inner membrane, focusing on the membrane's mechanical properties. Our model system contains three types of lipids that replicates the main aspects of E. coli's lipidomic, and their relative proportion is adjusted to mimic the transition temperature of native membranes. Analyzing this model by combining atomic force microscopy (AFM) and differential scanning calorimetry (DSC), we comparatively track its phase transition kinetics, comparing it when supported and unsupported. The results show that the main influence is coming from contact with a support, which does not only shift the transition temperature dramatically, but it can also induce a global re-arrangement of the lipids. The result is a phase transition that partially follows classical nucleation on short timescales (<1h) but moves on to a spinodal decomposition on the longer term.

Poster Presentations
– Session 2 –

P-492
Examinations of cellular uptake of cell penetrating peptides in vitro and in vivo
Gabriella Tóth[1], Gyula Batta[1,2], Levente Kárpáti[3], Árpád Szöőr[1], István Mándity[3], Péter Nagy[1]
[1] University of Debrecen, Faculty of Medicine, Department of Biophysics and Cell Biology, Hungary [2] University of Debrecen, Faculty of Science and Technology, Institute of Biotechnology, Department of Genetics and Applied Microbiology, [3] Semmelweis University, Faculty of Pharmacy, Institute of Organic Chemistry

Cell-penetrating peptides (CPPs) are peptides that enter cells by endocytosis and/or directly through the cell membrane. CPPs in general have been considered potential carriers of molecules that have difficulties entering cells. This is the feature that we would like to exploit and thereby establish the opportunity for CPPs to have therapeutic applications in the long term. Our previously published results have shown that we can increase the cellular uptake and endosomal release of CPPs with statins. Our goal was to modify them and test if it is possible to make them enter the cells more efficiently. We also aimed to test the biodistribution of CPPs in mice after intravenous administration. We examined the cellular uptake and endosomal release by flow cytometry and confocal microscopy in SKBR-3 and MDA-MB-231 cell lines, while for the in vivo experiments a mouse model was applied. Fluorescently-labeled CPPs were used both in the in vivo and in vitro experiments. We compared the differences in the biophysical properties of the original and the modified CPPs, and we found that the cellular uptake of the modified version is more effective. There is a difference between the enhancement in the uptake of CPPs labeled by the pH-sensitive naphthofluorescein or Alexa Fluor 532. In the case of in vivo experiments, we found that peptides enter the mouse organs, including the liver, for which we have shown that CPPs is present in the intracellular space of hepatocytes. CPPs hold promise for increasing the efficiency and specificity of drug delivery to cells.

P-493
One Ring to Rule Them All: Lugdunin's Disruptive Effects
Marius F. W. Trollmann[1,2], Dominik Ruppelt[3], Claudia Steinem[3,4], Rainer A. Böckmann[1,2]
[1] Computational Biology, Department of Biology, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany, [2] Erlangen National Center for High-Performance Computing (NHR@FAU), Erlangen, Germany, [3] Institute of Organic and Biomolecular Chemistry, Georg-August- Universität Göttingen, Göttingen, Germany , [4] Max Planck Institute for Dynamics and Self-Organization, Göttingen, Germany

Antimicrobial resistance represents a growing threat to global public health, underscoring the urgent need for novel strategies to counteract the spread of multi-resistant bacterial strains. Antimicrobial peptides (AMPs) have emerged as a promising alternative to common antibiotics for inhibiting bacterial growth without inducing new forms of resistance. Recently, the cyclic peptide lugdunin was isolated from nasal Staphylococcus lugdunensis and has shown a strong antimicrobial activity against several Gram-positive bacteria. Lugdunin consists of six D,L-amino acids and a thiazolidine moiety. While maintaining membrane integrity, lugdunin was shown to enable proton translocation across the membrane. However, the mechanistic mode of action of lugdunin on membranes is hardly understood. Here, we applied atomistic molecular dynamics simulations to investigate lugdunin's differential interaction with a range of model membranes. Our results suggest that lugdunin easily penetrates the membrane interface region. We decipher the driving forces for lugdunin membrane embedment and in particular the role of the thiazolidine moiety and discuss possibilities for channel formation. The presented simulations provide a basis for the future rational design of new macrocyclic thiazolidine peptide antibiotics with enhanced efficacy and safety profiles.

P-494
Single-molecule studies of an osmoregulatory transmembrane transporter
Katarzyna (Kasia) Tych[1], Lyan van der Sleen[1], Marco van den Noort[1], Bert Poolman[1]
[1] University of Groningen, Netherlands

Using the optical tweezers is, by now, a well-established technique for the study of protein (un)folding and for the observation of function-related protein motions. In my lab, we seek to expand the capabilities of this method to enable (amongst other things) simultaneous mechanical and fluorescent readouts of inter- and intra-molecular distances and dynamics, characterisation of the function-related motions of membrane proteins, and assessment of the impact of lipid membrane composition on protein function. Here, I present our most recent work: the single-molecule characterisation of the bacterial osmoregulatory ATP-binding cassette transporter OpuA. Adaptation to stress is an essential feature of living cells, and a hypertonic environment leads to a potentially dangerous loss of turgor of the cell, due to outflow of water. OpuA restores the cell volume by accumulating large amounts of compatible solute, glycine betaine. Despite being extensively studied, molecular-level details of the transport mechanism, such as the interactions between the substrate binding domains and the transmembrane domain, are lacking. Using a combination of single-molecule FRET and optical tweezers measurements we are unravelling these functionally-relevant details with the goal of obtaining a quantitative overview of the functional cycle of OpuA.

P-495
Laser Irradiated Chlorpromazine - Application in Breast Cancer Treatment
Ana-Maria Udrea[1,2], Angela Staicu[1], Speranta Avram[3], Madalina-Andreea Badea[2,4], Ionut-Relu Andrei[1], Mihail-Lucian Pascu[1], Patricia Preduna[4], Mihaela Balas[4]
[1] National Institute of Laser, Plasma and Radiation Physics, 409 Atomistilor Str., 077125 Magurele, Romania., [2] Research Institute of the University of Bucharest, Earth, Environmental and Life Sciences, Section-ICUB, 050663 Bucharest, Romania., [3] Department of Anatomy, Animal Physiology and Biophysics, Faculty of Biology, University of Bucharest, 91-95 Splaiul Independentei, 050095 Bucharest, Romania, [4] Department of Biochemistry and Molecular Biology, Faculty of Biology, University of Bucharest, 91-95 Splaiul Independentei, 050095 Bucharest, Romania.

In 2020, cancer was the leading cause of death worldwide, and the most common type was breast cancer, with 26 million cases (1). The usual breast cancer treatments include chemotherapy, radiation, surgery, or a combination. This work may contribute to reducing the cancer research gap by proposing a new method and compound that, after laser irradiation, may be used in breast cancer treatment. The reported study, which employs in- silico techniques combined with laser radiation and in vitro validation, offers an innovative strategy for repositioning medications in cancer treatment. Molecular docking predicts chlorpromazine's biological activity before and after laser irradiation in interactions with several receptors targeted in cancer therapy. Chlorpromazine was irradiated using a pulsed laser beam (10 ns) with 6.5 mJ average pulse energy at 266 nm wavelength and a pulse repetition rate of 10 pps for various periods of time. To evaluate the cytotoxic effect of the treatment on the breast cancer cell line MCF-7, we used the MTS assay and live/dead staining techniques. Our predictions reveal that chlorpromazine photoproducts produced by laser irradiation exhibit biological activity similar to chlorpromazine. Our in vitro studies, however, show that irradiated chlorpromazine has a higher cancer-inhibitory effect than the non-irradiated drug, either through the action of the photoproducts individually or as cocktails.

Poster Presentations
– Session 2 –

P-496
Correlative spectral STED nanoscopy and STED-FCS elucidate the origin of anomalous diffusion in cellular membranes

Iztok Urbančič[1,2], Falk Schneider[1,3], Silvia Galiani[1], Erdinc Sezgin[1,4], Christian Eggeling[1,5,6]
[1] MRC Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, UK, [2] Jožef Stefan Institute, Ljubljana, Slovenia, [3] Translational Imaging Center, University of Southern California, Los Angeles, USA, [4] Science for Life Laboratory, Karolinska Institutet, Solna, Sweden , [5] Friedrich□Schiller□University Jena, Jena, Germany, [6] Leibniz Institute of Photonic Technology e.V., Jena, Germany

Advances in our understanding of the nanoscale architecture of the plasma membrane heavily rely on the development of non-invasive experimental methods, particularly of advanced fluorescence microscopy and spectroscopy techniques with high spatio-temporal resolution and sensitivity to local molecular properties. By integrating a spectral detector into a super-resolution stimulated emission depletion (STED) microscope, we unlocked further potential for multifaceted characterisation of membrane heterogeneities. First, we show that compared to the standard ratiometric detection, the spectrally resolved acquisition (together with spectral fitting or phasor analysis) can improve the spectral sensitivity of experiments with STED-compatible polarity-sensitive probes several fold. We further demonstrate that this acquisition scheme allows the use of such probes in combination with other dyes with overlapping spectra, enabling co-localisation of the membrane order maps with other cellular structures of interest, e.g. fluorescently labelled proteins. Finally, we can correlate the obtained membrane order with the detailed diffusion properties, reported by STED fluorescence correlation spectroscopy (STED-FCS). We find that for fluorescent lipid analogues with inhomogeneous partitioning between ordered and disordered membrane domains, including sphingomyelin-based probes, the most apparent trapping sites locate at the boundaries of ordered domains.

P-497
Rotation of ATP synthases as membrane structure modulator

David Valdivieso González[1,2], Marcin Makowski[1,3], M. Pilar Lillo[4], Francisco J. Cao-García[5,6], Manuel N. Melo[3], Víctor G. Almendro-Vedia[1,2], Iván López-Montero[1,2,7]
[1] Universidad Complutense de Madrid, Spain., [2]Hospital Doce de Octubre (imas12), Spain, [3] Universidade Nova de Lisboa, Portugal., [4] Instituto de Química-Física "Rocasolano" (CSIC), Spain., [5]Universidad Complutense de Madrid, Spain, [6] IMDEA Nanociencia, Spain., [7] Instituto Pluridisciplinar. Paseo Juan, Spain.

ATP synthases are proteins that catalyse the synthesis of ATP through rotation of their membrane-spanning subunit. In mitochondria, ATP synthases organise as dimers at the rim of crista due to specific interactions between some subunits that are not present in bacteria. We explore a direct link between the rotatory movement of ATP synthases and their preference for curved membranes. We find an active curvature sorting of reconstituted Escherichia coli 's ATP synthases in lipid nanotubes pulled from giant vesicles. Coarse-grained simulations confirmed the curvature- seeking behaviour of rotating ATP synthases, promoting reversible and frequent protein-protein contacts. The formation of transient protein dimers relies on the membrane-mediated attractive interaction of the order of kBT produced by a hydrophobic mismatch upon protein rotation. Transient dimers are sustained by a conic-like arrangement of a wedge angle of $\theta \approx 50^{o}$, producing a dynamic coupling with membrane curvature. Our results suggest a new role of the rotational movement of ATP synthases for their dynamic self-assembly in biological membranes.

P-498
K+ transport from channel subunit to pump subunit in kdpFABC

Hridya Valia Madapally[1], Himanshu Khandelia[1], David Stokes[2]
[1] University of Southern Denmark, Denmark [2] NYU Grossman School of Medicine

Survival of bacteria in low K+ environments necessitate functioning of a K+ transporter called kdpFABC. This unique channel-pump hybrid complex consists of a channel subunit, kdpA and a P-type ATPase pump subunit, kdpB. Data from cryo-EM studies suggests that a 40 Å long tunnel connecting the channel and pump subunit is the course of K+ passage to the kdpB ion-binding site. However, the identity of the densities found in the tunnel is disputed; it is unclear whether they correspond to K+ ions or water molecules. Employing molecular dynamics simulations, we observe that both K+ ions and water molecules co-exist in the tunnel. Moreover, we notice that there is predominantly a single stable ion binding site in the tunnel beyond the selectivity filter and the channel-pump interface. Free energy of ion transport along the intramembrane tunnel calculated using well-tempered metdadynamics shows that a large barrier prevents spontaneous diffusion of K+ ion from the channel to the pump subunit in the E1-ATP state. Further investigations are ongoing.

P-499
Hydrophobic substituents convert the α7 nicotinic receptor agonist ether quinuclidine into a type I positive allosteric modulator.

Franco Viscarra[1,2], Juan Facundo Chrestia[3], Yaima Sanchez[4], Edwin G. Pérez[5], Cecilia Bouzat[3], Isabel Bermudez[1], Philip C. Biggin[2], John J. López[4]
[1] Department of Biological and Medical Sciences, Oxford Brookes University, Oxford, UK., [2] Department of Biochemistry, University of Oxford, Oxford, UK., [3] Instituto de Investigaciones Bioquímicas de Bahía Blanca, Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur-Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Bahía Blanca, Argentina., [4] Facultad de Ciencias Químicas, Departamento de Química Orgánica, Universidad de Concepción, Concepción, Chile., [5] Departamento de Química Orgánica, Pontificia Universidad Católica de Chile, Santiago, Chile.

The α7 nicotinic acetylcholine receptor (nAChR) is a homomeric receptor that contributes to cognition, neuroprotection, immunity, and inflammation. Drugs that enhance α7 nAChR function are considered as valid therapeutic tools for the treatment of inflammatory diseases and pain, and the cognitive deficit present in Alzheimer's disease and schizophrenia. In this study, six new ligands (4-9) containing a 3-(pyridin-3- yloxy)quinuclidine moiety (ether quinuclidine), which is present in highly selective and potent α7 agonists, were synthesised. To determine the pharmacological activity of the ligands, two-electrode voltage-clamp and single-channel recordings were performed. Surprisingly, only ligand 4 retained agonist activity. Of the remaining ligands, 5 and 9 had no activity at α7 nAChR, but ligands 6, 8 and 9 potentiated the currents evoked by ACh with varying efficacy. Ligand 6 was the most potent and efficacious of the ligands, with an estimated EC50 for potentiation of 12.6 ± 3.32 μM and a maximal potentiation of EC20 ACh responses of 850 ± 120 %. The potentiation exerted by ligand 6 was clearly evidenced by the appearance of bursts that were prolonged with respect to those of the control, consistent with the profile of type I PAMs. Furthermore, computational studies revealed the preference of ligand 6 for an intersubunit site in the transmembrane domain and highlighted some putative key interactions that explain the different profiles of the synthesised ligands.

Poster Presentations
– Session 2 –

P-500
Insights into the dynamics of human neuropeptide Y1 receptor with solid-state NMR spectroscopy
Matthias Voitel[1], Holger A. Scheidt[1], Anette Kaiser[1], Daniel Huster[1]
[1] Leipzig University, Germany

Four high resolution structures of the human neuropeptide Y1 receptor (Y1R) in the antagonist bound state and in the G-protein bound state are published so far. But they represent only the most stable conformations. To obtain a detailed view on the dynamics of receptor activation, we investigate local conformational changes of Y1R upon interactions with intra- and extracellular molecules using solid-state NMR spectroscopy with site specific 13C labelling. Y1R was expressed using a continuous exchange cell free system based on E. coli extract allowing the introduction of 13C labelled tryptophan. The receptor, expressed as precipitate, was functionally refolded in DMPC membranes. Local conformational changes upon binding with NPY, G-protein or arrestin can be monitored as chemical shifts changes using 13C-13C DARR experiments. In order to simplify the spectra, some tryptophans were mutated into phenylalanine. The functionality of Y1R variants was proven in cell culture assays.

P-501
HDL-MEMBRANE-INTERACTIONS ARE HIGHLY INFLUENCED BY THE TARGET MEMBRANE-LIPID COMPOSITION
Florian Weber[1,2], Herbert Stangl[3], Taras Synch[1], Birgit Plochberger[2], Erdinc Sezgin[1]
[1] Department of Women's and Children's Health, Karolinska Instituted, Solna, Sweden, , [2] Medical Engineering, Univ. of Applied Sciences Upper Austria, Linz, Austria, [3] Center for Pathobiochemistry and Genetics Department of Medical Chemistry, Medical University of Vienna, Vienna, Austria

Lipid transfer from lipoprotein particles to cells is essential for lipid homeostasis. High-density lipoprotein (HDL) particles are part in an important mechanism in cholesterol homeostasis in the body and are mainly captured by cell membrane-associated scavenger receptor class B type 1 (SR-B1) from the bloodstream. Recently we were able to demonstrate an additional concept of a receptor-independent interaction between lipoprotein particles and lipid membranes. The role of the different lipid composition, properties, and organization of the target lipid membrane itself has not yet been investigated. This work addresses the question of how the interaction of HDL with synthetic membranes is regulated by varying the chain length, the degree of saturation as well as the binding groups of the head-tail region of the target membrane lipids. Total internal reflection microscopy and fluorescence correlation spectroscopy allowed us to demonstrate that target lipid membrane composed of ether lipids, significantly influences the preferred interaction region in phase separated membranes. In addition, when HDL particles were incubated with single-phase membranes containing ether lipids, we observed that the change in the diffusion coefficient of a fluorescent tracer lipid was significantly higher than for membranes without ether lipids. The receptor-independent interaction of lipoprotein particles with membranes is a fairly new concept and these new findings will help to refine the knowledge of lipoprotein particle biology as well the treatment in dyslipidemia diseases.

P-502
Co-translational membrane insertion of the voltage-gated potassium channel KvAP
Justin Westerfield[1], Gunnar von Heijne[1]
[1] Department of Biochemistry and Biophysics, Stockholm University, Sweden

Despite great advances in predicting membrane protein structures, there is still a wide gap in understanding how membrane proteins fold. In particular, how forces acting on the nascent chain during insertion contribute to the final topology in the membrane is not known. Recent studies have shown that forces exerted on the nascent chain during membrane protein insertion are not necessarily maximal precisely when a hydrophobic transmembrane segment (TMH) enters the membrane. Rather, forces can be modulated during insertion by the presence of other protein segments. In force profile analysis (FPA), this results in apparent shifts of force peaks to shorter longer chain lengths than expected from hydrophobicity alone. Here we study the co-translational folding of the voltage-dependent K+ channel KvAP using FPA and glycosylation-based topology mapping. We find that the KvAP nascent chain dynamics depend strongly on the sequence context. Specifically, we find that the shape and location of the force peak generated by a given transmembrane helix can depend on the presence or absence of up to two of its upstream TMHs. Therefore, our findings suggest that both nearby and more distant interactions may influence the translocon-dependent co-translational insertion of TMHs into the E. coli inner membrane. These results have implications in the evolutionary conflict between foldability and functionality.

P-503
Mode of action of marginolactones on bacterial membranes
Christina Wichmann[1], Christian Eggeling[1,2]
[1] Institute of Applied Optics and Biophysics, Jena, Germany, [2] Leibniz Institute of Photonic Technologies, Jena, Germany

Azalomycin F (AzaF) is a macrolactone produced by Streptococcus spp. While its antimicrobial properties against gram-positive bacteria are known, its exact mode of action is not clear yet. This would be important to understand the extent to which macrolactones can be used in clinical applications. One hypothesis is that it might cause damage to the cell membrane. We therefore investigated the interaction of AzaF and other macrolactones with model membranes and bacterial cultures. We found that the growth of several bacteria is significantly negatively affected by different concentrations of AzaF. Furthermore, we assessed the interaction of different amounts of AzaF and other macrolactones on lipid vesicles via time course fluorescence spectroscopy and microscopy. The results demonstrate the influence on lipid membranes at different concentrations of macrolactones, contributing to understanding the mode of action of newly discovered natural products on their antimicrobial mode of action.

Poster Presentations
– Session 2 –

P-504

SYNTHESIS OF LARGE LIPID MEMBRANES WITH INTEGRATED MEMBRANE PROTEINS FROM GAS PHASE

Matthias Wilm[1]
[1] University College Dublin, Ireland

I am presenting a new method to generate large, transportable membranes with integrated membrane proteins. These membranes can be used as an in-vitro system to study membrane protein function, investigate their interaction with lipids or study membrane protein complex assembly in a system whose composition is controlled on a molecular basis. The large membranes have the biochemical activity of their membrane proteins. The method lends itself to produce anisotropic membranes in lipid composition and protein orientation. The only established method to self-assemble transportable bio-membranes is in nano-discs. However, they are limited to about 17 nm in diameter. Here, large bio-membranes self-assemble in a thin sheet of glycerol generated on a liquid surface. A nano-electrospray ion source is used to lay down a series of molecular monolayers on a liquid surface from gas phase in which the membrane self-assembles. The target area is the liquid surface of a container filled with buffer solution and some SM-2 Biobeads. The preparation steps: 1. formation of a lipid bi-layer; 2. formation of a thin glycerol layer; 3. formation of lipid mono-layer as template for the membrane assembly; 4. adding detergent-solubilised membrane proteins and some more lipid; 5. sealing the layers off by some glycerol; 6. detergent extraction from the surface by SM-2 Biobeads over several days. When the detergent is extracted, the membrane self assembles in the glycerol layer. Since this layer is very thin the membrane can grow only laterally and a large, transportable membrane forms.

P-505

GPMV based bottom-up approaches for the investigation of CaV1.3 ion channel clusters

Maximilian Winkler[1], Nikolas K. Teiwes
[1] Georg-August-Universität Göttingen, Germany

Calcium ions play a major role in signal transmission in eukaryotic cells and thereby they are regulating many physiological processes. CaV1.3 is a voltage-gated Ca2+ ion channel, which naturally occurs in heart cells as well as inner hair cells of the cochlea. Here they form ion channel clusters in the cell membrane and play a pivotal role in pacemaking activity and signal transmission at the synaptic cleft. This is achieved by the voltage dependent opening and closing of the ion channels, however many biophysical properties of the clusters still need to be investigated. Here, we describe a bottom-up approach, in which giant plasma membrane vesicles (GPMVs) are generated from transfected HEK-293 cells to investigate said CaV1.3 clusters. By spreading of the GPMVs on either solid substrate to generate supported plasma membrane bilayers (SPMBs) or porous substrate to generate pore spanning plasma membranes (PSPMs), different aspects of the ion channel clusters can be examined. SPMBs provide a higher attractive force to the membrane, leading to a decreased mobility of membrane compartments. Thus, they can be used for structural and mechanical analysis of the clusters. In contrast PSPMs allow a nearly free movement of ion channel clusters in the pores and are used to address dynamic processes, like cluster diffusion in the cell membrane.

P-506

The power of combining NMR experiments and MD simulations: long, hydrophobic molecules in phospholipid membranes

Anika Wurl[1], Tiago Mendes Ferreira[1]
[1] NMR group, Inst. of Physics, Martin-Luther Universität Halle-Wittenberg, Germany

The organization of hydrophobic and amphiphilic molecules inside cellular membranes is of importance for a variety of biophysical processes, such as the formation of lipid droplets or the design of lipid nanoparticles. However, little is known about the molecular structure of even simple molecules such as long n-alkanes in a lipid membrane environment. The combination of MD simulations and solid-state NMR measurements is an extremely powerful approach to address such problems: We have performed 2H and 1H-13C dipolar recoupling NMR experiments for a number of phospholipid membranes containing hydrophobic molecules, e.g. long n-alkanes and triglycerides, and performed MD simulations of these systems with two force-fields (CHARMM36 and Slipids). The experimental observables enabled us to validate the structures obtained from the MD simulations and to choose the most appropriate force-field, which depends on the size of the molecules studied. In turn, the simulations allowed us to interpret the experimental results in terms of miscibility, partitioning and conformation of the hydrophobic chains. Our findings highlight that studying such simple molecules in lipid bilayers is an important step towards understanding the behaviour of more complex hydrophobic molecules inside cell membranes, and that these systems are highly valuable for optimizing the available MD force-fields for lipid membranes.

P-507

Symmetry-Adapted Markov State Models of Closing, Opening, and Desensitizing in α7 nicotinic acetylcholine receptors

Yuxuan Zhuang[1], Rebecca Howard[1], Erik Lindahl[1,2]
[1] Science for Life Laboratory, Department of Biochemistry and Biophysics, Stockholm University, Solna, Sweden., [2] Department of Applied Physics, Swedish e-Science Research Center, KTH Royal Institute of Technology, PO Box 1031, Solna, 171 21 Sweden

The α7 nicotinic acetylcholine receptors (nAChRs) are pentameric ligand-gated ion channels gated by the neurotransmitter acetylcholine. They allow cations to cross the postsynaptic membrane, thereby controlling electrical signaling in the nervous system. Recent research has resolved and functionally annotated closed, open, and desensitized states of α7 nAChRs, providing insight into ion permeation and lipid modulation effects. However, the process by which α7 nAChRs transition between states remains unknown. To better understand gating and modulation, we generated two ensembles of molecular dynamics simulations of α7 nAChRs with or without cholesterol. Using symmetry-adapted Markov state modeling, we developed a five-state gating model in which the channel predominantly resides in its closed state, as predicted in the absence of agonists. Kinetics of the transition from open to a non-conductive “flipped” state corresponded to experimentally measured open durations. The addition of cholesterol led to stabilization of the desensitized state and increased coupling between various states. These results allow us to define functionally important states, build plausible transition pathways, and assess lipid modulation effects in the conformational cycle of α7 nAChRs. They also provide an ensemble of structural models that can be utilized for future rational lipidic drug design.

Poster Presentations
– Session 2 –

P-508
Functional impact of specific N-terminal phosphorylation on TRPC5 ion channel activity
Michal Mitro[1,2], Alexandra Ptakova[1,2], Viktorie Vlachova[1], Lucie Zimova[1]
[1] Institute of Physiology of the Czech Academy of Sciences, Prague, Czech Republic, [2] Faculty of Science, Charles University in Prague, Prague, Czech Republic

TRPC5 is a non-selective calcium permeable ion channel expressed in sensory neurons, kidney, and brain. It has been linked to inflammatory, mechanical, and spontaneous pain, making it a potential target for new pain treatments. However, its mechanism of activation in the context of cellular processes is not well understood. We focused on two phosphorylation sites at the membrane-proximal part of the N-terminus previously identified by mass spectrometry. Using patch-clamp technique, we evaluated the membrane currents induced by voltage and an agonist. Substitution of serines with aspartate significantly slowed the onset of agonist response compared to wild-type TRPC5. Alanine substitution of S193 caused a gain-of-function phenotype. Molecular dynamics simulations of the phosphorylated state of the channel showed that p-S193 alters the interactions with neighbouring subunits in close proximity to this residue. Our results suggest that the N-terminus of TRPC5 contains a previously unrecognized site of functional regulation, which when phosphorylated attenuates TRPC5 channel activity.

P-509
Impact of Psychedelic Tryptamines on Bilayer Lipid Membrane
Fateme Zohairi[1], Himanshu Khandelia[2], Ali Asghar Hakami Zanjani[2]
[1] University of Luxembourg, [2] University of Southern Denmark

Psychedelic compounds have a long history of use in religious, recreational settings, and remedies. Some of them have therapeutic potential to safely treat a wide range of diseases such as anxiety and depression. It is hypothesized that psychedelics like tryptamines can exert their effects by binding to the serotonin receptor and/or modifying the properties of the neuronal membrane that can alter the conformational equilibrium and desensitize receptors. Using all-atom molecular dynamics simulations we investigated the impact of three different tryptamine compounds with a tertiary amine (DMT, bufotenine, and 5-MeO-DMT) in both neutral and charged forms on a model bilayer lipid membrane. We analyzed the tendency of these compounds to partition into the membrane using free energy calculations. Neutral tryptamines partition into the bilayer almost completely. DMT and 5-MeO-DMT cross the membrane spontaneously, but bufotenine does not, although it has the maximum effect on the structural properties of the membrane. However, protonated compounds partition partially into the bilayer and cannot pass across the membrane during the simulation time. We found that subtle alteration of chemical structure can play a significant role in the improvement or deterioration of partitioning of these compounds into the bilayer and their passage across the membrane.

P-510
Super resolution techniques combined with image spatial correlation analysis to study Angiotensin II type 1 receptor´ dynamic and organization in renal cells.
Yenisleidy de las Mercedes Zulueta Diaz[1], Camilla Andersen[1], Jakob L. Kure[1], Mathias Hessellund Eriksen[1], Adam Leslie Lovatt[1], Christoffer Lagerholm[2], Sebastian Morales[3], Simon Sehayek[3], Thomas M. D. Sheard[4], Paul W. Wiseman[3], Eva C. Arnspang[1]
[1] SDU Biotechnology, Department of Green Technology, University of Southern Denmark, Denmark, [2] Kennedy Institute for Rheumatology, University of Oxford, U.K, [3] Department of Physics and Department of Chemistry, McGill University, Montreal Canada, [4] School of Biosciences, University of Sheffield, U.K

Deepening of the understanding of membrane receptors has recently been accelerated by current advances in super-resolution fluorescence microscopy (SRFM) techniques which have overcome the spatial diffraction limit and thereby allowed for unprecedented sensitive visualization and quantification of cellular processes with high temporal and spatial resolution in living cells. Specifically, the mechanism by Angiotensin II type 1 Receptors (AT1Rs) are organized and diffused in the PM remains unclear despite its crucial role in various intracellular signaling pathways resulting in hypertension, endothelial dysfunction and vascular remodeling. In this study, we used newly- developed bioimaging methods that allowed the visualization of AT1R in renal cells; as well as obtaining information on the dynamics and organization of the AT1Rs and their corresponding receptor-ligand complexes. This was achieved by using Photoactivated Localization Microscopy (PALM) combined with Spatial- temporal Correlation Image Analysis and Expansion microscopy (ExM). This study showed 1) an increase in the lateral diffusion of AT1R after Angiotensin II (AngII) treatment. 2)The receptor diffusion was found transiently confined in the PM. 3) Clustering of AT1R in the PM followed by a decreased in the cluster size after AngII treatment. For a deeper understanding of the AT1 receptor distribution, Cryofixation with ExM experiments were performed. This novel technique enabled us to obtain 3D images of cells with high levels of cellular architecture preservation at nanoscale. This study opens up a path into a new level of understanding regarding the mechanism which regulates AT1R.

P-511
Uncovering the Hidden Role of 2-Oxoglutarate/Malate Carrier in Uncoupling: Implications for Metabolic Disorders and Cancer
Kristina Žuna[1], Tatyana Tyshchuk[1], Taraneh Beikbaghban[1], Elena E. Pohl[1]
[1] Institute of Physiology, Pathophysiology and Biophysics, University of Veterinary Medicine, Vienna, Austria

The mitochondrial 2-oxoglutarate/malate carrier (α-ketoglutarate/malate carrier, OGC) transports 2-oxoglutarate for malate across the inner mitochondrial membrane and plays a crucial role in the malate-aspartate shuttle. OGC is ubiquitously expressed in healthy and cancerous tissues, and its gene knockout is embryonically lethal. Despite sharing a high homology with other proteins from the solute carrier 25 family (adenine nucleotide translocase 1 and uncoupling proteins 1-3 that facilitate proton transport mediated by free fatty acids (FAs), the role of OGC in uncoupling has never been studied in a well-defined system. We hypothesized that OGC can directly enhance proton transport in the presence of FAs. To test this, we produced OGC in E. coli and reconstituted it in liposomes. Our experiments show that reconstituted OGC increases the specific membrane conductance of lipid bilayer membranes only in the presence of FAs, proving its involvement in proton transport. OGC's substrates and substrate analogs inhibited this effect, suggesting competition for the same binding site in the protein's cavity. Site-directed mutagenesis revealed that R90 is involved in the FA-mediated proton leak, suggesting a common binding site for substrates and FAs. Understanding the molecular mechanism and role of OGC in uncoupling could have important implications for the development of targeted drug delivery systems for the treatment of metabolic disorders, obesity, and cancer.

Poster Presentations
– Session 3 –

P-600
napari-live-recording: embedding camera acquisition in napari

Jacopo Abramo[1], Pia Pritzke[1,2], Felix Wanitschke[1,2], Francesco Reina[1], Christian Eggeling[1,2]
[1] Leibniz-Institute for Photonic Technology, Jena, Germany, [2] Friedrich-Schiller-Universität, Jena, Germany

Custom-built microscopes often require to control hardware objects directly. A high-complexity device group is cameras. Moreover, camera producers provide softwares which often lack the features needed to properly perform measurements as intended. The scientific community responded to this need by providing open-source solutions such as Micro-Manager, who pioneered the field, or python-microscope to control cameras using a common interface. In this context, we present napari-live-recording, a plugin for napari. The plugin aims to converge acquisition and analysis together, so to bring data analysis directly to the workbench for quick data evaluation. We aim to integrate a real-time image processing pipeline taking frames directly from cameras, showing results on image viewer or storing them using file formats such as TIFF and HDF5. Moreover, the plugin aims to converge existing camera control options into a single solution, by integrating the afore-mentioned Micro- Manager and python-microscope device layers within the plugin itself. The plugin architecture also allows scientists to integrate their own custom camera devices quickly and easily, minimizing the time to deploy new cameras or expanding the control over existing equipment. The plugin development is founded by the Chan-Zuckerberg Initiative through the "napari Plugin Foundation Grants (Cycle 2)" program.

P-601
Stay viscoelastic: Mechanical properties of minimal actin cortices accessed by atomic force microscopy

Elena Ackermann[1]
[1] Georg-August-Universität Göttingen, Germany

Viscoelasticity of cells is enabled by an actin shell underlying the inner face of the plasma membrane. During deformation caused by external cues it feedbacks to maintenance the cell shape and leads to mechanic stability and integrity of the cell as a whole. The precise force forwarding by this actin cortex attached directly to the membrane via ERM proteins is still elusive. In vivo attempts to fathoming the elastic response is challenging, even nearly impossible, due to the highly complex intertwining of numerous components. Here, we established an in vitro bottom-up approach using solely the minimum of proteins and lipids to mimic and conquer the structural and mechanical properties of actin cortices. We are building minimal actin cortices by first spreading vesicles consist of the anchor lipid PIP2 on activated substrates with a high porosity, forming pore- spanning lipid bilayers followed by the addition of ezrin and fluorescently labeled non-muscle actin. With this at hand we can investigate the mechanical response of the actin cortex on free-standing pore-spanning membranes to deformations via indentation experiments by means of atomic force and fluorescence microscopy. This technique is beneficial since the porosity of the substrates enables the elucidation of mechanistic properties in absence of the solid support and show that artificial actin cortices response in a viscoelastic manner comparable to the cellular cortex.

P-602
Targeting α-synuclein amyloid aggregates with the BRICHOS domain – basis for treatment of Parkinson's disease

Laurène Adam[1], Rakesh Kumar [1], Willem Molenkamp [1], Rami Alkenayeh[1], Henrik Biverstål[1], Jan Johansson[1], Axel Abelein[1]
[1] Karolinska Institutet, Sweden

Parkinson's disease (PD) is the second most common form of neurodegenerative disorder. Its pathogenesis is linked to the aggregation of α- synuclein protein (αSyn) leading to accumulation of toxic oligomers and amyloid fibrils, which are found in patients' brains. Identifying and controlling the pathways of aggregation have been challenging yet is the key for a detailed understanding of the molecular mechanisms of protein aggregation in neurodegenerative diseases. Due to repetitive failure of treatment in the past, new disease-modifying treatment strategies are currently highly desired. Naturally occurring chaperones, such as the BRICHOS protein domain, is part of the new class of endogenous inhibitors of amyloid toxicity and holds great promise for the development of new therapeutic tools. Here, we use in vitro aggregation kinetics assays and show that recombinant human Bri2 BRICHOS inhibits αSyn aggregation by specifically inhibiting elongation and secondary nucleation, preventing the formation of toxic oligomers. Our electron microscopy images unveiled the presence of BRICHOS on the αSyn fibril surface and surface plasmon resonance measurements revealed binding constants to the fibrils. In contrast, no binding of BRICHOS to monomeric αSyn could be detected by solution NMR and isothermal titration calorimetry. We are currently evaluating the potential inhibition of αSyn-associated toxicity using different cell toxicity assays, to establish a link between aggregation mechanism and toxicity. These results provide a detailed understanding of the molecular mechanisms of αSyn aggregation and the inhibitory effect of BRICHOS, which could potentially facilitate novel treatment approaches.

P-603
Combinational Techniques for the Separation of Clusters and singlets/doublets of Group A Streptococcus using Deterministic Lateral Displacement and Filter Membrane-Sonication

Elham Akbari[1], Jason P. Beech[1], Sebastian Wrighton[2], Pontus Nordenfelt[2], Jonas O. Tegenfeldt[1]
[1] Division of Solid State Physics, department of Physics, Lund University, [2] Department of Clinical Sciences, Division of Infection Medicine, Faculty of Medicine, Lund University, Lund, Sweden

Streptococcus pyogenes (GAS) is a human pathogen that causes disease with an estimated 700 million infected people each year. With no developed vaccines, there is an urgent need for a better understanding of GAS pathogenicity. An intriguing characteristic of GAS is that they may grow in a wide range of morphologies and clusters of various sizes. This is believed to be part of a strategy to adapt to a diverse set of conditions that the bacteria face during infection. Separating clusters and singlets/doublets bacteria into subpopulations based on their physical properties would therefore provide new opportunities to analyze the different phenotypes' pathogenicity in isolation. We used deterministic lateral displacement (DLD) for microfluidic separation of GAS clusters. In addition to the DLD technique, a filter membrane-sonication setup was used for the separation of singlets and doublets bacteria. Larger particles were retained by the membrane and singlets and doublets could be collected downstream of the membrane. Analysis of the separated bacteria and clusters showed significant differences between the separated subpopulations, one with large clusters of the cross-sectional area of 900 – 1500 μm2 and one with smaller clusters with areas ranging from 29 -400μm2. The subpopulation obtained by the filter-sonication setup included mostly singlets and doublets with areas ranging from 2-11 μm2. The two separation setups together were able to separate the bacteria and bacteria clusters into three morphologically distinct subpopulations with a purity of over 80%. Future work will focus on exploring the interaction of the different subpopulations with host cells and immune cells.

Poster Presentations
– Session 3 –

P-604
Diffusion Analysis of NAnscopic Ensembles: A Tracking-Free Diffusivity Analysis for Nanoscopic Ensembles in Biological Samples and Nanotechnology

Ulrike Alexiev[1], Alexander Wolf[1], Pierre Volz-Rakebrand[1], Jens Balke[1], Fereshteh Ghazisaeedi[2], Marcus Fulde[2]

[1] Freie Universität Berlin, Physics Department, Arnimallee 14, 14195 Berlin, Germany, [2] Freie Universität Berlin, Centre for Infection Medicine, Institute of Microbiology and Epizootics, Robert-von-Ostertag-Str. 7, 14163 Berlin, Germany

With the advance of single molecule microscopy and its various applications there is a growing demand for high accuracy analysis tools to extract information from fluorescent probes in tissue, cells, or membranes. Unfortunately, the accuracy of diffusive parameters derived by conventional tracing methods (particle tracking) decreases with higher molecule densities, blinking/bleaching and fast diffusion, thereby imposing possible unnatural limitations for experimental designs. We developed a new particle image-based approach (DANAE) using a tracking-free fully-statistical approach that solves all these obstacles. Importantly, DANAE requires no approximations or any a priori input regarding unknown system-inherent parameters, such as background distributions. We show DANAEs superiority compared to classical tracing-based methods. Applications to the distribution of drugs in nanostructured lipid carriers, diffusion and interactions of an activated GPCR in lipid membranes, and the constricted movement of bacteria and the use of background analysis in the mucus of epithelial cells are highlighted.

P-605
Functionalization of Gold Nanoparticles for Specific Detection of Biological Nanoparticles using Twilight Holographic Microscopy

Julia Andersson[1], Erik Olsén[1], Anders Lundgren[2], Daniel Midtvedt[2], Fredrik Höök[1]

[1] Chalmers University of Technology, Gothenburg, Sweden, [2] University of Gothenburg, Gothenburg, Sweden

Optical detection of biological nanoparticles has become vital in several research fields, including both basic science and medical diagnostics, and there is a constant strive for more sensitive analytical methods. It was recently demonstrated that holographic microscopy equipped with a low frequency attenuation filter can be used to identify virus particles via specific binding of gold nanoparticles using the relationship between the complex-valued optical signal and the material composition of suspended dielectric-metal NP complexes. However, while the limit of detection was observed to approach femtomolar levels, a substantial amount of nonspecific aggregation was observed in complex biological media, severely limiting the applicability of the method. We have in this work investigated how functionalization of gold nanoparticles can decrease the level of nonspecific aggregation. Gold nanoparticles were synthesized using seed-mediated synthesis and functionalized with thiol-modified polyethylene glycol (PEG), a fraction of which being biotin-terminated. The PEG-modified gold nanoparticles were then functionalized with streptavidin to enable specific binding to biotinylated vesicles, subsequently detected using twilight holography from the dielectric and metallic signal of the complex. It was found that PEGylation reduces the level of nonspecific aggregation both with respect to number of aggregates and metallic signal, enabling the implementation of a clear threshold for specific detection limits. Furthermore, dissolving the PEG-thiol in 95 % ethanol during functionalization was observed to reduce the dielectric debris previously observed in the gold nanoparticle solution. It was also concluded that a limit of detection approaching femtomolar levels appears to remain also in complex media.

P-606
Cell biophysical properties unlock a new dimension in defining functional state of cells in health and diseases.

Luca Andronico[1], Yidan Jang[2], Sofia Iskrak[1], Franziska Ragaller[1], Patrick Sandoz[3], Marcus Buggert[1], Petter Brodin[1], Erdinc Sezgin[1]

[1] Karolinska Institutet, Sweden [2] EMBL Heidelberg, [3] KTH Royal Institute of Technology

Functional states of cells are classically defined based on the expression level of a specific proteins and/or gene subset. Many of these proteins reside in the plasma membrane (PM), which represents the first hub for cellular signalling and plays a key role in preserving cellular homeostasis. Although the increasing evidence of a cause/effect relationship between cellular states and collective biophysical properties of PM (e.g., stiffness, fluidity, deformability), the implications of the latter in health and diseases remain mostly unexplored due to the lack of enabling technologies. Here, we fill the gap and describe an instrumental pipeline based on flow cytometry and environment-sensitive probes to study cellular biophysical properties in a high-throughput and quantitative manner. By using our methodology, we were able to measure the PM's biophysical properties of human-derived immune cells and show differences among the main subpopulations under physiological conditions and in diseases. Specifically, we compared results from healthy donors with patients who experienced acute or chronic inflammatory response. Lastly, we show how dysfunctional cells undergo biophysical remodelling of PM without notable changes in their proteins profile. In conclusion, our results suggest that cell biophysical properties represent a new, so far unexplored, dimension in cell phenotyping and could be potentially used for prediction of disease states and for designing of more efficient therapies based on physical insight.

P-607
Effect of Hofmeister anions on α-lactalbumin amyloid fibrillization

Andrea Antosova[1], Miroslav Gancar[1], Jozef Marek[1], Zuzana Gazova[1]

[1] Department of Biophysics, Institute of Experimental Physics Slovak Academy of Sciences, Kosice, Slovakia

The design of amyloid-based templates with desired properties for biotechnology requires detailed knowledge about amyloid self-assembly mechanisms. In general, amyloid aggregation of proteins can be readily modulated by the presence of salts. Using a multi-technique approach, we have compared the effect of 300 mM anions (SO_4^{2-}, CH_3COO^-, $H_2PO_4^-$, Br^-, NO_3^-, ClO_4^-) on the amyloid formation of α-lactalbumin (α-LA). The aggregation kinetics, the secondary structure content, and amyloid fibrils' morphology have been studied using ThT and intrinsic fluorescence, FT- IR spectroscopy, and atomic force microscopy. We found out that the effect of anions on kinetic parameters of α-LA amyloid formation and morphology of α-LA fibrils depends strongly on the salt's position in the Hofmeister series. These findings are essential to uncover the general mechanism of amyloid fibrillation and the possible future application of such fibers in biotechnology.

POSTER # 3

Poster Presentations
– Session 3 –

P-608
Prenylated Chalcones Exhibiting the Aggregation-Induced Emission Enhancement with Bioimaging Potential

Marta Arczewska[1], Iwona Budziak-Wieczorek[2], Daniel Kamiński[3], Alicja Skrzypek[2]
[1] Department of Biophysics, University of Life Sciences in Lublin, Akademicka 13, 20-950 Lublin, Poland, [2] Department of Chemistry, University of Life Sciences in Lublin, Akademicka 15, 20-950 Lublin, Poland, [3] Institute of Chemical Sciences, Maria Curie-Skłodowska University, Pl. Marii Curie-Sklodowskiej 3, 20-031 Lublin, Poland

Chalcone-based fluorophores with proven biological activities have recently attracted attention because of their potential applications in bioimaging. The spectroscopic and structural features of prenylated chalcones: xanthohumol (XN), isobavachalcone (IBC) and licochalcone A (LIC) were studied using the spectroscopic and structural methods, namely UV-Vis spectroscopy, steady-state fluorimetry, and single-crystal X-ray diffraction. In addition, the fluorescence studies were carried out in the solvent system with different viscosity and the solid state to prove the presence of aggregation-induced emission enhancement (AIEE) effect. The results of spectroscopic analyses revealed that IBC and LIC exhibited effective AIEE behaviour with good photostability, high fluorescent quantum yields and large Stokes shifts (>100 nm) in the aggregated forms and solid state. Moreover, the chalcone-lipid interactions were examined in phosphatidylcholine model membranes to investigate the structure-binding ability relationship. Finally, this study may provide an example of a chalcone-based biosensor, which can be further developed to synthesise new compounds with desirable spectroscopic properties for real-time monitoring of biomolecules and biological processes.

P-609
DNA Microscopy: A Novel Technique for Sequencing-Based Spatial Transcriptomics

Igor Baars[1], Alexander Kloosterman[1]
[1] Karolinska Institutet, Sweden

Current spatial transcriptomics techniques rely on complex microscopy setups, that are limited in target numbers and require significant expertise. Here, we present DNA microscopy, a method utilizing sequencing information to infer spatial organization without optical constraints. Our approach involves PCR-based amplification and concatemerization of DNA or RNA molecules, followed by sequencing to reveal their local adjacencies. From this information, a spatial reconstruction of the sample is then obtained. Using this method, we successfully obtained reconstructions of cell cultures that were similar to microscopy images. In conclusion, this novel technique aims to overcome limitations of traditional optical-based methods and pave the way for advancements in spatial transcriptomics and biological research.

P-610
Non-fluorescent transient states of tyrosine - a basis for label-free protein conformation and interaction studies

Niusha Bagheri[1], Hongjian Chen[1], Joachim Piguet[1], Mihailo Rabasovic[2], Jerker Widengren[1]
[1] Experimental Biomolecular Physics, Department of Applied Physics, KTH Royal Institute of Technology, Stockholm 10691, Sweden, [2] Photonic Center, Institute of Physics, Belgrade, University of Belgrade, Serbia

The aromatic amino acids tryptophan, tyrosine, and phenylalanine have been extensively used for different label-free protein studies, where information about conformations and interactions of the proteins is obtained from the intensity, wavelength and/or polarization of their emitted fluorescence. Like most fluorescent organic molecules, these amino acids also tend to undergo transitions into dark meta-stable states, such as triplet and photo-radical states. While this may be perceived as a problem, these transitions are also highly environment-sensitive and can be used as an additional set of parameters, reflecting interactions, folding states, and immediate environments around the proteins. In this work, we applied the transient state monitoring (TRAST) technique, analyzing the average intensity of tyrosine emission under different excitation modulations, to characterize the photophysics of tyrosine for such readout purposes. By investigating how the dark state transitions of tyrosine varied with excitation intensity, solvent pH, and redox conditions we established a photophysical model for tyrosine. Next, we studied Calmodulin (containing two tyrosines), a calcium-binding protein which is also used as a calcium sensor. From these TRAST experiments, performed with 280nm time-modulated excitation, we show that tyrosine dark state transitions represent a useful source of information for (label-free) analyses of protein conformations and interactions.

P-611
Sulfation patterns of heparan sulfates regulate the interactions of human papillomavirus to glycosaminoglycan for entry

Fouzia Bano[1], Laura Soria-Martinez[2], Dominik van Bodegraven[2], Kerstin Seier[1], Dario Conca[1], Konrad Throsteinsson[1], Anna M. Brown[3], Nicole L. Snyder[3], Mario Schelhaas[2], Marta Bally[1]
[1] Department of Clinical Microbiology and Wallenberg Centre for Molecular Medicine, Umeå University, Sweden , [2] Institute of Cellular Virology, ZMBE, University of Münster, Germany, [3] Department of Chemistry, Davidson College, Davidson, USA

Human papillomavirus 16 (HPV16) is the leading cause of cervical cancer and is of great medical significance. The interaction between HPV16 and heparan sulfate (HS) is essential for both the initial attachment to host cells and activating a crucial conformational change in the viral capsid termed structural activation. In this context, the role of specific sulfation groups of HS in regulating these processes is currently unknown. Here, we used biophysical assays to investigate the characteristics of the HPV16-HS interactions and a set of biochemical and cell-based assays to probe the functional role of individual sulfation in virus binding and structural activation. Our HPV16-HS interactions studies, relying on single molecule force spectroscopy (SMFS) to probe the monovalent virus-HS interaction and on single particle tracking to study the multivalent attachment/detachment kinetics of individual virions to HS, reveal the crucial role of N-sulfation in HPV16 binding and 2O-sulfation is dispensable. Also, SMFS data shows that 6O-sulfation mechanically strengthens the interactions between HS and HPV16 to facilitate binding. Our functional assays, in excellent agreement with biophysical assays, demonstrate that N-sulfation is crucial but alone insufficient for binding and structural activation of HPV16 and is likely aided by 6O-sulfation. Collectively, these findings identify the direct involvement of sulfation patterns of HS to HPV16 binding and structural activation and reveal how the distinct sulfation groups of HS synergize to facilitate the viral attachment which may influence entry and tropism of HPVs.

Poster Presentations
– Session 3 –

P-612
13C, 15N-labeling distinguishes different polypeptides in nanoscale images of the infrared absorption. Test case: interaction of the amyloid-β peptide with an inhibitory peptide

Andreas Barth[1], Suman Paul[1], Elina Berntsson[1,2], Cecilia Mörman[1], Adéla Jeništová[1], Jüri Jarvet[1,3], Astrid Gräslund[1], Sebastian K. T. S. Wärmländer[1]
[1] Stockholm University, Sweden [2] Tallinn University of Technology, [3] National Institute of Chemical Physics and Biophysics

Interactions between molecules are fundamental in biology. They occur also between amyloidogenic polypeptides that are associated with different amyloid diseases, which makes it important to study their properties in mixed samples. However, addressing such research questions with imaging techniques is hindered by the problem to distinguish different polypeptides without adding artificial probes for detection, which might modify their properties and interactions. Here we present a new method for this purpose. We show that 13C, 15N-labeling can be used to distinguish peptides in nanoscale images of their infrared absorption, even when they have similar secondary structure. We studied different aggregation states of the amyloid-β peptide (Aβ) and its interaction with an inhibitory, cell-penetrating peptide (NCAM1-PrP) using scattering-type scanning near field microscopy. Labeled and unlabeled peptides could be discriminated by comparing images of the optical phase taken at wavenumbers characteristic for either the labeled or the unlabeled peptide. NCAM1-PrP seems to be able to dissolve or associate with existing Aβ fibrils because "naked" Aβ fibrils are not detected after mixing.

P-613
A lever-like anti-Aβ fibrils potential of azobenzene molecules

Zuzana Bednarikova[1], Dorota Niedzialek[2], Grzegorz Wieczorek [2], Przemyslaw Kozminski[3], Zuzana Gazova[1]
[1] Institute of Experimental Physics, Slovak Academy of Sciences, Watsonova 47, Kosice, Slovakia, [2] Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawinskiego 5A, Warsaw, Poland, [3] Centre of Radiochemistry and Nuclear Chemistry, Institute of Nuclear Chemistry and Technology, Dorodna 16, Warsaw, Poland

Amyloid fibrils of amyloid β (Aβ) peptides are a neuropathological feature of Alzheimer's disease (AD), one of the world's fastest-growing incurable neurological diseases. Therefore, the exploration of novel treatment approaches is in high demand. We have employed the lever-like potential of azobenzene molecules (reversible photo-induced isomerization between the cis (nonplanar, metastable) and trans (planar, thermodynamically stable) conformations) to dissociate fibrillar aggregates of Aβ peptides. We have integrated in vitro, in silico, and cell workflow to study the activity of azobenzene molecules consisting of one or two molecules of azobenzene with a DTPA ligand. The compound with 2 molecules of azobenzene connected via DTPA ligand was able to dissociate Aβ fibrils in low micromolar concentrations. The dissociation activity increased upon photo- induced isomerization by 10-folds. The compounds were able to dissociate the Aβ fibrils into non-cytotoxic species. Based on our results, we assume that the cis-trans switch of the initially excited azo-molecule put mechanical stress on the β-strands, which increases the potential of compounds to dissociate the Aβ fibrils.

P-614
Utilizing DNA origami-based molecular platforms for accessing ion-dependent conformational dynamics of guanine-rich DNA motifs

Aleksandra Bednarz[1], Ganesh Agam[2], Steffan Sønderskov[1], Tim Schroeder[2], Mingdong Dong[1], Philip Tinnefeld[2], Don Lamb[2], Victoria Birkedal[1]
[1] Aarhus University, Denmark [2] Ludwig-Maximilians-Universität

DNA origami enables self-assembly of custom-designed nanodevices and molecular breadboards. These nanostructures offer the possibility of predictable arrangement on the nanoscale of a plethora of components such as fluorophores, DNA, or proteins. They are thus compelling platforms for numerous applications where spatial control is key, provided that their structure remains unaltered by the imaging solution or environment. Here, we utilize DNA origami-based structures for anchoring and subsequent investigation of conformational dynamics of non-canonical secondary structures of DNA known as G-quadruplexes (G4s) with the aid of single-molecule FRET spectroscopy. G4 motifs are widely involved in regulation of essential cellular processes and often exhibit rough conformational energy landscape, which leads to frequent instances, where several topologies of similar free energies are populated. One of the essential factors determining the predominant and coexisting topologies are type and concentration of metal ions present in the solution. We provide quantitative information on the influence of various ionic conditions on the stability and applicability of the molecular breadboards as well as on the structural dynamical switching of the G4s.[1,2] With the aid of the developed system, we observed slow conformational interconversion on a timescale of seconds as well as fast dynamics in the microsecond range, providing insights into G4 folding and dynamics.

P-615
Molecular Mechanisms of Genetically Encoded Fluorescent Biosensors

Melike Berksöz[1], Canan Atılgan[1]
[1] Sabanci University, Turkey

Genetically encoded fluorescent biosensors are molecular tools that couple ligand-induced conformational changes to a fluorescence output. Structural determinants of successful biosensors are difficult to predict which makes the development of new biosensors a long trial-and-error process. Therefore, we set out to establish a methodology to predict biosensor performance based on ligand-induced conformational changes and resulting hydrogen bond dynamics observed in molecular dynamics (MD) simulations. We modeled several reported biosensors with Colabfold in apo and holo forms and performed 600 ns long all-atom MD simulations. Protonation state of the chromophore ultimately determines the fluorescence state; therefore, we modelled the chromophore in both neutral (dark) and anionic (bright) states. For control purposes, we also simulated bright and dark forms of green fluorescent protein (GFP) and red fluorescent protein (RFP) without the sensor domains. Hydrogen bond occupancies of equilibrated trajectories were analyzed and hydrogen bonded residue pairs whose occupancies differ by more than 40% between holo and apo states are reported as fingerprints of on/off states of the biosensors. We find that a percolating hydrogen bond network communicates the binding event in the sensing domain to the chromophore of the fluorescent protein; as a result, the anionic chromophore in the bright state is typically stabilized by a proton donor nearby. This continuous network is found to be broken in the off state. Our results suggest that hydrogen bond dynamics can be a good predictor of biosensor efficiency. We believe this approach can help accelerate development of a broader range of fluorescent biosensors.

Poster Presentations
– Session 3 –

P-616
Live-cell super-resolution imaging of actin using LifeAct-14 with a PAINT-based approach
Haresh Bhaskar[1,2], Mathew Horrocks[2], Lynne Regan[2], Sonia Gandhi[1]
[1] Francis Crick Institute, London, UK, [2] University of Edinburgh, United Kingdom

Protein localisation and organization are highly dynamic processes that have historically been studied using fluorescence microscopy techniques. However, diffraction-limited methods such as fluorescent protein fusions, lack the resolution to uncover detail at the nanoscale. We present direct- LIVE-PAINT, an easy-to-implement approach for the nanoscopic imaging of protein structures in live cells using labeled binding peptides. We demonstrate the feasibility of direct-LIVE-PAINT with an actin-binding peptide fused to EGFP, the location of which can be accurately determined as it transiently binds to actin filaments. We show that direct-LIVE-PAINT can be used to image actin structures below the diffraction-limit of light and have used it to observe the dynamic nature of actin in live cells. We envisage a similar approach could be applied to imaging other proteins within live mammalian cells. We are currently working on simultaneous approaches to target aggregating proteins involved in neurodegenerative disease.

P-617
A Refined All-Atom Model for Accurate Electrostatic Interactions of Glycans
Denys Biriukov[1,2], Miguel Riopedre Fernández[2], Hector Martinez-Seara[2]
[1] CEITEC – Central European Institute of Technology, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic, [2] Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Flemingovo namesti 2, 160 00 Prague, Czech Republic

All-atom molecular dynamics (MD) simulations are a potent tool to study molecular details of vital biological processes and guide drug development. Many of such processes occur at the cellular surface, where glycans play a crucial role in host-pathogen identification. However, glycans, especially charged, are typically insufficiently accurate in the most common MD force fields due to missing electronic polarization. We will present our recent progress in developing "charge-scaled" MD models for glycans that include electronic polarization in a mean-field way. We optimized the models for D-glucuronic and D-galacturonic acids, principal building blocks of larger glycan-containing (bio)molecules. Our models do not suffer from the common problems of nonpolarizable force fields like unphysical aggregation and exaggerated ion pairing. Using new models, we constructed larger polysaccharides called glycosaminoglycans and investigated their interactions with positively charged peptides. We found that short arginine-rich peptides have a higher affinity for hyaluronan (the only non-sulfated glycosaminoglycan) compared to lysine-rich peptides, in agreement with NMR experiments. As the next step in optimizing glycan interactions, we examined the binding of calcium, a divalent cation of paramount biological importance, to sulfonates, the dominant functional group of sulfated glycosaminoglycans. We designated the solvent-shared ion pairing as the preferential binding mode, in agreement with ab initio MD simulations. Our work offers a simple recipe to improve electrostatic interactions in all-atom MD models for glycans, which can be used for accurate MD simulations of biological systems, including computational design of antibacterial and antiviral treatments.

P-618
DNA binding enhances activation domain accessibility in pioneer factor Sox2
Sveinn Bjarnason[1], Jordan McIvor[2], Andreas Prestel[3], Kinga Demény[1], Birthe Kragelund[3], Davide Mercadante[2], Pétur Heidarsson[1]
[1] Department of Biochemistry, Science Institute, University of Iceland, Sturlugata 7, 102 Reykjavík, Iceland., [2] School of Chemical Science, University of Auckland, Auckland, New Zealand., [3] Structural biology and NMR Laboratory, Department of Biology, Faculty of Science, University of Copenhagen, 2200 Copenhagen N, Denmark.

Transcription factors are key players in controlling gene expression and cell fate decisions in humans. Transcription factor function is conveyed through intrinsically disordered effector domains, yet descriptions of their conformational ensembles and how they are modulated by DNA binding are largely missing. Our study focused on Sox2, a pioneer transcription factor involved in early development, and used a combination of single- molecule FRET, NMR experiments, and molecular simulations to obtain insight into the protein's structural ensembles. The C-terminal effector domain of Sox2 is highly disordered and dynamic, but its conformational properties are influenced by weak electrostatic interactions with the structured DNA binding domain. Binding to DNA or nucleosomes leads to a significant expansion of the effector domain, which increases the accessibility of the transcriptional activation domains. General sequence features of transcription factors indicate that transient and charge-driven interactions between domains are common, enabling tuning of activation domain accessibility without necessarily compromising DNA binding affinity.

P-619
Monte Carlo simulations for the evaluation of oligomerization data in TOCCSL experiments
Clara Bodner [1], Dominik Kiesenhofer[2], Gerhard J. Schütz[1], Mario Brameshuber[1]
[1] Biophysics Group, Institute of Applied Physics, TU Wien, Lehárgasse 6, 1060 Vienna, Austria, [2] Quantum Optics and Spectroscopy, Institut für Experimentalphysik, Universität Innsbruck, Technikerstraße 25, 6020 Innsbruck, Austria

The stoichiometry of cell-surface proteins and lipids is fundamental to cellular signaling and function. Among others, the single-molecule fluorescence microscopy technique "Thinning Out Clusters while Conserving Stoichiometry of Labeling" (TOCCSL) is used to study the stoichiometry of proteins and lipids in the plasma membrane of live cells and in model systems at physiologically high surface densities. In TOCCSL, an aperture-defined subregion of the plasma membrane is photobleached. At the onset of the recovery process, individual cell-surface proteins can be imaged as diffraction-limited single-molecule signals and their oligomerization state can be determined via brightness analysis (one-color TOCCSL) or co-localization analysis (two-color TOCCSL). Due to finite photobleaching times, finite laser intensities and diffraction of the laser at the aperture edges some oligomers undergo non-stoichiometric/partial photobleaching. Hence, not all oligomeric subunits are detectable after recovery and the number of higher order oligomers is underestimated. To characterize the influence of partial photobleaching, oligomerization state-dependent mobility differences and false oligomer detection due to random encounters on the detected oligomeric composition, we used Monte Carlo-based in silico TOCCSL experiments. Our simulations clearly show, how the selection of experimental parameters (photobleaching time, recovery time, size of analysis region) as well as the intrinsic characteristics of the investigated system (diffusion coefficient, cell size, fractions of oligomers of different order) affect the interpretation of TOCCSL data.

Poster Presentations
– Session 3 –

P-620
Spectral signatures of excess-proton waiting and transfer-path dynamics
Florian Brünig[1], Manuel Rammler[1], Ellen Adams[2], Martina Havenith[2], Roland Netz[2]
[1] Freie Universität Berlin, Physics, Berlin, Germany, [2] Ruhr-Universität Bochum, Chemistry, Bochum, Germany

Signatures of solvated excess protons in infrared difference absorption spectra, such as the continuum band between the water bend and stretch bands, have been experimentally known for a long time and have recently been used to analyze protonation dynamics in photoactive proteins. However, the theoretical basis for linking spectral signatures with the microscopic proton-transfer mechanism so far relied on normal-mode analysis. We analyze the excess-proton dynamics in ab initio molecular-dynamics simulations of aqueous hydrochloric acid solutions by trajectory-decomposition techniques. The continuum band in the 2000 cm to 3000 cm range is shown to be due to normal-mode oscillations of temporary H O complexes. An additional prominent peak at 400 cm reports on the coupling of excess-proton motion to the relative vibrations of the two flanking water molecules. The actual proton transfer between two water molecules, which for large water separations involves crossing of a barrier and thus is not a normal mode, is characterized by two time scales: Firstly, the waiting time for transfer to occur in the range of 200 fs to 300 fs, which leads to a broad weak shoulder around 100 cm, consistent with our experimental THz spectra. Secondly, the mean duration of a transfer event of about 14 fs, which produces a rather well-defined spectral contribution around 1200 cm and agrees in location and width with previous experimental mid- infrared spectra.

P-621
Studying sub-cellular molecular organization in bacteria through single-molecule co-localization
Chiara Caldini[1], Sara Del Duca[2], Alberto Vassallo[2], Renato Fani[2], Francesco Saverio Pavone[1,3,4], Lucia Gardini[1,3,4]
[1] LENS – European Laboratory for Non-linear Spectroscopy, Via Nello Carrara 1, 50019 Sesto Fiorentino, Italy., [2] Department of Biology, University of Florence, Via Madonna del Piano 6, Sesto Fiorentino, 50019 Florence, Italy., [3] Department of Physics and Astronomy, University of Florence, Via Sansone 1, 50019 Sesto Fiorentino, Italy., [4] National Institute of Optics – National Research Council, Largo Fermi 6, 50125 Florence, Italy.

Despite bacteria apparently lack any kind of subcellular organization several studies on metabolic pathways have been raising the evidence for a certain degree of intracellular organization aimed at bringing enzymes involved in the same metabolic pathways in close proximity to drive the reactions to a faster kinetics. Molecular biology provides different techniques for the study of molecular interactions, such as the two-hybrid systems, but an imaging approach is needed to obtain spatial information about those interactions. Studying spatial localization at the molecular level requires super-resolution techniques that can reach the nanometer scale. In this work we show how by combining PhotoActivated Localization Microscopy (PALM) imaging process with Expansion Microscopy (ExM) treatment of biological samples it is possible to achieve the resolution required to perform simultaneous colocalization of single molecules and measure the relative distances between them with few nanometers precision. Ex-PALM is applied to study the spatial distribution of two enzymes (HisF, HisH) of the histidine metabolic pathway in E. Coli bacteria. By inserting a plasmid, bacteria were transformed so that each protein of interest was tagged with a photoactivable fluorescent protein (HisF- PAGFP, HisH-PAmCherry) which could be imaged and localized with nanometer precision. Here we show preliminary results where, after correcting for crosstalk and chromatic aberrations, an analysis method based on a combination of local density co-localization index and a nearest neighbours search was applied to identify colocalizing pairs of molecules and estimate the distance between them. Preliminary results show an average co-localization distance of 9.7 nm.

P-622
Fluorescence Labelling influences differently the aggregation of misfolded peptides; correlative nanoscopy applications.
Claudio Canale[1], Paolo Bianchini[3], Alberto Diaspro[1,3]
[1] Department of Physics, University of Genova, Italy, [2] Materials Characterization Facility, Istituto Italiano di Tecnologia, Italy, [3] Nanoscopy, CHT Erzelli, Istituto Italiano di Tecnologia, Italy

Fluorescence microscopy and spectroscopy are primary in biological and biophysical investigation. These techniques work perfectly in liquid in physiological temperature, pH, and ionic strength conditions, characterizing dynamic intermolecular processes in an optimal environment. Despite this, some issues are inherently related to the use of fluorescent dye molecules. The labeling procedure, i.e., the binding of a fluorescent tag to the investigated molecule, represents a small structural change that, in some cases, modifies the molecule's ability to interact with other molecules. Misfolded protein aggregation represents a particular intermolecular process case and is associated with several pathologies, such as Alzheimer's and Parkinson's disease. Several works focused on in-vitro aggregation are performed by using fluorescence techniques. A challenge is to check if the presence of fluorophores influences aggregation and fibrillation. We used a correlative microscopy technique based on integrating an atomic force microscope (AFM) and a stimulated emission depletion (STED) super-resolution microscope. We first investigated the aggregation of insulin and of the Alzheimer's disease- related Aβ1-42, and Aβ1-40 peptides. We then investigated the aggregation process of α-synuclein, a peptide involved in the neurodegenerative cascade in Parkinson's disease. In all the cases, the aggregation started from a solution of partially labeled monomers. We demonstrated that the labelling procedure, and in particular the binding site of the fluorophore along the polypeptide chain, induces changes in the aggregation propensity of the peptides. We also demonstrated that by targeting the dye in a particular region of the polypeptide chain the influence of the fluorophore is minimized.

P-623
α-catenin regulates mechanotransduction at cell-cell junctions by a directionally asymmetric and cooperative catch bond with F-actin
Marco Capitanio[1,2], Marios Sergides[1,2], Claudia Arbore[1,2], Lucia Gardini[2,3], Giulio Bianchi[1,2], Anatolii, V. Kashchuk[1,2] [1] University of Florence, Italy, [2] LENS (European Laboratory for Non-linear Spectroscopy), Italy, [3] CNR-INO, Italy

Cell adhesions dynamically tune their mechanical properties during tissue development and homeostasis. Fluid connections required for cell motility can switch to solid links to maintain the mechanical rigidity of epithelial layers. Changes in the composition and clustering of adhesion molecules have been proposed to modulate cell junction fluidity, but the underlying mechanisms are unclear. α-catenin has been shown to play a fundamental role in different adhesion sites. At adherens junctions (AJ), α-catenin forms heterodimers with β-catenin that are believed to resist force on actin. Outside AJ, α-catenin binds itself to form homodimers that regulates the actin cytoskeleton network and promote adhesion and migration, but its mechanosensitive properties are inherently unknown. We used ultra-fast laser tweezers to investigate the load-dependence of α- catenin interaction with F-actin at high temporal and spatial resolution. Surprisingly, we found that a single α-β-catenin heterodimer does not resist force but instead slips along actin in the direction of force. Conversely, the action of 5 to 10 α-β-catenin heterodimers together with force applied to the -end of an actin filament engaged a molecular switch in the α-catenin molecules, which unfolded and strongly bound actin as a cooperative catch-bond. Similarly, a single α-catenin homodimer formed a strong asymmetric catch-bond with actin triggered by protein unfolding under force applied to the -end of F-actin. Our data reveal an unconventional mechanism that allows α-catenin to regulate the mechanical properties of AJ and indicate that α-catenin clustering together with intracellular tension engage a fluid-to-solid phase transition at the membrane- cytoskeleton interface.

Poster Presentations
– Session 3 –

P-624
alphaS1-Casein administration to a C. elegans model of Alzheimer's disease through proteoliposome delivery
Rita Carrotta[1], Angela Paterna[1], Pamela Santonicola[2], Giulia Di Prima[3], Estella Rao[1], Samuele Raccosta[1], Giuseppina Zampi[2], Oscar Moran[4], Mauro Manno[1], Elia Di Schiavi[2], Fabio Librizzi[1]
[1] CNR–Institute of Biophysics, Palermo 90146, Italy, [2] CNR–Institute of Biosciences and BioResources, Napoli 80131, Italy, [3] UNIPA–STEBICEF, Palermo 90128, Italy, [4] CNR–Institute of Biophysics, Genova 16149, Italy

In the early phases of Alzheimer's disease (AD) highly reactive and toxic species form spontaneously and transiently in the amyloid building pathway. A therapeutic strategy consists in the recruitment of these intermediates, thus preventing their aberrant interaction with cell membrane components. Milk αs1-Casein is an intrinsically disordered protein, that is able to inhibit A amyloid aggregation in vitro, by sequestering transient species. In order to test αs1-Casein for the treatment of the AD, it needs to be delivered in the place of action. Here we demonstrate the use of large unilamellar vesicles (LUV) as nanocarriers for αs1-Casein. After proteo-LUVs characterization and αs1-Casein loading quantification, we report the effectiveness of the proposed delivery strategy in vivo on a C. elegans AD model. Proteo-LUVs allow efficient administration of the protein, exerting a positive functional readout at very low doses, while avoiding the intrinsic toxicity of αs1-Casein. Proteo-LUVs of αs1-Casein represent an effective proof of concept for the exploitation of partially disordered proteins as therapeutic strategy in mild AD conditions.

P-625
Untangling the Disaggregation Mechanism of the Hsp100 Protein Machine ClpB
Remi Casier[1], Gilad Haran[1]
[1] Weizmann Institute of Science, Israel

Protein disaggregases are nano-sized machines capable of reversing protein aggregation and are vital for maintaining cell homeostasis. Hsp100 disaggregases, such as ClpB, are believed to liberate proteins from aggregates by actively pulling a strand of a substrate into a large central pore utilizing the energy of ATP hydrolysis. However, the exact mechanism and timescale of this process remains uncertain. To address this issue, we use single-molecule FRET spectroscopy to directly observe the dynamic interactions between ClpB and a model substrate, casein. The real-time monitoring of the efficiency of energy transfer between fluorescently tagged casein and ClpB revealed that, in the presence of ATP, casein is fully translocated through the central pore of ClpB on the millisecond timescale.

P-626
Protein charge transfer far from equilibrium: a theoretical perspective
Mike Castellano[1], Christoph Kaspar, Michael Thoss, Thorsten Koslowski
[1] University of Freiburg, Germany

Potential differences for protein-assisted electron transfer across lipid bilayers or in bio-nano setups can amount to several 100 mV, they lie far outside the range of linear response theory. We describe these situations by Pauli-Master equations that are based on Marcus' theory of charge transfer between self-trapped electrons and that obey Kirchhoff's current law. In addition, we take on-site blockade effects and a full non-linear response of the local potentials into account. We present analytical and numerical current-potential curves and electron populations for multi-site model systems and biological electron transfer chains. Based on these, we provide empirical rules for electron populations and chemical potentials along the chain. The Pauli-Master mean-field results are validated by kinetic Monte Carlo simulations.

P-627
Short truncated tau fragment 321-391 aggregates despite the lack of VQIxxK sequence
Ondrej Cehlar[1], Stefana Njemoga[1,2], Simona Slusna[1,2], Petra Majerova[1], Juraj Piestansky[3], Zuzana Bednarikova[4], Rostislav Skrabana[1]
[1] Institute of Neuroimmunology, Slovak Academy of Sciences, Dubravska cesta 9, 845 10 Bratislava, Slovakia, [2] Faculty of Natural Sciences, Comenius University, Ilkovicova 3278/6, 841 04 Bratislava, Slovakia, [3] Faculty of Pharmacy, Department of Galenic Pharmacy, Comenius University, Odbojárov 10, 832 32 Bratislava, Slovakia, [4] Institute of Experimental Physics, Department of Biophysics, Watsonova 47, 040 01 Kosice, Slovak Republic

Microtubule (MT) associated protein tau belongs to the class of intrinsically disordered proteins. Under pathological conditions of tauopathy, tau dissociates from microtubules to form insoluble filaments with disease specific fold. It was previously shown that the presence of two hexapeptide sequences (VQIxxK) can trigger protein aggregation and it was long believed that these aggregation prone sequences were responsible for tau aggregation. It begins to be apparent that sequences in R' region that follow tau MT binding repeats play a crucial role in MT binding and potentially also in aggregation process. Recombinant truncated tau proteins tau306-391, tau316-391, tau321-391 and tau326-391 were aggregated under different conditions (presence of heparin and DTT). Results of aggregation were monitored using different techniques: ThT fluorescence, DLS, AFM, FTIR and capillary electrophoresis. We have observed in vitro aggregation of several tau proteins, mainly tau 321-391 which lacks the aggregation prone sequence VQIVYK. Early signs of oligomer formation were observed by measurements using capillary electrophoresis. The results will further widen the knowledge about pathological aggregation of tau proteins. Acknowledgement. This work was supported by the Slovak Research and Development Agency Grant No. APVV-21-0479.

Poster Presentations
– Session 3 –

P-628
Understanding Morphology and Kinetics of Self-assembly of Intrinsically Disordered Protein beta-Casein
Saikat Chakraborty[1], Tatiana Morozova[2], Laura MATEO MINARRO[2], Tilo Seydel[2], Jean-Louis Barrat[1]
[1] Université Grenoble Alpes, [2] Institut Laue-Langevin, France

P-629
Simulation of Liquid Jet Explosions and Shock Waves Induced by X-Ray Free-Electron Lasers
Leonie Chatzimagas[1], Jochen S. Hub[1]
[1] Saarland University, Germany

X-ray free-electron lasers (XFELs) produce X-ray pulses with very high brilliance and short pulse duration. These properties enable structural investigations biomolecular nanocrystals, and they allow resolving the dynamics of biomolecules down to the femtosecond timescale. To deliver the samples rapidly into the XFEL beam, liquid jets are used. The impact of the X-ray pulse leads to vaporization and explosion of the liquid jet, while the expanding gas triggers the formation of shock wave trains traveling along the jet, which may affect biomolecular samples before they have been probed. Here, we used atomistic molecular dynamics simulations to reveal the structural dynamics of shock waves after an X-ray impact. Analysis of the density in the jet revealed shock waves that form close to the explosion center and travel along the jet. A trailing shock wave formed after the first shock wave, similar to the shock wave trains in experiments. Although using purely classical models in the simulations, the resulting explosion geometry and shock wave dynamics closely resemble experimental findings, and they highlight the importance of atomistic details for modeling shock wave attenuation.

P-630
Engineering electroosmotic flow for protein capture in nanopore sensing devices
Mauro Chinappi[1], Adina Sauciuc[2], Matteo Baldelli[1], Giovanni Di Muccio[3], Blasco Morozzo della Rocca[1], Matthijs Tadema[2], Francesco Viola[4], Sébastien Balme[5,6], Giovanni Maglia[2]
[1] Università di Roma Tor Vergata, Italy [2] University of Groningen, [3] Sapienza Università di Roma, [4] Gran Sasso Science Institute, [5] Institut Europen des Membranes, [6] University of Montpellier

Nanopores are powerful tools for single molecule sensing of biomolecules and nanoparticles. The signal associated to the molecule to be analyzed strongly depends on its interaction with the narrower section of the pore (constriction) that may be modified to improve the sensing accuracy.
Moreover, modification of the pore interior have been also commonly used to induce an electroosmotic flow that is able to favour the capture of the molecules in the pore under a voltage bias. This is particularly relevant for the analysis of weakly charged molecules, such as proteins, where electrophoretic force alone cannot be used to capture and translocate the molecule. Here, using experiment and simulations, we show how mutation to the pore interior are responsible of an electroosmotic flow able to induces the unidirectional transport of polypeptides, even against relatively strong electrophoretic forces. Finally, additional simulations indicates that it is possible to achieve intense an electroosmotic flow, without altering the pore constriction. This strategy provides much more flexibility in the pore design since electroosmosis can be controlled modifying the pore far from the constriction while the constriction can be optimized to improve sensing accuracy.

P-631
RAG DNA recombinase mechanism investigated by single molecule assays; novel reaction mechanism derived from thermodynamics and statistical analysis.
Mihai Ciubotaru[1], Elena Ioniță[1], Peter Koo[2]
[1] Colentina Clinical Hospital Bucharest,Dept Immunology, Şoseaua Ştefan cel Mare, Nr. 19-21,sector 2,020125,Bucharest, Romania, [2] Simons Center for Quantitative Biology, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, USA;

Introduction: RAG recombinase rearranges the genes encoding the variable regions of the specific lymphocyte receptors (T cells-TCRs, B cells Immunoglobulins) via V(D)J recombination. In all jawed vertebrates this is the central process generating the acquired immunity. Methods: Using the single molecule TPM technique, we investigate at molecular level the dynamics, affinity and stability of RAG-DNA interactions. The TPM studies are complemented by assessments of DNA-RAG intermolecular forces using the laser optical tweezers measurements. Results: Among these interactions and central to our studies is that of the RAG-DNA 12/23 synaptosome (PC) assembly which vastly determines the combinatorial diversity of the Immune repertoire. Single site RAG-DNA binding (SC) occurs rapidly and with high affinity. On the contrary, synapsis and post- synapsis DNA coupled cleavage are inefficient, occur with slow rates and such effects are poorly understood at molecular level. The TPM measured parameters are used first to refine RAG sequence of events in its reaction mechanism whereas the interplay between the calculated enthalpic/binding and entropic/diffusion controlled components help us define the rate and efficiency of PC formation and DNA catalysis. Transient unstable states observed either between SC and synapsis or after PC formation were thermodynamically assessed. Conclusions: A molecular model of interactions is built which integrates the assessed single-molecule events to a newly proposed RAG reaction mechanism.

Poster Presentations
– Session 3 –

P-632
A biophysical model of early embryogenesis: the role of surface tension in compaction and the polarisation vs position argument
Alaina Cockerell[1], David Richards[1]
[1] University of Exeter, United Kingdom

Despite decades of research, the earliest stages of mammalian embryogenesis remain poorly understood. The first few days of development, before blastocyst implantation, give rise to a host of fascinating, unsolved questions, including the physical principles that govern cellular self- organisation, how system robustness is achieved, and ways of quantifying embryo quality. These questions are crucial for understanding human fertility, improving IVF treatment (where the majority of human embryos fail to develop beyond implantation) and tackling global food security (via economically-important animals such as pigs, cows and sheep). Here, I will discuss my recent work to create a novel vertex model to simulate the very first few days of development. After describing the key biophysical forces involved, I will start by explaining how the model was developed and implemented. I will then present my key initial findings, including the factors that drive cell compaction and internalisation, the role of membrane tension and cell-to-cell adhesion, and key ways in which early embryogenesis can fail. I will also discuss my results from testing competing theories of how the trophectoderm cells are decided (the position, polarisation, and combined hypotheses). I will finish by discussing future plans including experimental tests and how the model can be extended to later stages of development.

P-633
The role of membrane complexity in the early entry stages of SARS-CoV-2 variants
Dario Valter Conca[1,2], Fouzia Bano[1,2], Julius von Wirén[1,2], Lauriane Scherrer[1,2], Justas Svirelis[3], Konrad Thorsteinsson[1,2], Andreas Dahlin[3], Marta Bally[1,2]
[1] Department of Clinical Microbiology, Umeå University, Sweden, [2] Wallenberg Centre for Molecular Medicine (WCMM), Umeå University, Sweden, [3] Department of Chemistry and Chemical Engineering, Chalmers University of Technology, Sweden

The highest density of mutations in SARS-CoV-2 variants is located on the spike glycoprotein (S), which is responsible for receptor ACE2 engagement. This suggests that SARS-CoV-2 is evolving to optimize viral entry. Several molecular studies report differences in the affinity between isolated S and ACE2 among variants. However, overall ACE2 affinity poorly correlates with the increased infectivity of recent variants. We address this discrepancy by considering the virus interaction with the whole plasma membrane and study the role of avidity and membrane complexity in modulating virus-host binding. To this end, we employ an in-vitro model system combining single-particle tracking and native supported lipid bilayers (nSLBs) made from lung epithelial cells. As virion mimics, we developed S-decorated liposomes that allow for direct comparison between variants and BSL-1 handling. S-liposome interaction with nSLBs showed a significant increase in avidity for Omicron compared to Delta and Wuhan strains. Further, using single-molecule force spectroscopy, we reveal a higher affinity for Omicron and Delta S to sensor immobilise heparan sulfate (HS). Our results indicate a shift in the variants' attachment strategy towards more efficient use of coreceptors and the role of HS as an initial docking site that facilitates virus accumulation at the membrane and ACE2 engagement.

P-634
Localization-dependent changes of lipid profile in various perivascular adipose tissues upon progression of atherosclerosis studied with Raman spectroscopy
Krzysztof Czamara[1], Zuzanna Majka[1], Mateusz Wawro[2], Agnieszka Kaczor[3]
[1] Jagiellonian Centre for Experimental Therapeutics (JCET), Jagiellonian University, 14 Bobrzynskiego Str., 30-348 Krakow, Poland., [2] Department of Cell Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, 7 Gronostajowa Str., 30-387 Kraków, Poland , [3] Faculty of Chemistry, Jagiellonian University, 2 Gronostajowa Str., 30-387 Krakow, Poland.

Introduction: Recent decades have shown that the perivascular adipose tissue (PVAT) surrounding the aorta contributes to cardiovascular and lifestyle diseases i.e., atherosclerosis. PVAT adipocytes secrete vasoactive substances that, after diffusion into the vessel wall, affect the endothelial layer leading to its dysfunction. Furthermore, phenotypic and functional differences in PVAT have an impact on susceptibility to atherosclerosis. In this work, we analyzed alterations in the lipid marker, the lipid unsaturation degree, used to determine the PVAT status upon the progression of atherosclerosis. Methods: Raman spectroscopy was applied to estimate the PVAT chemical composition based on lipid unsaturation degree (I1660/I1444). We investigated isolated primary adipocytes from thoracic and abdominal PVAT and PVAT samples from aorta, femoral and mesentery arteries compared to common white and brown adipose tissue depots from ApoE-/-/LDLr-/- mice at age of 5-72 weeks. Results: Lipid profile of the thoracic PVAT is homogeneous along aorta and distinguishable in contrast to the significantly mixed composition of the abdominal PVAT. Lipid unsaturation degree reveals location- and time-dependent alterations in PVATs lipid profile indicating, inter alia, earlier changes in the abdominal aorta, visible for 16-week-old animals, than in the thoracic aorta, while for mesenteric PVAT remained constant. Conclusions: PVATs lipid profile is heterogeneous and changes upon atherosclerosis development are manifested more in white-like adipose tissue depots that enable to shed more light on the biochemistry of PVAT.

P-635
Metal-binding and circular permutation-dependent thermodynamic and kinetic stability of azurin
Debanjana Das[1], Priya Yadav[1], Soumyajit Mitra[1], Sri Rama Koti Ainavarapu[1]
[1] Tata Institute of Fundamental Research (TIFR), Mumbai, India

Native topology is known to determine the folding kinetics and the energy landscape of proteins. Circular permutation (CP) of proteins alters the order of the secondary structure connectivity while retaining the three-dimensional structure, making it an elegant and powerful approach to altering native topology. Previous studies elucidated the influence of CP in proteins with different folds such as Greek key β-barrel, β-sandwich, β- α-β, and all α-Greek key. CP mainly affects the protein stability and unfolding kinetics, while folding kinetics remains mostly unaltered. However, the effect of CP on metalloproteins is yet to be elaborately studied. The active site of metalloproteins poses an additional complexity in studying protein folding. Here, we investigate a CP variant (cpN42) of azurin—in both metal-free and metal-bound (holo) forms. As observed earlier in other proteins, apo-forms of wild-type (WT) and cpN42 fold with similar rates. In contrast, zinc-binding accelerates the folding of WT but decelerates the folding of cpN42. On zinc-binding, the spontaneous folding rate of WT increases by >250 times that of cpN42, which is unprecedented and the highest for any CP to date. On the other hand, zinc-binding reduces the spontaneous unfolding rate of cpN42 by ~100 times, making the WT and CP azurins unfold at similar rates. Our study demonstrates metal binding as a novel way to modulate the unfolding and folding rates of CPs compared to their WT counterparts. We hope our study increases the understanding of the effect of CP on the folding mechanism and energy landscape of metalloproteins.

Poster Presentations
– Session 3 –

P-636
Avoiding photoblueing-induced artefacts in confocal and STED microscopy

Anindita Dasgupta[1,2], Agnes Koerfer[2], Boštjan Kokot[3], Iztok Urbančič[3], Christian Eggeling[1,2], Pablo Carravilla[4]
[1] Leibniz Institute of Photonic Technology, Germany [2] Institute of Applied Optics and Biophysics, Friedrich Schiller University, [3] Jožef Stefan Institute, [4] Science for Life Laboratory, Karolinska Institutet

Confocal and stimulated emission depletion (STED) super-resolution microscopy are scanning microscopy techniques widely used in life sciences. Unfortunately, commonly used laser excitation powers can induce photochemical reactions of fluorescent dyes. A particularly concerning reaction is photoblueing, i.e. the photoconversion of dyes to molecules showing a blue-shifted spectrum, which can introduce artefacts in quantitative microscopy. Here, we use a combination of advanced microscopy techniques, such as spectral STED, fluorescence correlation spectroscopy and fluorescence lifetime imaging to 177haracterize photoblueing of popular organic dyes and its dependency on the excitation and depletion lasers. We report that some widely used fluorescent dyes undergo a significant blue shift upon laser irradiation in confocal measurements, an effect further amplified when in STED mode. Spectral shifts are not only dye-dependent, but also greatly affected by common modifications such as antibody conjugation. We precisely quantify the number of molecules that undergo photoblueing which is as high as 25% for some popular dyes. Even if the brightness of photoblued dyes is significantly lower than that of the original dyes, we show that they can lead to biased estimations of membrane biophysical parameters in live cell experiments when using lipid packing sensors. Finally, we demonstrate that artifacts can be circumvented by using exchangeable dyes and we also identify STED-compatible dyes showing negligible photoblueing tendency.

P-637
Dynamic single particle measurements with a high throughput iSCAT-TIRF setup

Giovanni De Angelis[1,2], Mariia Miasnikova [1,2], Jacopo Abramo[1], Anne Marie Scholz [1,2], Christian Eggeling [1,2], Francesco Reina[1]
[1] Leibniz Institute of Photonic Technology (IPHT), [2] Friedrich Schiller University, Germany

Interferometric Scattering Microscopy (iSCAT) enables label free, prolonged and high frame rate image acquisition. Typically, iScat setups employ scanning illumination schemes to achieve uniform sample illumination. However, this implementation limits the field of view (FoV) and maximum sampling rate, while increasing hardware requirements and setup size. We demonstrate that it is possible to achieve a large (80µm x 80µm) uniformly illuminated FoV through a passive refractive optical element in the iSCAT illumination path. Thus, we developed a scanning-free Interferometric Scattering Microscopy setup in combination with an objective based Total Internal Reflection Fluorescence Microscopy (TIRF) channel. This setup has the additional advantage of a compact footprint and a freely available software implementation. We show that we can acquire images with a uniform contrast and a ≤10 nm localisation precision throughout the FoV. The instrument is capable of performing quantitative single molecule measurements as Single Particle Tracking and imaging Fluorescence Correlation Spectroscopy. We demonstrate these measurements on model membrane systems. This illumination-detection scheme for iSCAT has the potential to drive further innovation and applications of iSCAT microscopy in the life sciences.

P-638
Controlling protein orientation using strong electric fields: perspectives for single particle imaging

Emiliano De Santis[1,2], Erik Marklund[1], Carl Caleman[2,3], Maxim Brodmerkel[1]
[1] Department of Chemistry – BMC, Uppsala University, Box 576, 75123 Uppsala, Sweden, [2] Department of Physics and Astronomy, Uppsala University, 75120 Uppsala, Sweden, [3] Center for Free-Electron Laser Science, DESY, Hamburg - Germany

Single particle imaging is a set of emerging techniques that utilize ultrashort and ultraintense X-ray pulses to generate diffraction from single isolated particles in the gas phase to determine their structures. One of the largest difficulties in realizing this goal is the unknown orientation of the individual sample molecules at the time of exposure. The orientation recovery process requires computationally demanding algorithms that in some cases are unable to find the correct solution, making structure determination impossible in those cases. Preorientation of the molecules using external electric fields has been identified as a possible solution to this problem. Using classical and ab initio molecular dynamics simulations, we identify a range of electric field strengths that allow for successful orientation of proteins without compromising their structure. Additionally, we demonstrate that orientation information is crucial for successful structure determination in various experimentally relevant cases. Our findings suggest that non-destructive field orientation of intact proteins is a viable method that opens up new avenues for structural investigations using single particle imaging.

P-640
Understanding the signal transduction mediated by Mincle receptor

PASCAL DEMANGE[1], Maxime NORIEGA[1], Adrien SCHAHL[1], Guillaume FERRE[1], Olivier SAUREL[1], Matthieu CHAVENT[1], Andrew ATKINSON[1], Georges CZAPLICKI[1]
[1] Institute of Pharmacology and Structural Biology (IPBS-CNRS), France

Mincle is a C-type lectin receptor that plays key roles in the innate immune system by acting as a sensor of pathogen-associated molecular patterns. Mincle is a transmembrane protein that consists of a short cytosolic N-terminal sequence and an extracellular domain including a C-terminal carbohydrate recognition domain (CRD). The extracellular and cytosolic domains are connected by a single-pass transmembrane domain. The extracellular binding of trehalose dimycolate (TDM), a glycolipid from mycobacteria, to human Mincle leads to intracellular activation of NF-κB via the Syk-Card9-Bcl10-Malt1 pathway. Signal transduction requires FcRγ, a single-pass transmembrane protein bearing cytosolic motifs that are phosphorylated by Src family kinases in the first step of this signalling pathway. We aim to express the isotopically labelled protein using constructions of various sizes, including the whole protein and its partner in a membrane context using lipid nanodiscs. We are currently carrying out ligand interaction studies and characterisation of the dynamic properties of Mincle CRD through 15N relaxation measurements. In parallel, a theoretical approach employing multi-scale molecular dynamics is used. To capture various conformations of the protein, an adaptive sampling protocol has been employed in combination with the Amoeba polarizable force field. The philosophy behind this project is to implement an integrative approach involving these multiscale simulations and a range of biophysical techniques in order to understand the molecular mechanisms underlying this signaling event and, potentially, to contribute to the development of new therapies against tuberculosis.

Poster Presentations
– Session 3 –

P-641
The ILL Deuteration Laboratory (ILL D-Lab), a platform for isotope labelling of biological molecules.
Juliette Devos[1], Valérie Laux[1], Martine Moulin[1], Michael Haertlein[1], Trevor Forsyth[1,2]
[1] Life Sciences Group, Institut Laue-Langevin, Grenoble, France, [2] Faculty of Medicine and LINXS, Lund, Sweden

The ILL Deuteration Laboratory is dedicated to the deuteration of biomolecules for neutron applications, such as neutron scattering, protein crystallography, dynamics and reflectometry. The D-Lab is a user platform available to all neutron users. Access to the platform is by a rapid electronic peer-review system, available at any time. In neutron experiments in biology, the replacement of the common hydrogen isotope protium (1H) by its stable isotope deuterium (2H) is of crucial importance for biomolecules. Depending on the neutron experiment, various levels of deuteration of these molecules are necessary. Microorganisms such as bacteria and yeasts have been successfully adapted to growth in deuterated minimal media. Large-scale protein deuteration by recombinant expression in high-cell density cultures was initially developed in the ILL D-Lab. The production of various labelled biomolecules required for the study of proteins, protein-nucleic acid complexes, protein-lipid complexes, glycoproteins, membrane proteins and stealth lipid nanodiscs will be presented. The in vivo deuteration of small biomolecules of major functional importance will also be highlighted, as well as recent advances and method developments for the deuteration of biomolecules in vivo and in vitro. For further information, you can consult the webpage on the ILL website dedicated to the platform (https://www.ill.eu/users/support-labs-infrastructure/deuteration-laboratory).

P-642
Memristive Hydrophobic Nanopores for Neuromorphic Computing
Goncalo Paulo[1], Giovanni Di Muccio[1], Ke Sun[2], Alberto Gubbiotti[1], Blasco Morozzo della Rocca[3], Giovanni Maglia[4], Mauro Chinappi[5], Alberto Giacomello[1]
[1] Sapienza Università di Roma, Italy [2] Sichuan University, [3] Università di Roma Tor Vergata, [4] University of Groningen, [5] Unviersità di Roma Tor Vergata

Under certain conditions of geometry and surface chemistry, a vapor bubble may spontaneously nucleate in hydrophobic nanopores at pressures and temperatures which would not allow the same process in bulk water. Hydrophobic gating is the mechanism by which the ionic current lowing through a nanopore can be hindered or allowed by the drying or wetting of the pore, and it is relevant in both biological and synthetic pores. The change of ionic conductance due to drying and wetting is stochastic and reversible for sufficiently small pores, and its dynamics may span a broad range of timescales depending on the pore features and, importantly, on the applied external voltage. This can lead to the emergence of long-term memory behaviors. Using analytical models and atomistic simulations, we build a coarse grained model of the pore wetting and drying to explore the time scales at which the memory effects arise. This analysis clearly show that biological nanopores perfectly match the geometrical features needed to exploit hydrophobic gating in technologically achievable devices. As a proof of principle, we show that an engineered FraC nanopore, with a hydrophobically mutated constriction, acts as a“memristor”, in accordance with the theoretical predictions. These results pave the way for a brand-new class of nanofluidic devices, based on engineered hydrophobic nanopores, which can be used, e.g., in neuromorphic computing applications.

P-643
Artificial Intelligence-Driven Prediction of Drug Metabolism and Drug Transport Inhibition Integrating Protein Dynamics- and Ligand-based Information
Balint Dudas[1,2,3], Youcef Bagdad[1], Daniel Toth[1,4], Erika Balog[4], Edina Rosta[3], David Perahia[2], Maria A. Miteva[1]
[1] Inserm U1268 MCTR, CiTCoM UMR 8038 CNRS - University of Paris, Paris, France, [2] Laboratoire de biologie et pharmacologie appliquée, Ecole Normale Supérieure Paris-Saclay, Gif-sur-Yvette, France, [3] Department of Physics and Astronomy, University College London, London, U.K., [4] Department of Biophysics and Radiation Biology, Semmelweis University, Budapest, Hungary

The complex process of drug elimination is governed by drug metabolizing enzymes (DMEs) and transporters. The inhibition of these crucial actors can directly lead to increased cytosolic toxicity levels and undesirable drug-drug interactions. At the same time, drug candidates that are high- affinity DME or exporter substrates often turn out to be of unsatisfactory efficiency, while hazardous metabolites have also been reported. The in- silico prediction of interactions with DMEs and drug transporters can help reduce the rate of drug candidate failure at an early stage of drug development, thereby increasing the safety of health treatments and reducing associated costs. Conformational changes are driving forces for the accommodation of the diverse ligands of DMEs and for the substrate translocation of transporters due to their large promiscuity. However, current machine learning (ML) models predicting ligands of DMEs and transporters mostly neglect protein structure and dynamics. We developed and employed different enhanced molecular dynamics simulation tools, such as our kinetically excited targeted molecular dynamics approach, and revealed the transport mechanism of the ABC transporter ABCG2, and the ligand binding mechanisms of the phase II DMEs, SULT and UGT. We collected experimentally validated ligands, substrates and inhibitors of the target proteins, and we integrated protein structure- and dynamics- based information together with ligand-based information and information on protein-ligand interactions in supervised ML algorithms. We created high-throughput, efficient, and high-accuracy in-silico inhibition prediction models, which are now ready for use to prefilter drug candidate molecules.

P-644
The MOlecular-Scale Biophysics Research Infrastructure (MOSBRI): a transnational initiative to structure and consolidate the European molecular biophysics community
Patrick ENGLAND[1]
[1] Molecular Biophysics platform, Institut Pasteur, Paris, France

The MOSBRI project (www.mosbri.eu) aims at structuring and consolidating the European molecular biophysics community and is funded until June 2025 by the European Commission through its Horizon 2020 INFRAIA scheme. Its integrated consortium of 13 academic centres of excellence and two industrial partners from 11 European countries carries out three types of activities: 1) Joint Research Activities enabling the emergence of novel methodologies and technologies (in partnership with instrument and software developers), the design, production and dissemination of standard reference proteins, the coining of proficiency accreditation schemes for biophysical techniques, and the establishment of data and metadata archival standards and a universal database for molecular-scale biophysics data; 2) Networking Activities such as the organization of international conferences and courses, and the creation with synergies with like-minded networks and associations, and 3) free of charge Trans-national access (TNA) provision to cutting-edge instrumentation and expertise. By summer 2023, MOSBRI has notably already held two conferences (Paris and Zaragoza), organized 8 training schools, accepted more than 100 TNA projects and prototyped the first database for molecular biophysics data.

Poster Presentations
– Session 3 –

P-645

Transient state characterization of cyanine fluorophores to take next generation super-resolution imaging into the near-IR. Hanie Esmaeeli[1], Chinmaya Venugopal Srambickal[1], Joachim Piguet[1], Mark Bates[2], René Siegmund[2], Lenny Reinkensmeier[2], Alexander Egner[2], Jerker Widengren[1]
[1] Experimental Biomolecular Physics, Department of Applied Physics, Royal Institute of Technology (KTH), Stockholm, Sweden, [2] Institut für Nanophotonik Göttingen, Germany

Fluorescence imaging in the near-infrared (NIR, 700-1700nm) is receiving increasing interest in life science, offering major merits, including lower phototoxicity, reduced light scattering and autofluorescence, deeper imaging depths and an extended wavelength range for multiplexing. Essentially all fluorescence-based single molecule detection (SMD) and super-resolution microscopy (SRM) methods rely on photo-physical properties of the fluorophores. To successfully extend SRM techniques such as STED, PALM, STORM and MINFLUX into the NIR, a systematic characterization of suitable fluorophores and imaging conditions is needed. In this study, we applied Transient State (TRAST) spectroscopy, a method originating from our lab, to perform this characterization. In TRAST, reversible transitions of long-lived dark states of fluorescent molecules can be characterized, from how the time-averaged fluorescence intensity from the fluorophores varies with the modulation of the laser excitation intensity. In the set of NIR cyanine fluorophores studied, we found prominent blinking in the µs range, attributed to trans-isomerization, and in the ms timescale due to photo-reduction. Additionally, we identified how these kinetics depend on different sample and excitation conditions. By this systematic characterization we could identify suitable NIR fluorophores, as well as sample and excitation conditions which will facilitate the extension of SRM/MINFLUX imaging into this spectral region.

P-646

Physicochemical optimization of lipid-based nanocarriers for topical ophthalmic therapeutic delivery towards the posterior segment of the eye

Maria João Faria[1], M. Elisabete C. D. Real Oliveira[1], Gonzalo Carracedo[2], Marlene Lúcio[1,3]
[1] Centre of Physics of Minho and Porto Universities (CF-UM-UP), University of Minho, Portugal [2] Department of Optometry and Vision, Faculty of Optics and Optometry, University Complutense of Madrid, [3] CBMA - Centre of Molecular and Environmental Biology, Department of Biology, University of Minho

The impact of age-related retinal diseases (ARRD) is increasing worldwide, along with the aging of the population. Besides the adverse consequences on human quality of life, ARRD mostly affects the posterior segment of the eye, and their treatment often relies on invasive procedures with associated risks and economic burdens. Thus, the development of novel topical strategies to increase therapeutic biodistribution in intraocular tissues is urgent, and nanotechnology-based approaches are reliable options. In this study, cationic lipid-based nanocarriers (LN) enriched with monoolein (LN@MO) or phytantriol (LN@PHY) were developed and optimized to increase their interactions with the ocular surface while enhancing ocular penetration. To attain the ideal physicochemical properties, LN were evaluated regarding their size, polydispersity index, zeta potential, and thermodynamic behavior by dynamic and electrophoretic light scattering. Furthermore, the deformability index of LN@MO and LN@PHY was determined, and the diffusion coefficients were tested in aqueous, mimetic tear film, and mucin-enriched media, respectively. Transmission electron microscopy was used to confirm LN shape and mean size. Overall, both LN@MO and LN@PHY showed adequate sizes and surface charges for increased interaction with the corneal surface. Additionally, LN@PHY showed better diffusion properties in all media and both LN@PHY and LN@MO displayed good deformability indexes, indicating potential penetrating properties towards posterior ocular tissues.

P-647

Fucose binding induces strain-dependent changes in the flexible norovirus P domain

Yuzhen Feng[1], Ronja Pogan[2], Lars Thiede[2], Jürgen Müller-Guhl[2,3], Charlotte Uetrecht[2], Wouter Roos[1]
[1] Molecular Biophysics, Zernike Institute for Advanced Materials, University of Groningen, Netherlands [2] CSSB Centre for Structural Systems Biology, Deutsches Elektronen-Synchrotron (DESY) & Leibniz Institute of Virology (LIV), [3] Partner Site Hamburg-Lübeck-Borstel-Riems, Bernhard Nocht Institute for Tropical Medicine and German Center for Infection Research (DZIF)

The majority of nonbacterial gastroenteritis in humans and livestock is caused by norovirus. Norovirus variants have a strain-dependent binding profile to fucose, which is supposed to facilitate norovirus infection. It remains unclear, however, what the molecular mechanism behind the strain- dependent functioning is. In this study, we studied norovirus-like particles (noroVLPs) of three norovirus variants. The P domain of these variants exists in either the rising or the resting conformation. By applying atomic force microscopy (AFM) nanoindentation technology, we found the two different P domain conformations have opposite effects on the mechanical properties of the noroVLPs. When compared to noroVLPs with the resting P domain, the noroVLPs with the rising P domain showed differences in mechanical properties. The noroVLPs were then subjected to fucose treatment. The results of noroVLPs after fucose treatment imply that the noroVLPs of these variants adopt a strain-dependent conformational switch following fucose binding, which reduces the previously found differences in their mechanical properties. These findings suggest that fucose binding promotes norovirus infection via the strain-dependent conformational changes of the flexible P domain to boost immune evasion or exposing additional binding sites.

P-648

Broadband Cavity Enhanced UV-VIS Absorption Spectroscopy for Picolitre Liquid Samples

Imogen Fermor-Worth[1], Catalin Chimerel[1,2]
[1] University of Exeter, United Kingdom [2] Transilvania University of Brasov

Absorption spectroscopy is a widely used analytical technique due to its label-free nature. However, its application to small liquid samples is hampered by the associated short absorption pathlengths, limiting sensitivity. A concept for the development of an ultrasensitive broadband absorption spectrometer optimised for thin liquid films is presented. We implemented an optical cavity within a fibre-based absorption spectrometer, to enhance sensitivity of the absorbance measurements. In the setup, light propagates multiple times through the sample of interest resulting in greatly increased sensitivity. The bandwidth of the instrument is determined by the choice of two dielectric mirrors forming the optical cavity and, in this implementation, has been set to be optimised for UV detection (250-450 nm). The sensing volume of the spectroscope is prescribed by the choice of optical fibres employed to deliver light to the sample, here we employed 400 µm diameter fibres, giving a sensing volume of 630 picolitres for a thin film of 5 µm in thickness. As a proof-of-concept, we have used our platform for the ultrasensitive detection of the antifungal drug Amphotericin B. Cavity enhancement factors, the equivalent pathlength increase over classical absorption spectroscopy, in the range of 200X have been achieved across a broad wavelength range. Taking advantage of the extended path length the limit of detection for Amphotericin B in a 5 µm thick aqueous film has been dropped from ~125 µg/ml to ~20 µg/ml. We envision multiple applications of our technology ranging from low concentration nucleic acid quantification to label-free cellular drug uptake.

Poster Presentations
– Session 3 –

P-649
Biophysical insights of an innovative lipid-engineered Stratum Corneum model for compound permeation studies

Eduarda Fernandes[1], Irene López-Sicilia[2], Vanessa Cardoso[1,3], Senentxu Lancerós-Mendez[1,4], Juan Giner-Casares[2], Maria Teresa Martin-Romero[2], Marlene Lúcio[1]
[1]Universities of Minho and Porto, Portugal, [2] Universidad de Córdoba, Spain, [3] University of Minho, Portugal, [4] BCMaterials, Spain

The main penetration barrier for topically administered compounds is provided by the Stratum Corneum (SC), the outermost layer of skin composed by corneocytes embedded in an intercellular lipid matrix (ILM). Actually, the permeation barrier function of SC is highly associated with the complex organization of ILM. ILM is mainly composed by ceramides (CER), cholesterol (CHOL) and free fatty acids (FFAs) with different chain lengths organized in two crystalline coexisting lamellar phases with repeated distances of 13 and 6 nm – long periodicity phase and short periodicity phase, respectively. Recently, ethical, technical and reliability concerns with human and animal experiments for testing cosmetic/pharmaceutical products have led to a huge demand for alternative skin surrogates for in vitro permeation studies. Additionally, lipid biomimetic models have gained increased attention due to their simplicity yet ability to mimic the biophysics of physiological barriers. Herein, a newly developed lipid SC biomimetic model CER[I]:CER[IV]:CHOL:FFA was biophysically characterized before and after its inclusion in two types of filter scaffolds used as a skin surrogates for permeation studies. The complex biophysics of SC model as multi-stacked bilayers, monolayers and liposomes were characterized through small and wide-angle X-ray scattering, surface-pressure isotherms coupled with Brewster Angle Microscopy and UV-VIS reflection spectroscopy, as well as Fourier-transform infrared spectroscopy (FTIR), dynamic light scattering and differential scattering calorimetry. The lipid-engineered SCs were characterized by scanning electron microscopy, FTIR and contact angle, and were further used to evaluate the permeation of three model compounds as proof-of-concept of its ability as skin surrogate.

P-650
AFM-IR characterization of tau fibrils and aggregates obtained with different cofactors

Cecile Feuillie[1], Vicky Ury-Thiery[1], Téo Leboucq[1], Clara Piersson[1], Yann Fichou[1], Sophie Lecomte[1], Michael Molinari[1]
[1] Univ. Bordeaux, CNRS, Bordeaux INP, CBMN, UMR, France

Aggregates of the tau protein are involved in several neurodegenerative diseases, called tauopathies, including Alzheimer's disease. Cryo-EM structures of brain-extracted tau filaments have revealed distinct conformations depending on the tauopathy but homogeneous ones within each disease (Scheres et al., 2020, Current Opinion in Structural Biology). Recent findings suggest aggregation cofactors, and notably membrane lipids, might dictate tau aggregates properties and pathological activity. Central questions about tau aggregation remain unanswered. In particular, are the cofactors incorporated in the fibrils? Does the secondary structure of an aggregate depend on the cofactor used for aggregation? A previous study used TERS (tip enhanced Raman spectroscopy) to show that tau construct K18 aggregated in the presence of PIP2 lipids suggested lipid recruitment inside the fibers (Talaga et al., 2018, Angew. Chem. Int. Ed). In this study, we use AFM-IR (atomic force microscopy coupled to infrared spectroscopy) to obtain local IR spectra and chemical mapping of tau aggregation products with nanoscale resolution (~20 nm). In addition to morphological characterization, this method therefore allows for chemical characterization of single amyloid species, allowing us to assess structural and chemical heterogeneity of a sample at the nanoscale (Feuillie et al., 2020, Front. Mol. Biosci.). After a thorough optimization of the AFM-IR signal on heparin-induced tau fibers and aggregates, we notably focused on POPS- and arachidonic acid-driven aggregation of tau. We compare obtained morphologies, chemical signatures and structural heterogeneities.

P-651
The development of rulers to characterize fluorescent DNA topology sensors

Filip Filipović[1,2], Karl Duderstadt[1,2]
[1] Max Planck Institute of Biochemistry, [2] Technische Universität München, Germany

All cellular organisms use DNA for storage and transmission of genetic information. This is advantageous as it provides a universal code for information retrieval, however accessing the information-rich bases during essential cellular processes leads to topological changes, such as overwinding and unwinding. Many cellular machineries and nucleoid-associated proteins have specifically evolved to recognize, transform, and harness DNA topology. Additionally, the changes in the pitch of the DNA helix have been found to provide more favorable binding sites and increased fluorescence for many DNA-specific fluorophores. To characterize and quantify DNA topology dynamics, a toolbox of fluorescent DNA topology sensors will be developed. First, a library of positively and negatively supercoiled plasmids with precise topoisomer distributions will be created to serve as standard rulers to determine the properties of candidate sensors. Next, promising sensors will be used in quantitative measurements of DNA topology changes during replication, transcription, and recombination. These sensors will ultimately elucidate a basic understanding of topology-dependent processes, as well as be used for biotechnological applications.

P-652
Evaluation of the biophysical properties and drug delivery capacity of serum albumin nanoparticles

Monica Florescu, Melinda David , Claudia G. Chilom , Sorina Iftimie , Adriana E. Balan, Adrian Enache, Daniela Oprea , Monica Enculescu
[1] Transilvania University of Brasov, Romania

Serum protein-based systems are studied as drug-delivery vehicles due to their advantageous properties. Two nanoparticles (NPs), with bovine serum albumin (BSA) and human serum albumin (HSA) as precursors, were synthesized by nanoprecipitation protocol using ethanol as desolvat and glucose as reticulation agent. BSA-NPs and HSA-NPs were characterized by Scanning Electron Microscopy, Atomic Force Microscopy, and UV- Vis absorption spectroscopy. Artificial models based on DPPC liposomes reconstruct the natural functions of biological membranes, thus Surface Plasmon Resonance and Electrochemical Impedance Spectroscopy were used to evaluate the interaction of albumin NPs with the DPPC membranes' function of temperature. In vitro evaluation of NPs' effects on fibroblast L929 and colon HT-29 cell lines was assessed by MTS assay and fluorescence microscopy. Results showed that both NPs are uniform, have sizes below 100 nm, and have similar stability over time. Due to the fluidization of the lipid-NPs membranes with increasing temperature, the dielectric properties, along with the film thickness, changed. Cell toxicity studies showed that all NPs preserved cell viability and tended to concentrate on the cell surface. The results will be a starting point for the potential use of these NPs as drug carriers in biological systems that require rapid drug release.

Poster Presentations
– Session 3 –

P-653
The k-th nearest neighbour for biomolecular conformational, binding and solvation entropy estimation
Federico Fogolari[1], Roberto Borelli[1], Agostino Dovier[1], Gennaro Esposito[2]
[1] University of Udine, Italy [2] New York University at Abu Dhabi

Entropy calculation is an important step in the postprocessing of molecular dynamics trajectories or predictive models. In recent years the nearest neighbor method proposed by Demchuk and coworkers has emerged as a powerful method to deal in a flexible way with the high dimensionality of the problem. The method estimates the entropy from the density of other samples in a hyperball centered on each sample. In order to apply the method to the space of rotation-translations we defined a metric in this six-dimensional space. The method has been extended to the space of two rotation-translations. Applications to most important biomolecular processes, including folding, binding and solvation, have been presented demonstrating the usefulness of the approach even in large dimensional spaces. Two programs have been developed to compute conformational and rotational-translational entropies from biomolecular ensembles of conformations and they are available on a github repository. Data structures suited for the k-th nearest neighbour method have also been reviewed. A sample of useful applications of the method will be presented.

P-654
Using super resolution imaging to optimize DNA origami nanostructure functionalization
Iris Rocamonde Lago[1], Ferenc Fördös[1], Björn Högberg[1]
[1] Karolinska Institutet, Sweden

DNA origami has become a widely used tool in the field of molecular biology due to the relative ease and high accuracy with which one can use it to create nanometer-scale patterns of bio molecules such as proteins. There has been a lot of progress in making DNA nanostructures more stable in biological conditions, making their components more easier to produce in large scales and higher precision. One of the remaining obstacles for the technology to reach its full potential is the relatively low yield of successful placement of molecules in the designed positions on individual nanostructures leading to a generally low overall yield of complete structures. This was partly due to the lack of single molecule techniques sensitive enough for characterizing functionalized DNA origami structures in a high throughput manner. In this work we are presenting a characterization technique using DNA PAINT super resolution imaging that allows the detection of individual biomolecules on single DNA origami structures and enables the optimization of origami design approaches to improve functionalization yields.

P-655
Conformational dynamics of Elongation Factor G: an in silico study
Sara Gabrielli[1], Helmut Grubmüller[1], Lars V. Bock[1]
[1] Department of Theoretical and Computational Biophysics, Max Planck Institute for Multidisciplinary Sciences, Germany

During protein synthesis, when the ribosome moves along the mRNA, the tRNAs bound to the ribosome translocate between different binding sites. Elongation Factor G (EF-G) is a GTPase that interacts with bacterial ribosomes and uses the energy from GTP hydrolysis to accelerate tRNA translocation. The final products of GTP hydrolysis are GDP and inorganic phosphate. Cryo-EM studies have shown that, after the release of the inorganic phosphate, EF-G undergoes a large overall reorientation in the ribosome. However, little is known about the intermediate conformations sampled during this rearrangement. The high flexibility of domain IV observed in Cryo-EM studies and its proximity to the A-site tRNA suggest that the dynamics of domain IV might play a fundamental role during translocation. To gain insight on the conformations that are favoured in the protein independently from the interactions with the ribosomal environment, we performed extensive all-atom Molecular Dynamics (MD) simulations of E. Coli EF-G in solution. The most pronounced inter-domain motion taking place in the simulations is the rotation of domains IV-V relative to domains I-III and, along this motion, two energetically favourable states of EF-G can be identified. Hence, we hypothesise that a movement of domain IV precedes the movement of the rest of the protein during the reorientation of EF-G in the ribosome. Here we integrate the results obtained in solution using atomistic correlation-driven MD (CDMD) simulations of the E. Coli ribosome and sampling early translocation intermediates that are not yet structurally resolved.

P-656
Neutron diffraction for deciphering lectin-glycan interactions involved in bacterial infection
Lukas Gajdos[1], Matthew Blakeley[1], Michael Haertlein[1], Trevor Forsyth[2], Juliette Devos[1], Anne Imberty[3]
[1] Institut Laue-Langevin, France, [2] LINXS, Sweden, [3] CNRS-CERMAV, France

Lectins are carbohydrate-binding proteins that play important roles in cell recognition and host-pathogen interactions. Bacteria produce lectins that are specific for glycans present on the host surface and that participate in adhesion and colonization. Lectin-carbohydrate interactions are mostly formed by hydrogen bonds. Other types of interactions include CH-π stacking, hydrophobic interactions, water-bridging, or metal coordination. Using neutron protein crystallography, we have unraveled details of protein-carbohydrate interactions in two fucose-specific lectins, with the unique feature of producing perdeuterated monosaccharide fucose using a glyco-engineered strain of E. coli bacteria. PLL lectin from Photorhabdus luminescens bacteria was chosen as a model system for a detailed description of the H-bonding network involved in sugar recognition, including direct and water-bridged hydrogen bonds and stacking interactions between fucose and aromatic amino acids. LecB lectin from Pseudomonas aeruginosa, a human opportunistic pathogen that causes lethal infections in cystic fibrosis patients, is viewed as a drug target for glycomimetic compounds. The neutron study enabled a complete description of the hydrogen-bonding network and the protonation state of charged amino acids involved in the sugar-binding including the observation of a low-barrier hydrogen bond between fucose and the protein. The structural data may help in the design of new potent glycomimetics.

Poster Presentations
– Session 3 –

P-657
Hierarchical domain motions control the catalytic properties of adenylate kinase
Nicole Galenkamp[1], Sarah Zernia[1], Giovanni Maglia[1]
[1] University of Groningen, Netherlands

The energy balance of the cell is mainly reflected by levels of adenosine nucleotides which are controlled by multiple enzymes mediating nucleotide synthesis and hydrolysis. In this concert, Mg-dependent adenylate kinase (AdK) takes a special role as it is reversibly converting ADP into AMP and ATP and therefore fine-tuning the nucleotide concentrations. The enzyme consists of a catalytic core domain and two flexible lids that close upon ligand binding to enable phosphotransfer. However, kinetics of the domain movements could not be elucidated so far. Here we use nanopore recording to monitor the lid movements, the binding of ligands and decouple the influence of Mg2+ on conformational transition and catalytic activity at the single-molecule level. We found that AdK is sculpted with multiple soft conformations connected through allosteric interactions that have different affinity for its ligands. The binding of the ligand to the ground-state conformation induces a conformational switch that initiates a precise hierarchy in the binding of ligands and domain motions to control the catalytic step. Our observations suggest that allosteric interactions evolved within AdK structure to use the free energy of cofactor- and ligand-binding to control the affinity of the enzyme for ATP, ADP and AMP; and, in turn, to precisely modulate the energy balance in the cell. This model proposes a straightforward connection between the role of allostery, enzyme dynamics and conformational changes, which can accommodate the observations and physical models proposed in the past.

P-658
Herbal extracts' influence on the amyloid aggregation of human insulin
Miroslav Gancar[1], Elena Kurin[2], Zuzana Bednarikova[1], Jozef Marek[1], Silvia Bittner Fialova[2], Pavel Mucaji[2], Milan Nagy[2], Zuzana Gazova[1]
[1] Department of Biophysics, Institute of Experimental Physics Slovak Academy of Sciences, Watsonova 47, 040 01, Kosice, Slovakia, [2] Department of Pharmacognosy and Botany, Faculty of Pharmacy, Comenius University Bratislava, Odbojarov 10, 832 32, Bratislava, Slovakia

The phenomenon of protein amyloid aggregation poses a major health risk. A therapeutic agent for diabetes, insulin, forms undesirable insoluble deposits containing fibrillar structures at its administration site leading to iatrogenic injection amyloidosis. Herbal extracts may serve as a foundation in searching for effective drugs to relieve patients' symptoms and ultimately treat amyloidoses. The in vitro effect of seven compounds from Camellia sinensis and Origanum vulgare, including their equimolar mixtures, against the insulin amyloid aggregation, has been evaluated and compared. The anti-amyloid activity was analyzed by the ThT fluorescence assay, ATR-FTIR, AFM, and the collected results were supported by in silico calculations. Extracts from Origanum vulgare proved superior in terms of effective anti-amyloid concentration; however, we observed minimal synergy between the studied compounds. On the other hand, Camellia sinensis constituents postponed the onset of insulin amyloid aggregation much more significantly at given conditions, and multiple potentiating synergies were recognized. We have subsequently explained in vitro examinations using molecular docking. To summarize, we have identified several individually effective natural compounds, defined how they affect insulin amyloid aggregation, and interpreted synergistic or antagonistic behavior induced by their equimolar mixtures.

P-659
Probing Post-translational Modifications of Amyloidogenic Proteins
YING GE[1], Jingzhou Yang[1], Francesco Aprile[1]
[1] Imperial College London, United Kingdom

Protein aggregates called amyloids underly many neurodegenerative diseases (NDs). Proteomics revealed that post-translational modifications (PTMs), such as acetylation, are enriched in the brains of ND patients. Accordingly, certain PTMs alter aggregation kinetics and increase cytotoxicity. Probing PTMs will facilitate the development of diagnostics and therapeutics against NDs. Our work presents methods to generate and probe PTMs in vitro. Amyloid-β (Aβ), associated with Alzheimer's diseases, is particularly hard to modify, due to its high hydrophobicity and tendency to precipitate. Using a solubility-enhancing fusion construct, we successfully applied chemical mutagenesis to Aβ and introduced diverse mimetics of PTMs. Kinetic and structural analyses demonstrated the effects of these PTM mimetics to be comparable to authentic PTMs. Our method uses commercially available chemicals and can be easily performed on other proteins. To target PTM-containing Aβ, we used display technologies of synthetic camelid-derived antibodies and identified hits that show strong specificity for PTM-Aβ. We also test the effect of antibodies on aggregation and select those targeting specific conformations of Aβ.

P-660
Study of the quality of the humoral immune response following COVID-19 vaccination in immunocompromised people
Daphnée Georges[1], Romain Malempré[1], André Matagne[1], Arnaud Marchant[2]
[1] University of Liège - Centre for protein Engineering, Belgium [2] University of Brussels - Institute for Medical Immunology

Following the COVID-19 pandemic, various vaccines have been developed aimed at combating the SARS-CoV-2. To induce protection, vaccines must stimulate production of neutralizing antibodies directed against the Spike protein, responsible of the infection. Vaccine responses are frequently determined by a quantitative assessment, the neutralizing antibody titers. Here, we propose to assess new parameters to measure the quality of the immune response: the antibody avidity and the epitope repertoire diversity. Avidity is determined using biolayer interferometry (BLI) allowing to obtain real-time kinetics measurements of the polyclonal antibody-antigen interaction. Our results show differences in maturation and avidity level in immunocompromised versus immunocompetent individuals following mRNA vaccination. Moreover, difference in naive individuals compared to those who contracted the disease before vaccination were observed independently of their immunocompetency, emphasizing the importance of including quality-oriented parameters to evaluate the immune response. Epitope repertoire diversity is determined by hydrogen-deuterium exchange monitored by mass spectrometry (HDX-MS). This approach is widely used with monoclonal antibody, and we adapted it with polyclonal antibodies to get the global qualitative view of the immune response. Preliminary results show an alternative conformation of the Spike and potential epitopes.

Poster Presentations
– Session 3 –

P-661
A Novel Mechanism of Action of Antidepressants Based on Allosteric Modulation of TRKB Neurotrophin Receptors

Mykhailo Girych[1], Giray Enkavi[1], Rafael Moliner[2], Eero Castren[2], Ilpo Vattulainen[1]
[1] Department of Physics, Faculty of Science, University of Helsinki, Helsinki, Finland, [2] Neuroscience Center, HiLIFE, University of Helsinki, Helsinki, Finland

Amazingly, the exact mechanism by which antidepressants exert their therapeutic effects has remained enigmatic. What is known for certain is that responses to antidepressants are related to neuronal plasticity and its principal gateway, tropomyosin receptor kinase B (TRKB), the receptor for brain-derived neurotrophic factor (BDNF). Here, we reveal how the transmembrane domains of TRKB play the most central role in the action of antidepressants. By studying several antidepressants (e.g., Prozac), we show that both conventional and fast-acting antidepressants directly interact with the transmembrane domains of TRKB, which increases its synaptic localization and BDNF activation. Using several computational and experimental methods, including atomistic molecular dynamics simulations combined with machine learning analysis, we identify precise drug- binding sites in the transmembrane region of TRKB dimers. We show that the action of the explored antidepressants is based on the allosteric modulation of TRKB in a way that simultaneously senses the cholesterol level of the cell membrane. We show that mutagenesis of TRKB's antidepressant-binding motif results in the attenuation of cellular, behavioral, and plasticity-enhancing responses to antidepressants in vitro and in vivo. Our results suggest that the binding of antidepressants to TRKB and the subsequent allosteric facilitation of BDNF signaling represent a conserved mechanism of antidepressant action. The findings provide a possible reason to understand why the effect of conventional antidepressants is typically slow. The results create opportunities for further research and development of therapeutic substances intended for serious neurological diseases.

P-662
Interactions that differentiate the flavins in bifurcating electron transferring flavoprotein

María González-Viegas[1,2], Rajiv K. Kar[2], Anne-Frances Miller[2,3], Maria-Andrea Mroginski[2]
[1] Department of Physics, Freie Universität Berlin, Berlin, Germany, [2] Department of Chemistry, Technische Universität Berlin, Berlin, Germany, [3]Department of Chemistry, University of Kentucky, Lexington KY, U.S.A.

The two flavin cofactors present in bifurcating electron transferring flavoproteins (Bf-ETFs) display contrasting roles. We used hybrid quantum mechanical molecular mechanical formalisms to study the non-covalent interactions between each cofactor and the protein environment. Our computations replicated the different reactivities of the flavins. The electron transferring flavin (ET flavin) displayed a stable anionic semiquinone (ASQ), while the bifurcating flavin (Bf flavin) disfavored the ASQ state more than free flavin. The stability of ET flavin ASQ was partly attributed to H-bond donation to the flavin O2 atom from a His side chain, by comparing models with different His tautomers. Reduction of ET flavin to the anionic hydroquinone (AHQ) state resulted in side chain reorientation, backbone displacement and reorganization of its H-bond network, involving a side chain from another domain of the protein. In contrast, the binding site of the Bf flavin was less responsive overall. However, formation of the Bf flavin AHQ allowed a nearby Arg side chain to adopt an alternative rotamer, building a new H-bond to the Bf flavin O4. Therefore, our computations have provided insights on states and conformations that cannot be characterized experimentally, providing explanations for observed residue conservation and raising possibilities that can now be tested.

P-663
Protein vibrations – localized or extended ? A numerical scaling analysis.

Felix Guischard[1], Jetmir Haxhija[1], Jan Kaiser[1], Thorsten Koslowski[1]
[1] University of Freiburg, Germany

Investigating the spatial extension of protein normal modes, we address a fundamental physical problem – the nature of elementary excitations in complex systems – and its biophysical and biochemical implications. For a set of eighteen proteins that cover five classes of increasing size, we compute the participation ratio as a measure of the spatial extent of protein vibrations. In this scaling analysis, we find extended low-frequency far- infrared and Terahertz modes, in contrast to localized high-frequency near-infrared vibrations. These regimes are separated by a crossover at 260 cm-1. The vibrational localization properties are compared to those of amorphous solids.

P-664
Exploiting the path independence of free energies to study potential inhibitors for β-lactamases

J. Jasmin Güven[1], Antonia S.J.S. Mey[1]
[1] EaStCHEM School of Chemistry, The University of Edinburgh, United Kingdom

Ensuring reliability for pharmaceutically relevant protein targets and ligand series remains a challenge. An example of this is metallo-β-lactamase (MBL) enzymes, where active-site zinc ions directly interact with ligands and thus influence their binding. The production of beta-lactamases by bacteria is the most common mechanism against antibiotics, such as penicillin. Thus, building reliable and easy-to-use AFE methods is essential for using MBLs as drug targets.Here, we present a set of baseline results of existing AFE methods calculated for a series of known inhibitors across serine- (KPC-2) and metallo-β-lactamases (VIM-2) [2]. Conventional methods can be used for KPC-2 to achieve accuracies of around 0.8 – 1.2 kcal/mol, depending on the forcefield, simulation engine and perturbation network used. For KPC-2, we obtain an MUE of 0.80 kcal/mol and a Spearman's rank correlation coefficient of 0.60. Preliminary results for both KPC-2 and VIM-2 suggest that improvements to the methodology are needed in multiple areas. For AFE calculations to become computationally competitive for metalloproteins, streamlining the setup and calculations is essential. Overall, we highlight the need for new methodologies and optimised AFE methods to make them more practical for metalloproteins.

Poster Presentations
– Session 3 –

P-665
The dimensions of an ensemble of disordered protein molecules under folding conditions
Elisha Haas[1], Dan Amir[1], Gil Rahamim[1], Osman Bilsel[2]
[1] Bar Ilan university, Israel [2] university of Massachusetts

What are the dimensions of ensemble of protein molecules at the initiation of the folding transition? Does an ensemble of disordered molecules of foldable globular protein under folding conditions follow Flory's theory of collapsed polymers? Using E. coli Adenylate kinase (AK) as a model protein and microfluidic mixing device combined with time resolved FRET measurements ("the double kinetics" method) we tested the hypothesis that at the initiation of refolding, the dependence of the mean of the segmental end-to-end distance on the segment length, n, is weak (n>~30). We measured the transient distributions of segmental end to end distances of seven segments of the AK molecule, with n from 45 to 196 residues at 50 s after initiation of mixing. The means of these distributions range from 45±2 Å, for the short segment, to 70±2 Å for the long segment. The Flory exponent for the n dependence of the mean segmental end to end distance is 0.32±0.01. Thus, this transient ensemble is indeed a collection of disordered molecules. We now have a benchmark for the segmental end to end distance distribution of unfolded AK molecules under folding conditions (poor solvent). Any intramolecular mean distance that would be significantly shorter can be considered as an indication of an early formed intramolecular contact. This experiment enables systematic detection of the time sequence of formation of non local contacts in the refolding protein molecules. A step which is essential for deciphering the mechanism of folding of globular proteins.

P-666
DNA-based platforms for nanoparticle-fluorophore assembly to study plasmonic light modulations
Niklas Hansen[1,2], Jakub Čopák[3], David Roesel[1], Petr Cígler[3], Vladimíra Petráková[1]
[1] J. Heyrovsky Institute of Physical Chemistry, Prague, [2] University of Chemistry and Technology, Prague, [3] Institute of Organic Chemistry and Biochemistry, Prague, Czech Republic

Here, we present a versatile platform for the controlled assembly of plasmonic nanoparticles and fluorophores to investigate the modulations of light emitted by the plasmon-coupled fluorophore. Various photonic phenomena rely on precise positioning of photon sources in close arrangements with nanometer precision. DNA nanotechnology offers solutions for this, taking advantage of the programmable nature of DNA spatial configuration. Moreover, it offers opportunities to assemble additional light-modulating elements, like plasmonic nanoparticles, in defined distances next to photon sources. In this work, we extend the traditional use of organic fluorophores in the DNA origami assembly, to non-traditional solid states fluorophores – nitrogen vacancy centers in nanodiamonds. These fluorescent defects represent attractive features for broad photonics and biosensing applications. We prepared DNA origami nanostructures and decorated them with different configurations of nanoparticles. We demonstrate attaching nanodiamonds and gold nanoparticles and investigate their optical coupling. We confirmed the assembly of a variety of nanostructures using Atomic Force Microscopy and Transmission Electron Microscopy. We show the potential of DNA origami to position solid-state fluorophores with nanometer precision for further examinations. The next steps include investigations into the plasmonic enhancement mechanism of coupled NV centers and the shifts in their projected position based on the assembly design.

P-667
Solution structure of the SARS-CoV-2 pseudoknot from SAXS-guided molecular dynamics
Weiwei He[1,2], Josue San Emeterio[3], Michael Woodside[4], Serdal Kirmizialtin[1], Lois Pollack[3]
[1] Chemistry Program, Science Division, New York University Abu Dhabi, UAE, [2] Department of Chemistry, New York University, USA, [3] School of Applied and Engineering Physics, Cornell University, USA, [4] Department of Physics, University of Alberta, USA

SARS-CoV-2 depends on -1 programmed ribosomal frameshifting (-1 PRF) to express proteins essential for its replication. The RNA pseudoknot stimulating -1 PRF is thus an attractive drug target. However, the structural models of this pseudoknot obtained from cryo-EM and crystallography differ in some important features, leaving the pseudoknot structure unclear. We measured the solution structure of the pseudoknot using small- angle x-ray scattering (SAXS). The measured profile did not agree with profiles computed from the previously solved structures. Beginning with each of these solved structures, we used the SAXS data to direct all atom molecular dynamics (MD) simulations to improve the agreement in profiles. In all cases this refinement resulted in a bent conformation that more closely resembled the cryo-EM structures than the crystal structure. Applying the same approach to a point mutant abolishing -1 PRF revealed a notably more bent structure with reoriented helices. This work clarifies the dynamic structures of the SARS-CoV-2 pseudoknot in solution and elucidates that linear conformations of the SARS-CoV-2 pseudoknot are disfavored, with the dominant conformers featuring a notable bend at the ring through which the 5' end of the transcript is threaded.

P-668
Protein misfolding in Glutaric Aciduria-type I: from protein defects to function regulation by cofactor and substrate
Bárbara J. Henriques[1], Joana V. Ribeiro[1], Cláudio M. Gomes[1]
[1] Faculty of Sciences University of Lisbon, Lisbon, Portugal, BioISI – Biosystems and Integrative Sciences Institute, Lisbon, Portugal

Inborn Errors of Metabolism (IEM) comprise a group of genetic diseases in which the vast majority of phenotypes are associated to single gene mutations that lead to impaired cellular metabolism, often as a consequence of defective protein folding. The era of genomics permit to identify an increased number of cases, though a clear correlation between genotype and phenotype has been difficult to establish, and moreover therapeutic options are limited. Here we report our studies in Glutaric Aciduria-type I (GA-I), an IEM caused by deficiency of glutaryl-CoA dehydrogenase (GCDH). We have investigated disease-related variants combining biochemical, biophysical and structural methods to establish the effects of point mutations on protein folding, stability and function. Moreover, we have evaluated the effect of small molecules, cofactor and substrate, in GCDH structure and function. We established genotype/phenotype correlations for two missense variants by showing that although both variants retain the overall protein fold they present different conformational stabilities, and the observed compromised enzymatic activity depend on different features. Further, we were able to provide a molecular rationale for the potential benefit of riboflavin supplementation in GA-I patients. By the other hand, we showed that increasing amounts of glutaryl-CoA, GCDH substrate, lead to enzyme glutarylation diminishing is enzymatic activity. Moreover, this negative effect could be reverted by sirtuin5, a known regulator of mitochondria acylation levels. Overall, our results clarify the molecular pathogenesis of GA-I, and open new avenues to the understanding of IEM.

Poster Presentations
– Session 3 –

P-669
Structural analysis of the influenza genome by high-throughput single virion DNA-PAINT
Christof Hepp[1], Qing Zhao[1], Ervin Fodor[2], Nicole Robb[3], Achillefs N Kapanidis[1]
[1] Kavli Institute for Nanoscience Discovery, [2] Sir William Dunn School of Pathology, [3] Warwick School of Medicine, United Kingdom

Influenza A, a negative-sense RNA virus, has a genome that consists of eight single-stranded RNA segments. During influenza co-infections, re- assortant virus strains containing gene segments from either strain can occur, occasionally leading to pandemic outbreaks with severe, worldwide consequences for human health. To better understand the formation of these potentially pandemic re-assortants, we analysed the selective packaging of all eight RNA segments into virions. To this end, we designed a multiplexed DNA-PAINT approach that is capable of a) detecting the presence or absence of all eight gene segments inside of more than 10,000 individual virus particles in one experiment and b) spatially resolving the individual segments inside complete virus particles with a precision of ~ 10 nm. With its high throughput and the capability of unambiguously identifying specific gene segments, this experiment complements data from previous electron microscopy studies. Our preliminary results show that 1) there is a cooperative effect in influenza genome assembly, with a tendency towards virions with higher segment counts, 2) certain segment pairs co-appear preferentially, indicating segment-specific interactions, while generally, all of the segments interact to some extent and 3) inter- segment distances and the spatial distribution of segments inside virions suggest a flexible arrangement. Overall, our data points to a flexible network of inter-segment interactions that form a robust genome assembly for influenza A, in agreement with data from previous studies. In the long term, we will develop our experimental approach for the structural and functional study of viral nucleoprotein complexes.

P-670
Computational decomposition of the thermodynamics of Hsp90 protein-ligand binding
Simon Holtbrügge[1], Lars Schäfer[1]
[1] Ruhr-University Bochum, Germany

The free energy of protein-ligand binding is one of the key factors that determine the potency of a small molecule drug. The binding free energy can be predicted from molecular dynamics (MD) simulations, which is routinely used in drug discovery to estimate if one ligand is more potent than another. To understand why one ligand is more potent, we go a step further and investigate the full thermodynamic picture of protein-ligand binding, including free energies and meaningfully decomposed enthalpies and entropies. For this purpose, we combine the multiscale cell correlation method for entropy computations with non-equilibrium based computations of relative binding free energies. Our investigation focuses on complexes involving Hsp90, a molecular chaperone involved in protein folding and a target for anticancer drugs.

P-671
Floating Lipid Bilayers and Neutron Reflection for Studying Membrane Biophysics
Arwel Hughes[1]
[1] Rutherford Appleton Laboratory, United Kingdom

In recent years, we have developed novel supported model membrane systems for investigating membrane processes using Neutron Reflectivity (NR). The systems, whilst robustly associated with the substrates, and insulated from their constraining effects by substantial cushions of water. These systems show appropriate phase behaviour expected from their compositions, and allow specific membrane types such as raft forming cholesterol membranes or asymmetric bacterial mimics to be formed and studied. They are also fully submerged, allowing bulk water properties (such as salt composition or pH) to be controlled, and enabling studies of binding of proteins from solution, for example, which cannot be done with membrane stacks in humid air. NR allows Angstrom level information about the membrane structures to be probed in-situ in a non- destructive manner, and when coupled with atomistic models for the membranes, allows the data (and hence the membrane structure) to be examined in great detail. Here we will review the historical progress made on the development of floating bilayers, from simple DPPC to complex gram-negative membrane mimics, discuss parallel enhancements in reflectivity data analysis using MD simulations, and also provide some future perspectives regarding the continued development of this technology for studying membrane biophysics.

P-672
Investigating Viral Adhesion on Surfaces Using Combined AFM and Coarse-Grained Simulation Techniques
Pablo Ibáñez-Freire[1], Aritz B. García - Arribas[1], Rafael Delgado - Buscalioni[1], Pedro J. de Pablo[1]
[1] Universidad Autonoma de Madrid, Spain

This study presents a comprehensive approach for investigating the adhesion of lipid-enveloped viruses on various surfaces using a combination of atomic force microscopy (AFM) and coarse-grained molecular dynamics simulations. We prepared solutions with varying virus concentrations and deposited them on different substrates, including mica and MOS HOPG, to assess adhesion. By allowing sufficient time for adsorption and measuring the number of viruses on the surface, we estimated the binding constants using Langmuir isotherms. In parallel, we developed a coarse- grained model of a lipid-enveloped virus, integrating different models for lipids and proteins, to simulate the adhesion process. Umbrella sampling was employed to calculate the binding constants between the virus and surface. Our AFM results initially showed a surprising observation that taller viruses were also more strongly adsorbed on the surface. This finding was explained through our simulations, revealing a tradeoff between lipid and protein interactions with the surface. Our study utilized innovative parallel GPU simulation techniques, making these simulations more accessible and efficient. This work demonstrates the potential of combining experimental and computational methods to understand the complex mechanisms of viral adhesion on surfaces, with potential implications for the design of antiviral surfaces and materials.

Poster Presentations
– Session 3 –

P-673
Nanoscale Strategies for Single-Molecule and Single-Cell Analysis

Aleksandar Ivanov[1], Joshua Edel[1], Ren Ren[1], Shenglin Cai[1], Binoy Paulose Nadappuram[1], Xiaoyi Wang[1], Caroline Koch[1], Benedict Reilly-O'Donnell[1]

[1] Imperial College London, United Kingdom

There is a significant drive to deliver nanotechnology-based solutions that enable the analysis of the fundamental components of life at the single- molecule and single-cell scale. Our study introduces nanoscale sensors and platforms that address some of the challenges in single-molecule and single-cell analysis. Our approach includes the use of nanopore-based single-molecule techniques that offer high sensitivity and selectivity for detecting trace analytes in biological fluids through the use of molecular carrier probes that have implications for next-generation biomarker analysis (Wang et al. JACS, 2023 145, 11, 6371; Cai et al., Nat. Commun. 2021, 12, 3515; Cai et al., Nat. Commun. 2019, 10 (1), 1797; Ren et al. Adv. Mater. 2021 33 (38), 2103067, 2023; Sze et al., Nat. Commun. 2017, 8, 1552) We also report on nanotweezers capable of trapping and extracting single entities such as DNA, RNA and single mitochondrion from a living biological cell that could help better understand the fundamentals of cellular processes and how these processes occur in real-time (Nadappuram et al., Nat. Nanotechnol. 2019 14, 80) This work bridges the gap between single-molecule/organelle manipulation and cell biology and can ultimately enable a better understanding of living cells.

P-674
GlycoShape3D: A database and toolbox for structural glycomics

Callum Ives[1], Ojas Singh[1], Carl Fogarty[1], Aoife Harbison[2], Akash Satheesan[1], Silvia D'Andrea[1], Beatrice Tropea[1], Elisa Fadda[1]

[1] Maynooth University, Ireland, [2] University College Dublin

To understand the architecture of glycan-mediated interactions, a detailed atomistic understanding of glycan structure is necessary. However, determining the 3D structure of glycans is difficult due to their intrinsic flexibility and micro/macro-heterogeneity. As a result, glycans represented within the Protein Data Bank (www.rscb.org) are often incomplete or presented in questionable and/or highly distorted conformations. The release of the AlphaFold (AF) Protein Structure Database (alphafold.ebi.ac.uk) has dramatically increased the availability of protein structure data, yet this information is incomplete as it does not include co- and post-translational modifications, metals and cofactors that are often essential to protein structure and function. Some of us found that AF predicted glycoprotein regions where glycans should have been present are preserved in these models. This facilitates the direct grafting of glycans onto these models, providing that the structure of the glycan is known. Here we will present the design and principles of GlycoShape3D, a unique online open-access repository of structural data on free (unbound) glycans from equilibrium MD simulations. GlycoShape3D will deliver complete and consistent structural information on glycan structure in a format that is accessible to glycobiology experts and non-experts alike, with a demonstration of some of the key potential applications and innovations of this novel repository.

P-675
Detection of citrulline modification in histone peptides using biological nanopore

Priyanka Jain[1], Tobias Ensslen[1], Jan C. Behrends[1]

[1] University of Freiburg, Germany

Citrullination, a particularly subtle (-1 Da) post-translational modification (PTM), has recently gained significant interest in the field of biomedicine because of its suspected involvement in human diseases like rheumatoid arthritis and tumors. Despite of minimal change in residue size introduction of citrulline in histone proteins is known to drastically change the structure and function. Hence, sensitive techniques are in demand for the detection of citrullination. Here, we demonstrate the use of a mutant of the protein nanopore Aerolysin (AeL) namely R220S-AeL to not only detect but also discriminate different sites of citrulline modification on a peptide derived from human histone H3 protein(H3f), (A1-K9), containing 2 citrullination sites at position 2(H3f.R2-cit) and 8(H3f.R8-cit). Peptides were characterized using the relative residual current (I/Io) of pore blocks resulting from analyte-pore interactions(4 M KCl & -120 mV trans). We found small but clearly detectable differences in I/Io values for all 4 peptides: H3f(uncitrullinated), H3f.R2-cit, H3f.R8-cit and H3f.R2,8-cit in the order of increasing I/Io which could be accounted for by change of arginine's guanidium group to urea. This is further evidence that single molecule nanopore techniques are a valuable solution for rapid and easy detection of PTMs.

P-676
Modelling age-associated tau aggregation in C. elegans

Maithili Joshi[1], Sanne van Falier[1], Jorieke Tiggelaar[1], Tessa Sinnige[1]

[1] Utrecht University, Netherlands

Protein aggregation is the hallmark of many neurodegenerative diseases. Aggregation diseases associated with Tau are known as taupathies and include Alzheimer's disease and frontotemporal dementia (FTD). The nematode Caenorhabditis elegans is a simple multicellular animal model whose optical transparency can be used to study protein aggregation in a living and aging organism. Currently, the vast majority of C. elegans models depend on overexpression of disease-related proteins, inducing rapid aggregation early in the life of the animals. This does not recapitulate the late onset of human diseases associated with aggregation. We created C. elegans strains expressing wild type and mutant (P301L) human tau in the nervous system from single gene insertions to address this problem. Our aim is to understand which circumstances trigger the onset of tau aggregation, including the intracellular concentration of tau, its subcellular localization, and the age of the organism. Using confocal microscopy, we observed that tau localizes predominantly to the neuronal processes of C. elegans, consistent with its function to bind to microtubules. Moreover, bright inclusions are apparent that increase in number as the animals age. The disease related tau mutation (P301L) features higher inclusion numbers, suggesting that the mutation increases the rate of aggregation. As a control we also created overexpression strains, which revealed a striking dependence of tau aggregation on the expression level. By performing further quantitative analysis as well as interaction studies in our C. elegans models, we aim to gain a mechanistic understanding of the onset and progression of tau aggregation in vivo.

Poster Presentations
– Session 3 –

P-677
Molecular basis of stability differences in right- and left-handed G-quadruplexes
Michał Jurkowski[1], Mateusz Kogut[1], Jacek Czub[1]
[1] Department of Physical Chemistry, Gdansk University of Technology, Gdansk 80-233, Poland

G-quadruplexes (G4s) are 4-stranded structures of nucleic acids formed by guanine-rich sequences, where guanine bases associate into planar tetrads through Hoogsteen hydrogen bonds. G4s have been shown to exist in various regulatory regions of the human genome, playing an important role in controlling many cellular processes. G4s are characterized by wide structural diversity due to multiple ways in which G4-forming sequence can fold to form a guanine core. One of the key structural features of G4s is the helicity of G-tracts, which, similarly to double helix, can be either right- or left-handed. Up to now, only few left-handed G4 structures were reported, which, in comparison to great verity of right-handed G4s, suggests their lower stability. Nevertheless, the molecular basis of this disproportion is yet to be discovered. In this work we used all-atom Molecular Dynamics simulations supported by quantum chemical calculations to reveal the structural and energetic differences between right- and left-handed G4s. Our results indeed show the higher stability of right-handed G4s over left-handed analogues. The fundamentals of such difference were found in the G-tracts themselves but also in the conformations of intervening loops. Additionally, we explained the interdependency of helicity and direction of strand propagation – another crucial feature of G4 structures.

P-678
Liquid Crystalline Behavior of Bifunctional Phage-Biosensor in Biomedical Detection
Vilhelmiina Juusti[1,2], Janne Kulpakko[2], Elizabeth Cudjoe[3], Linda E. Amoah[3], Ville N. Pimenoff[2,4,5], Pekka Hänninen[1]
[1] University of Turku, Tykistökatu 6A, 20520, Turku, Finland, [2] Aqsens Health Ltd., Itäinen Pitkäkatu 4B, 20520, Turku, Finland, [3] University of Ghana, P.O. Box LG581, Legon, Ghana, [4] University of Oulu, Aapistie 5B, 90220, Oulu, Finland, [5] Karolinska Institutet, 14186 Stockholm, Sweden

Biomedical applications of phages have been extensively researched during the past decade. The combination of in vitro selected phages and their intrinsic exhibition of liquid crystalline (LC) behavior can be utilized in novel biosensors. We have developed bifunctional phage-biosensors for optical detection of the target analytes with time-resolved fluorescence (TRF) and absorbance. Phages offer a tempting alternative to develop new biomedical detection methods. The Green Fluorescent Protein (GFP) was used as a model analyte due to its well known optical properties. The phage-derived GFP detection was measured and analyzed in relation to modulation in TRF signal and color formation with absorbance. The LC behavior during the GFP detection was studied by measuring refractive indexes and biosensor performance in lyotropic and thermotropic conditions. Furthermore, we developed a biosensor to detect Plasmodium falciparum in the saliva of symptomatic and asymptomatic malaria patients. We found that the optical properties of the phage-biosensor system changed accordingly with the GFP concentration. The results with TRF and absorbance reflected the changes in LC behavior. The biosensor reached the limit of detection of 0.24 µg/mL with GFP. Respectively, the accuracy of 90% in detection of Plasmodium falciparum infection from saliva was reached in 15 minutes. Our results provide valuable information about the optical changes of the phage-biosensor in relation to the presence of analytes. The LC behavior of the biosensor enables quantitative analyte detection as well as disease detection from saliva. Overall, we suggest that the method is suitable for rapid screening of biomedical matrices.

P-679
Chemically functionalised nanopores for protein trapping
Julia Järlebark[1], John Andersson[1], Andreas Dahlin[1]
[1] Applied Chemistry, Chalmers University of Technology, Sweden

Single molecule studies provide information about heterogeneity and dynamics on a molecular level that is not accessible through conventional ensemble measurements. One of the currently most used techniques to access single molecule information is nanopore sensing. We are using advanced nanofabrication tools, such as electron beam lithography and dielectric breakdown, to prepare new types of bi- or multi-layered nanopore sensors and nano-chamber structures. By material-specific chemistry, it is possible to selectively modify part of the nanopore with polymer brushes, which consist of end-grafted chains at such high surface density that they are forced to stretch into solution. Despite mainly consisting of water, a polymer brush inside a nanopore can be an extremely strong barrier towards proteins while still allowing ions, water and small molecules to pass. We are currently investigating the polymer brush barrier properties towards proteins when electrokinetic forces are present. One challenge with nanopore sensing is the short dwell time of proteins, making it difficult to use this approach for studies of e.g. protein dynamics and reaction mechanisms. To address this issue, we have fabricated a nano-chamber consisting of an attoliter sized nanocavity, which we are planning to use to realise long-time, non-intrusive trapping of proteins.

P-680
Predictable Morphology of Metal Nanocrystals Templated by Amyloidogenic Proteins/Peptides and Photonic Polymerase Chain Reaction using Triangular Nanoparticles
Pradeep Kadu[1], Satyaprakash Pandey[1], Laxmikant Gadhe[1], Suditi Neekhra[1], Ambuja Navalkar[1], Komal Patel[1], Rakesh Kumar[1], Murali Sastry[2], Samir K. Maji[1,3]
[1] Department of Biosciences and Bioengineering, IIT Bombay, Powai, Mumbai-400076, India, [2] Department of Materials Science and Engineering & Department of Chemical Engineering, Monash University, Clayton, VIC 3800, Australia , [3] Sunita Sanghi Centre for Aging and Neurodegenerative Diseases, IIT Bombay, Powai, Mumbai 400076, India

Biomolecules have the ability to interact with metals and produce nanostructured hybrid materials with diverse morphologies and functions. Despite significant advancements in biomimetics, the interplay between the physical properties of biomolecules, such as sequence, charge, hydrophobicity, and the predictable morphology of resulting nanomaterials remains largely unknown. In this study, we elucidated a defined principle for gold nanocrystal growth into triangular and supra-spherical shapes with high predictability using various amyloidogenic proteins/peptides and their corresponding fibrils, in combination with charge and hydrophobicity. By employing a combination of biophysical and structural techniques, we established the mechanism of nucleation and crystal growth of gold nanostructures and demonstrated the effective isolation of intact nanostructures of different shapes from amyloid templates using protein digestion. Among the various shapes, triangular gold nanoparticles were utilized as nanoheaters for photonic polymerase chain reactions (PCR), and their efficiency was compared with triangular silver nanoparticles. Remarkably, the higher PCR efficiencies observed with triangular silver nanoparticles were attributed to their direct binding and unfolding of double-stranded DNA. These findings not only shed light on the fundamental principles underlying the design of bioinspired materials but also provided valuable insights for rational design and fabrication of functional materials in various scientific and technological fields.

Poster Presentations
– Session 3 –

P-681
Discrete elastic rod model of the microtubule plus end tip dynamic
Maksim Kalutskii[1], Helmut Grubmüller[1], Maxim Igaev[1]
[1] Theoretical and Computational Biophysics, Max Planck Institute for Multidisciplinary Sciences, 37077 Göttingen, Germany

Microtubules (MTs) are a critical component of the cytoskeleton, a network of protein filaments that shapes cells, enables their movement, and organizes their contents. These structures comprise GTP-bound tubulin dimers that assemble into polar protofilaments (PFs), which in turn self- assemble into hollow microtubules. MTs are highly dynamic and exhibit stochastic oscillations between polymerization and depolymerization. This process, called "dynamic instability," allows MTs to exert force on other cellular components. The process of MT disassembly is tightly related to the nucleation state of the tubulins; however, the exact mechanism of mechanochemical transformation and force generation remains elusive. The highly dynamic and non-uniform structure of MTs hinders their experimental study. For example, the non-uniform structure of MTs' ends prohibits averaging between MT structures, limiting the imaging of MTs to nanoscale resolutions. Therefore, the nanoscale details of microtubule tips cannot be reliably resolved experimentally. In theory, missing information on the nanoscale can be obtained in silico, i.e., with the help of molecular dynamics simulation. In practice, all-atom and even coarse-grained simulations cannot reach relevant. To bridge this gap, we developed a new coarse-grained model based on the discrete elastic rod representation. The model is optimized against data obtained from extensive all-atom simulations (> 100 μs in total). Using this model, we performed ms-long coarse-grained simulations of MT tip. Our findings shed light on the complex interplay between elastic parameters of individual PFs (i.e., natural curvature, twist, etc.), the strength of lateral and longitudinal bonds between the PFs, and the dynamics of MT assembly.

P-682
Single cell optical manipulation with deformable microtools
Lóránd Kelemen[1], Gaszton Vizsnyiczai[1], Tamás Gergely Iványi[1], Botond Nemes[1], Jana Kubackova[2], Gregor Bánó[3], Zoltán Tomori[2]
[1] Biological Research Centre, Institute of Biophysics, Szeged, Hungary, [2] Slovakian Academy of Sciences, Institute of Experimental Physics, Kosice, Slovakia, [3] Pavol Jozef Šafárik University, Institute of Physics, Kosice, Slovakia

Task-specific microstructures can efficiently support single cell research helping their actuation, deformation or even culturing. When such microstructures are designed for mobility, optical tweezers provide a powerful tool to operate them as microtools providing precise spatial and temporal control. Here, we introduce a family of deformable single cell manipulating microtools made by laser microfabrication. The tools are deformed with optical tweezers via specific elastic elements incorporated to the otherwise rigid structure. The deformability enables them to take hold, carry and rotate non-adherent cells with the added benefit of being able to release them at will. The achievable cell manipulation schemes are demonstrated with three types of structures. The first selectively collects and transports cells in a suspension. The second one enables the precise microscopic observation of free-floating cells from any direction by minimizing their fluctuation. The third system is used for spatio-temporally controlled cell-cell interaction applying a pair of tools: one mounts the cell to the substrate while the other carries another cell and makes contact between them.

P-683
DNA origami patterning for AFM studies of lipid nanodiscs
Charlotte Kielar[1], Daniel Dornbusch[2], Madhumalar Subramanian[2], Lisa Nucke[2], Artur Erbe[1], Jana Oertel[2], Karim Fahmy[2]
[1] Institute of Ion Beam Physics and Materials Research Helmholtz–Zentrum Dresden–Rossendorf, Bautzner Landstraße 400, 01328 Dresden, Germany, [2] Institute of Resource Ecology, Helmholtz–Zentrum Dresden–Rossendorf, Bautzner Landstraße 400, 01328 Dresden, Germany

DNA origami is a versatile tool with great potential in many research areas such as nanotechnology, biophysics and single-molecule studies. Hybridizing single-stranded DNA scaffolds with specially designed oligonucleotides enables self-assembly of almost arbitrary nanostructures. Thus, regular patterns of chemical entities can be created for various biomedical applications. Their potential for new approaches in membrane protein (MP) research is currently explored. Recently, MPs were immobilized in lipid nanodiscs (NDs) on 2D streptavidin surfaces, however without control over the NDs' lateral spatial arrangement. Patterning of surfaces with NDs by DNA origami may allow superior control of the resulting 2D/3D-superstructures. Here, we use DNA origami to create defined arrangements of NDs in the origami lattice. We prepare highly hierarchical 2D lattices of triangular DNA origamis on solid-support surfaces and analyze occupation of the origami cavities with NDs at different concentrations. Using time-resolved Atomic Force Microscopy (AFM), we find that the NDs fill and orient in the cavities without chemical or functional modifications. Besides imaging the newly created surface, AFM ensures defined and controllable ND patterning. Marked differences in the ND-origami surface structures arise with increasing ND concentration. Our proof-of-principle experiments demonstrate that DNA origami serves as a 2D lattice for specific arrangement of large (several μm^2) ND-filled structured surfaces, enabling quantitative AFM analysis of parallelized MP structural and functional studies over long timescales.

P-684
Physical interaction of fast neutrons with biological tissues for radiological therapy – A theoretical perspective
FESTO KIRAGGA[1], KONSTANTIN BRAZOVSKIY[1]
[1] TOMSK POLYTECHNIC UNIVERSITY, TOMSK, RUSSIA

From the discovery of neutrons by James Chadwick in 1932, research into the clinical applications of fast neutrons has been going on for almost a century regardless of the several bottlenecks. Although it's still miles away from the present technological improvements in photon therapy, nevertheless, its practical use is inevitable as some tumors have presented unsatisfactory endpoints despite several interventions. The uncharged neutrons do not interact directly with electrons or electron cloud of atoms like it is in the case of X-rays or photons. They however interact with water or hydrogen in biomolecules, liberating the highly ionising protons which create dense ionisation chains along their paths. Along these paths, these protons transfer energy to the body in a process called Linear Energy Transfer (LET). It's this deposited energy that is responsible for the Double Strand Breaks (DSB) that are lethal and completely eradicate the tumor. The lack of proper understanding of the radiobiological phenomena that takes place when fast neutrons of interact with biomolecules is likely to be a major hindrance to the scientific progress. This is likely to lock out the 10 – 15% of patients in dire need of neutron therapy. This mini-review focuses on the biophysical interaction of fast neutrons and biological tissues to draw some lessons going forward and make some foundational principles plain to the clinical, theoretical and experimental scientists.

Poster Presentations
– Session 3 –

P-685
Inhibitory mechanism of the BRICHOS protein on α-synuclein aggregation related to familial Parkinson´s disease

Hannah Klute[1], Laurène Adam[1], Willem Molenkamp[1], Rakesh Kumar[1], Jan Johansson[1], Axel Abelein[1]

[1] Karolinska Institutet, Sweden

Aggregation of the intrinsically disordered α-synuclein protein (α-Syn) into amyloid fibrils is thought to be the main cause of the incurable neurodegenerative Parkinson's disease (PD). Hence, it is crucial to elucidate the still unknown molecular mechanisms underlying the progression of the disease. The hypothesis of α-Syn as main cause for PD is supported by the early onset of PD due to familial mutations (e.g. A53T, A30P). Of importance in the context of suppressing amyloid formation is the chaperone-like Bri2 BRICHOS protein, which has been shown to bind to diverse amyloid fibrils. Recent studies in our laboratory revealed a delaying effect of Bri2 BRICHOS on wild-type (WT) α-Syn aggregation. Here, we investigate the inhibitory effect of Bri2 BRICHOS on familial α-Syn mutants, which are known to differ in their aggregation behavior from WT α- Syn. Our most recent aggregation kinetics experiments on A53T α-Syn showed a concentration dependent inhibitory effect of BRICHOS where specific microscopic nucleation processes, like fibril-end elongation, were affected. By conducting additional experiments with other α-Syn mutants we aim to identify the general inhibitory effect on α-Syn variants, which will help to elucidate the generic mechanism-of-action of BRICHOS binding to amyloid structures.

P-686
Protein Production Sweden (PPS) and biological deuteration

Wolfgang Knecht[1,2,3]

[1] Lund University, Sweden, [2] Lund Protein Production Platform, [3] Protein Production Sweden

Protein Production Sweden (PPS; www.gu.se/pps) is a new national research infrastructure established in 2022 and focused on the production and purification of protein reagents for primarily Swedish researchers, both from academia and commercial entities. Well-established protein production platforms from five universities (the University of Gothenburg (host), Karolinska Institutet, KTH Royal Institute of Technology, Lund University, and Umeå University) collaborate to form the infrastructure and offer expert competence in 4 geographically distributed nodes (Gothenburg, Lund, Stockholm, Umeå). Researchers across Sweden can get access via a joint entry-point and have the possibility to get support based on their research needs throughout the whole process of protein production and purification, starting from project counselling and design to quality control, or for any single/multiple step(s) in the process. PPS does allow protein expression in 6 different expression systems (E. coli, P. Pastoris, Insect cells (BEVS), Plant cells, Mammalian Cells and Cell free expression). It also offers two Gateway modules, with one aiming at producing speciality reagents for use in X-ray crystallography. The other one aims at producing speciality reagents for and offer guidance to their use in neutron scattering experiments. (Per)deuteration of proteins is essential for neutron techniques such as neutron protein crystallography, neutron reflectometry and small angle neutron scattering. This module is thereby aiming to enable non-experts in protein production to get recombinant proteins fit for neutron scattering experiments.

P-687
Human dihydroorotate dehydrogenase and lipids revisited: enzymology and neutron reflectometry

Wolfgang Knecht[1,2], Juan Manuel Orozco Rodriguez[1,2], Hanna P. Wacklin-Knecht[1,3], Luke A. Clifton[4], Oliver Bogojevic[3], Anna Leung[3], Giovanna Fragneto[3,5], Ewa Krupinska [1,2]

[1] Lund University, Sweden, [2] Lund Protein Production Platform, [3] European Spallation Source ERIC, [4] ISIS Pulsed Neutron and Muon Source, [5]Institut Laue-Langevin

Human dihydroorotate dehydrogenase (DHODH) is an enzyme anchored to the inner mitochondrial membrane by a single N-terminal transmembrane helix and catalyzes the oxidation of dihydroorotic acid to orotic acid with the simultaneous reduction of ubiquinone Q10. DHODH is a target for anti-inflammatory drugs. Neutron reflectometry allowed us to determine in situ, under solution conditions, how the enzyme binds to lipid membranes and to unambiguously resolve the location of Q10. Q10 is exclusively located at the center of all of the lipid bilayers investigated, and upon binding, DHODH penetrates into the hydrophobic region of the outer lipid leaflet towards the Q10. We have also investigated the interaction between DHODH and lipids using enzymatic assays, thermal stability assays and Quartz Crystal Microbalance with Dissipation monitoring and extended the repertoire of our techniques to the characterization of mutant DHODHs giving rise to Miller Syndrome, a rare Mendelian disorder. Our assay results provide evidence that the N-terminal part of human DHODH is not only a structural element for mitochondrial import and location of DHODH but influences enzymatic activity and utilization of Q10 and ubiquinone analogues in in vitro assays. They point out tetraoleoyl cardiolipin as a lipid interacting with DHODH and have practical implications for DHODH inhibitor testing and design.

P-688
Investigating melanoma progression model based on cells, tissues and exosomes by applying biophysical characterization of lectin-glycan interaction

Tomasz Kobiela[1], Swamy Kasarla[1], Anna Sobiepanek[1], Karolina Staniak[1]

[1] Warsaw University of Technology, Poland

The analysis of biomolecular interactions has become essential for the development of innovative, more efficient techniques for the diagnosis and prognosis of cancer progression. Many studies revealed that cancer development and progression are accompanied by changes in glycosylation patterns of cell surface and secreted glycoproteins. The aim of the study was the evaluation of interactions between specific glycans, present on the surface of the primary tumor and metastases of melanoma at various stages of cancer progression, with appropriate lectins, using biophysical methods. The combination of quartz crystal microbalance (QCM) with other label-free methods like atomic force microscopy or surface plasmon resonance leads to the determination of several critical physicochemical parameters allowing the monitoring of biological systems at the interface. On the way to more personalized treatment regimens, we used commercial cell lines and patient-derived cell lines to distinguish cancer cells phenotype based on the cell-surface glycans lectin interaction. For the monitoring of melanoma treatment in patient samples, we developed a label- free method that allows for the direct and real-time assessment of molecular binding kinetics in situ on the paraffin-embedded tissue specimens using QCM. Finally, we applied QCM extended with a module enabling the simultaneous registration of changes in the refractive index (NanoPlasmonic Sensing) for the determination of differences in properties between exosomes secreted by different melanoma cells.

Poster Presentations
– Session 3 –

P-689
Novel screening platform for highly multiplexed biomarker detection
Caroline Koch[1], Benedict Reilley-O'Donnell[1], Richard Gutierrez[2], Carla Lucarelli[1], Fu Siong Ng[1], Julia Gorelik[1], Aleksandar P. Ivanov[1], Joshua B. Edel[1]
[1] Imperial College London, [2] Oxford Nanopore Technologies, United Kingdom

Traditionally, clinical diagnosis relies upon the detection of a limited number of biomarkers, often failing to provide enough clinical detail on the pathology. Therefore, a new diagnostic strategy is needed that allows simultaneous detection of numerous biomarkers in complex biological fluids. If successful, this method holds great promise to improve personalised diagnostics and monitoring disease progression. To overcome this challenge, we developed new biosensors capable of detecting and discrimination a panel of biomarkers in complex biological fluids. We combined nanopore sequencing with newly designed barcoded molecular probes, which selectively bind to different biomarkers including microRNAs, proteins, and small molecules. This highly multiplexed approach was validated in clinical samples, detecting 40 different miRNAs simultaneously. Our workflow is rapid, from sample preparation to result in less than 1 hour. The proposed method is easily adaptable, as the number and type of target can simply be changed, allowing for a highly personalised detection approach. Preliminary results suggest a clear advantage over existing technologies, as it is cheaper, faster and bears the opportunity for pooled patient sample testing. We believe this strategy could have an extensive impact on patient diagnostics and facilitate the way towards personalised medicine.

P-690
Can we fold G-quadruplexes efficiently?
Mateusz Kogut[1], Michał Jurkowski, Jacek Czub
[1] Gdańsk University of Technology, Poland

G-quadruplexes (G4) are unique secondary structures formed by guanine-rich nucleic acid sequences. These structures are composed of stacked G- quartets, which are planar arrangements of four guanine bases held together by Hoogsteen hydrogen bonding. G4 structures have gained increasing attention in recent years due to their diverse roles in cellular processes and their potential applications in biomedicine. G4s are characterized by great structural diversity due to the multiple ways in which G4-forming sequences can fold to form a guanine core and overall topology. By selecting appropriate G4 sequences, they can be engineered and functionalized to serve as building blocks for nanoscale devices, such as DNA origami, DNA nanocages, and DNA nanoswitches, with controlled size, shape, and properties. However, so far there is no theoretical model that allows for such precise design. In our work, we propose a general procedure for folding G4s from the fully unfolded DNA strand to formed G4 structure with any topology using steered molecular dynamics simulations. This allows us to determine the possibility of forming different G4 topologies and investigate the influence of loop length on their stability, measured as their lifetime for several experimental structures.

P-691
Developing a method for protein-based DNA microscopy
Simon Kolmodin Dahlberg[1], Ian T. Hoffecker[1]
[1] KTH Royal Institute of Technology, Sweden

Imaging-by-sequencing or DNA microscopy is a developing field using DNA's high information storage capacities to record information that would traditionally be acquired using light-based microscopy techniques. Light-based methods are often limited in their multiplexing capabilities without large effort, and DNA microscopy techniques overcome this limitation by enabling many distinguishable labels to be processed simultaneously, in addition to being natively 3D. We introduce custom DNA strands into a sample, with DNA covalently attached to affinity proteins for targeting, which can then connect to and record neighbouring strands' identity. These connections can be read by sequencing and relative locations computationally reconstructed. We have established a workflow for covalently attaching DNA to affinity proteins and have successfully performed the PCR reaction required for DNA localization, which are important milestones for realizing a protein-based imaging-by-sequencing method.

P-692
What the Phos? Using Force Matching to Generate Force Field Parameters for Phosphorylated Amino Acids
Viktoria Korn[1], Kristyna Pluhackova[1]
[1] University of Stuttgart, Germany

Post-translational modifications (PTMs), of which phosphorylation is the most common, enable cells to rapidly adapt and adjust the functionality of molecular machineries and thus of the biological processes. In detail, small molecules are covalently and reversibly attached to biomolecules, thus rapidly altering their structure and function. Misplaced, missing, or excessive PTMs are typically linked to disease pathology, including inflammation, cancer, and neurodegenerative diseases, where they are responsible for neurotoxicity and synaptic damage. PTMs modify the bulkiness and charges of chemical groups, triggering conformational changes of the structure and thereby altering the biomolecules' intermolecular interactions and biological function. Molecular dynamics (MD) simulations are an amazing tool that helps us study biochemical processes on an atomistic scale. In MD, molecules are described by a priori parametrized force fields. In order to adequately represent chemical structures and properties of biomolecules under natural conditions and reveal the molecular impact of PTMs, parameters for all kinds of atom groups, including PTMs, need to be known. Our goal is to develop accurate force field parameters of phosphorylated amino acids for both the CHARMM36 all-atom and the Martini coarse-grained force field. Using our own quantum-chemical calculations of solvated, phosphorylated amino acid analogs of different charge and counterions as well as experimental data from the literature, we generate new force field parameters using our own python code to do force matching.

Poster Presentations
– Session 3 –

P-693
Generalization of the Martini-Go Scheme for the Study of Large Multichain Protein Complexes
Ksenia Korshunova[1], Julius Kiuru[1], Juho Liekkinen[1], Giray Enkavi[1], Ilpo Vattulainen[1], Bart Bruininks[1]
[1] University of Helsinki, Finland

The dynamics of large-scale protein complexes is an active subject of study in computational biophysics. However, it is challenging to simulate such complexes on an atomistic scale, especially due to their high computational costs. To overcome this limitation, coarse-grained (CG) representations of biomolecules, such as Martini 3, are often employed instead. A critical challenge in these CG models is maintaining the proteins' tertiary structure in a way that would nevertheless allow for its dynamic conformational changes. Typically, this is accomplished by integrating additional interactions between non-local backbone CG beads into the CG model. The Martini-Go model (originally developed by Poma et al.) implements such interactions based on the contact map of the protein's native structure via pairs of Lennard-Jones interactions. This method has been proven to work for single-chain proteins both in water-soluble cases and in cell membrane environments. In this work, we present a method that implements an extension of the Martini-Go model (Open Access) for the simulation of much larger, multi-chain protein complexes in a way that allows the dynamic conformational changes of protein complexes to be investigated realistically. We demonstrate through several applications how this improved Martini-Go model retains the tertiary and quaternary structures while simultaneously preserving the overall flexibility of the complex structure. The method we present paves the way for the future simulation of the most challenging large-scale processes, such as the self-assembly of protein complexes.

P-694
Catalytic Mechanism of Formate Dehydrogenase: Computational Studies
Jiří Kozelka[1,2], Yevgen Yurenko[3], Jan Novotný[3,4], Radek Marek[3,4]
[1] Department of Condensed Matter Physics, Faculty of Science, Masaryk University, Kotlářská 2, 61137 Brno, Czech Republic, [2] Université de Paris, INSERM, BIGR, DSIMB Bioinformatics team, 75015 Paris, France, [3] National Center for Biomolecular Research, Faculty of Science, Masaryk University, Kamenice 5, 62500 Brno, Czech Republic, [4] Department of Chemistry, Faculty of Science, Masaryk University, Kamenice 5, 62500 Brno, Czech Republic

QM/MM simulations of formate oxidation by the NAD-dependent formate dehydrogenase from Candida boidinii were recently reported. Analyzing snapshot ensembles from umbrella sampling of the reactants and transition state regions we identified two reactants state conformations having formate-to-nicotinamide distances corresponding to the reaction onset and showing similar embedding of cofactor and substrate as that seen in the transition state, thus qualifying their structures as models for the Michaelis complex. Decomposition of their formate-nicotinamide interaction energies revealed a substantial orbital interaction component. Analysis of NOCV channels revealed charge transfer-related accumulation of electron density in the internuclear region between the transferred formate H-atom and nicotinamide C4-atom indicating genesis of the new bond. Interestingly, both structures have the formate O-atoms at similar positions, fixed there by H-bonds from neighbouring amino acids, but differ in the direction of the C-H vector. From these two geometries, the transition state can be accessed by a low-barrier rotation of the formate C-H bond by ~45° in opposite directions. We thank Prof. D.-T. Major for snapshot ensembles from a QM/MM simulation of Candida boidinii formate dehydrogenase. Computational resources were provided by the e-INFRA CZ project (ID:90140).

P-695
Voltage Gating Properties of Connexin36 Gap Junction Channels: The Role of N-Terminus Amino Acid Residues
Tadas Kraujalis[1,2], Lukas Gudaitis[1], Mindaugas Snipas[1,3], Lina Kraujaliene[1]
[1] Institute of Cardiology at Lithuanian University of Health Sciences, Kaunas, Lithuania, [2] Department of Applied Informatics, Kaunas University of Technology, Kaunas, Lithuania, [3] Department of Mathematical Modelling, Kaunas University of Technology, Kaunas, Lithuania

Connexin36 (Cx36) proteins form gap junction (GJ) channels in the brain and pancreatic β cells. Compared to other connexin family members, Cx36 channels exhibit unique biophysical properties such as low conductance of unitary channels and low sensitivity to transjunctional voltage (Vj). Previous studies showed that the N-terminus domain of Cx channels plays a critical role in determining main Vj gating properties of GJ channels. This study utilized electrophysiological recordings, site-directed mutagenesis, homology, and mathematical modeling to assess the impact of specific amino acid residues located in the N-terminus domain on the Vj gating properties of Cx36 channels. Cx36 mutations at N-terminus, such as E3Q, E8Q, A13K and H18K, were found to alter Vj gating properties in homotypic GJ channels. Effects varied among mutations, impacting Vj gating kinetics, residual conductance, and overall Vj sensitivity. In addition, by analyzing recordings from various heterotypic configurations of Cx36-OxCer and mutant Cx36, we were able to determine the Vj gating polarity of Cx36 channels. Our findings suggest that Cx36 hemichannels gate in response to relatively positive Vjs and that this gating polarity is retained in E8Q, A13K, and H18K channels, but is reversed in E3Q mutant.

P-696
Biomolecular simulations on the LUMI supercomputer
Rasmus Kronberg[1]
[1] CSC – IT Center for Science Ltd, Finland

LUMI is a European pre-exascale supercomputer hosted by CSC – IT Center for Science, Finland. Being the third fastest supercomputer in the world (Top500 ranking, November 2022) with a hefty GPU-partition, LUMI enables unprecedented high-performance computing, machine learning and data science workflows. Software for biomolecular simulations ported to AMD GPUs will also greatly benefit from the performant MI250X accelerators in addition to the auxiliary CPU partition of some 200 000 compute cores. LUMI provides thus a leading-edge computing environment for coupling traditional HPC applications with modern AI/ML pipelines. To overcome scaling limitations of common molecular dynamics and electronic structure software on GPUs, high-throughput task farming workloads present a pertinent avenue to deploying the computing resources as efficiently as possible. The capabilities of aggregate sampling and ensemble runs using multi-simulation tools available in software such as GROMACS and Amber will be showcased along with related LUMI- specific best practices and lessons learned so far. Finally, the process of getting access to LUMI will be outlined and the available support channels described.

Poster Presentations
– Session 3 –

P-697
Dual color NIR FCS and antibunching measurements using a single superconducting nanowire single photon detector
Abhilash Kulkarni[1], Niusha Bagheri[1], Jerker Widengren[1]
[1] Experimental Biomolecular Physics, Department of Applied Physics, KTH Royal Institute of Technology, Stockholm 10691, Sweden

Fluorescence fluctuation spectroscopy is widely used in biomolecular studies to extract useful information about molecular dynamics and interactions, including also monitoring of photophysical transitions in fluorophores. However, fluorescence correlation spectroscopy (FCS) ultimately relies on single-molecule detection conditions, and its application for cellular and in-vivo studies can be limited, mainly due to autofluorescence and scattering issues, resulting in low signal-to-background ratios (SBRs). In this investigation, we try to reduce such limitations by exploiting the benefits of Superconducting Nanowire Single Photon Detectors (SNSPDs) which include no after-pulsing (as seen in APDs), better detection efficiency in the near infrared region (NIR) and high time resolution. We have devised a novel setup which utilizes a single SNSPD channel to perform dual color cross correlation measurements by segmenting the signal temporally. We also show that the same strategy can be used for antibunching measurements, at the same time monitoring lifetimes and rotational diffusions on similar shorter time scales. We also investigate different emissive species of a NIR cyanine dye by spectrally separating them, thereby providing a specific signature for this dye which can be used to study exosomes and similar systems with a high SBR.

P-698
Development of the TMEM192-mKeima probe for evaluating lysophagy flux
Akiko Kuma[1], Takayuki Shima[1], Shuhei Nakamura[1], Tamotsu Yoshimori[1]
[1] Osaka University, Japan

P-699
Amyloid-β (1-42) oligomers conformational changes in membrane-mimicking environments: An FTIR study
Oleksandra Kurysheva[1], Faraz Vosough[1], Andreas Barth[1]
[1] Stockholm University, Department of Biochemistry and Biophysics, Sweden

Soluble Amyloid-beta (1-42) oligomers play a major role in developing neurodegenerative diseases, such as AD and Parkinson`s, as they are indicated as the most toxic species. However, the aggregation process of Aβ, and how it may be promoted by the cell membranes, is still under debate. A hypothesis states that membranes under certain conditions induce the structural transition from disordered state to the stable ordered species, dominated by beta-sheets. Our previous studies have shown that the membrane-mimicking detergent sodium dodecyl sulfate (SDS) stabilizes Aβ (1-42) oligomers` structure. Among existing experimental approaches for determining protein structure, FTIR spectroscopy is a powerful label-free tool for protein secondary structure analysis. Here, we report our "Aβ-model membrane" system preparation for FTIR spectroscopy, as well as our results on conformational changes of Aβ induced by lipid environment. This work contributes to a further understanding of the aggregation process of toxic Aβ species and their interaction with the model membrane system. Acknowledgements: This work was supported by a doctoral scholarship from The Sven and Lilly Lawski foundation to O.K.

P-700
Overcoming the High Concentration Barrier in Single-Molecule Fluorescence Experiments through Adaptable Fluorogenic ssDNA Label
Mirjam Kümmerlin[1], Achillefs Kapanidis[1]
[1] University of Oxford, United Kingdom

The "high concentration barrier" of ~50 nM fluorescent species is one of the main limitations of single-molecule (sm) fluorescence (SMF) measurements. Addressing this fundamental limit will enable several in vitro and in vivo single-molecule applications, including tracking in crowded environments, super-resolution imaging, and smFRET experiments. One way to achieve this is by employing fluorogenic labels. In this work, we engineer the quenching efficiency and fluorescence enhancement upon duplex formation of short ssDNA labels. Through the adjustment of fluorophore-quencher combinations, label lengths, and buffer compositions, we can perform SMF experiments at concentrations of up to 10μM fluorescent labels, which is a 200-fold improvement without the need for specialised optics. As one example application of high concentrations of fluorogenic labels, we perform smFRET measurements over extended observations spans of up to one hour, circumventing photo-bleaching through constant exchange of fluorogenic ssDNAs supplying donor and acceptor dyes (REFRESH-FRET). Highlighting the adaptability of our strategy, we are currently working on translating the REFRESH principles into live bacterial cells.

Poster Presentations
– Session 3 –

P-701
Atom Probe Tomography: computational challenges and perspectives
Gianluca Lattanzi[1,2], Simone Taioli[3], Giovanni Novi Inverardi[1,2], Francesco Carnovale[1,2]
[1] Department of Physics, University of Trento, Italy [2] NFN-TIFPA Trento Institute for Fundamental Physics and Applications, , [3] European Centre for Theoretical Studies in Nuclear Physics and Related Areas (ECT*), Bruno Kessler Foundation

A novel procedure for the application of Atom Probe Tomography (APT) to the structural analysis of biological systems has been recently proposed, whereby the specimen is embedded by a silica matrix and ablated by a pulsed laser source. Based on this technique, our group at the University of Trento is actively collaborating to the efforts of a recently funded EIC-pathfinder consortium, named MIMOSA, whose ultimate goal is to provide an alternative to 4D microscopy with a disruptive technology enabling a high spatial resolution and a chemical sensitivity analysis of bio-samples. However, this technique poses several challenges on the computational side. For instance, APT requires that the silica primer be properly inert and bio-compatible, keeping the native structural features of the system at hand while condensing into an amorphous, glassy-like coating. We have proposed a Molecular Dynamics protocol aimed at depicting and characterizing the earliest stages of the embedding process of small biomolecules in a solution of water and orthosilicic acid, here taken as a precursor of the silica matrix. In this first preliminary step, we observe a negligible influence of orthosilicic acid on the behavior of stable folded systems (such as ubiquitin), while it affects the fluctuations of intrinsically disordered and unstable peptides. However, the entire project presents several challenges that can be tackled (or at least addressed) with the available computational techniques, whether classical or ab initio. I will present our most recent applications in this direction and offer some perspectives for discussion.

P-702
Accurate rare-event kinetics from AI-assisted molecular dynamics simulations
Gianmarco Lazzeri[1,2], Hendrik Jung[3], Peter G. Bolhuis[4], Roberto Covino[1]
[1] Frankfurt Institute for Advanced Studies, Germany, [2] Goethe-Universität Frankfurt, Germany, [3] Max Planck Institute of Biophysics, Germany, [4] University of Amsterdam, The Netherlands

Molecular dynamics (MD) simulations track the evolution of molecular systems with femtosecond resolution at the atomic scale. They generate trajectories that, if long enough, disclose the mechanism and kinetics of the investigated phenomena. The growing computational power raised the size and complexity of the systems that MD simulations can tackle. However, high energy barriers remain a challenge, limiting transitions between molecular conformations to rare events. Molecular systems primarily dwell in low-energy metastable states, making rare events difficult and expensive to characterize. Transition path sampling (TPS) offers a solution by harvesting reactive trajectories as if they come from long equilibrium simulations. Still, TPS trajectories do not follow the Boltzmann distribution and necessitate further analysis to extract the underlying dynamics. We employed artificial intelligence (AI) to enhance TPS efficiency on-the-fly and simultaneously learn the transition mechanism. This information enabled the reconstruction of kinetics and free energy projected on arbitrary features. We tested our approach on the folding of the mini protein chignolin. We obtained accurate transition rates in a fraction of the time needed for observing a single event in an equilibrium simulation. These promising results pave the way for applications to larger systems.

P-703
A multiscale simulation approach to unravel cancer-related mutation patterns on conformational landscapes
Byung Ho Lee[1], Laura Orellana[1]
[1] Department of Oncology-Pathology, Karolinska Institutet, Stockholm, Sweden

Genomic data of cancer missense mutations have been accumulated rapidly, leading to significant advancement in the field of cancer research. However, gene sequence-based approaches are insufficient to directly characterize cancer-driving mutations whose patterns are distinct on conformational landscapes. In this regard, clustering of mutations depending on their impacts on conformational dynamics is essential to comprehend cancer-related mutation patterns. In this study, we propose a new multiscale simulation approach to efficiently screen hundreds of point mutations for cancer proteins. The fundamental concept is to quantitatively analyze the mutational impacts on conformational dynamics by using normal mode analysis, a powerful tool to describe intrinsic motion patterns of proteins based on harmonic approximation of the potential energy. To improve its computational efficiency, protein structures are simplified into coarse-grained mechanics systems, wherein backbone atoms are sampled as mass points and linked by springs. Using all-atom molecular dynamics trajectories for a dataset of proteins as benchmarks, we explore optimum values of modeling parameters (e.g., force constants and bond cutoff distances), which enhances sensitivity of our models to changes of conformational dynamics caused by mutations. For cancer-related proteins, we perform the proposed screening method and validate it by comparing the results with available experimental data. This simulation approach enables fast screening for a vast array of mutations, reveals mutation patterns on conformational landscapes, and by extension gives a deep insight into the intrinsic relationship between mutations, dynamics, and functions.

P-704
Functional characterization and structural basis of a dual-function flavonoid 2'-O-glycosyltransferase from Scutellaria baicalensis
Junhao Li[1], Zilong Wang[2], Xueqing Du[3], Ye Guo[2], Haotian Wang[2], Chenrui Liu[2], Fudong Li[4], Xue Qiao[2], Hans Ågren[1], Chao He[3], Dean Guo[5]
[1] Uppsala University, Sweden [2] Peking University, [3] Anhui University, [4] University of Science and Technology of China, [5] Shanghai Institute of Materia Medica, Chinese Academy of Sciences

Glycosylation is an important post-modification reaction in plant secondary metabolism, and contributes to structural diversity of bioactive natural products. In plants, glycosylation is usually catalyzed by UDP-glycosyltransferases (UGTs). In this work, UGT71AP2 was identified from the medicinal plant Scutellaria baicalensis as the first flavonoid 2'-O-glycosyltransferase. It could preferentially transfer a glycosyl moiety to 2'-hydroxy of at least nine flavonoids to yield six new compounds. Some of the 2'-O-glycosides showed noticeable inhibitory activities against 3CLpro of SARS- CoV-2 virus and cyclooxygenase 2 (COX-2). The crystal structure of UGT71AP2 (2.15 Å) was solved, and mechanisms of its regio-selectivity was interpreted by pKa calculations, molecular docking, MD simulation, MM/GBSA binding free energy, quantum chemical calculations, and hydrogen- deuterium exchange mass spectroscopy. Through structure-guided rational design, we obtained the L138T/V179D/M180T mutant with remarkably enhanced regio-selectivity (the ratio of 7-O-glycosylation byproducts decreased from 48% to 4%) and catalytic efficiency of 2'-O-glycosylation (kcat/Km, 0.23 μM-1 s-1, 12-fold higher than the native). Furthermore, we found UGT71AP2 possessed UDP-dependent de-glycosylation function, and that the His16-Asp107 dyad played a key role in proton transfer to initiate the reaction. This work provides an efficient biocatalyst to prepare flavonoid 2'-O-glycosides, and unravels mechanisms for the regio-selectivity and de-glycosylation activity of UGT71AP2.

Poster Presentations
– Session 3 –

P-705
Molecular Basis of Polyglutamine-Modulated ELF3 Aggregation in Arabidopsis Temperature Response
Richard Lindsay[1], Philip Wigge[2,3]
[1] Flatiron Institute - U.S., [2] University of Potsdam - Germany, [3]Leibniz-Institut für Gemüse- und Zierpflanzenbau - Germany

Arabidopsis thaliana possess a molecular mechanism for temperature sensing and responds to changes by modifying growth rate, photoperiod length and timing of flowering. Underlying this mechanism is a tripartite protein complex called the Evening Complex (EC) that functions as a DNA transcription repressor targeting growth-related genes. ELF3, a large disordered scaffolding protein, is the primary component of the EC, which sits over DNA targets occluding transcription machinery and halts growth. Rising temperature promotes ELF3 condensation which sequesters ELF3 allowing DNA transcription and plant growth to proceed. A prion-like domain (PrD) has been observed to be sufficient for ELF3 aggregation. Within this PrD region lies a poly-glutamine repeat of variable length, which has been found to modulate thermal responsiveness by hypocotyl (stem) elongation and condensate formation. We use a polymer chain growth approach to build large ensembles and characterize monomeric ELF3-PrD at a range of polyQ lengths and temperatures, and explore temperature-dependent dynamics. We find increased solvent accessibility of expanded polyQ tracts, promotion of temperature-sensitive helices adjacent to polyQ tracts, and exposure of a cluster of aromatic residues at increased temperature in wild-type, all three of which promote inter-protein interaction. We also find key differences in temperature-sensing mechanisms in absence of polyQ.

P-706
Single-protein diffusion in E. coli: heterogeneity in structure of cytoplasm and impact of (poly)ribosomes on mobility
Dmitrii Linnik[1], Michiel Punter[1], Bert Poolman[1]
[1] University of Groningen, Netherlands

Protein diffusion in bacteria is crucial for cellular homeostasis. Recently, we observed a decrease in the diffusion coefficient at the polar regions of E. coli, which exceeds the effects predicted by random-walk simulations in confined space. (Poly)ribosomes are excluded from the nucleoid to the cell periphery, so in this work we focused on determining the effect of ribosomes on the observed diffusion decrease. Single-molecule displacement measuring was used to determine the lateral diffusion coefficient (DL) of mEos3.2 fluorescent protein in the center and poles of E. coli. Erythromycin and rifampicin were used to alter the distribution of (poly)ribosomes, as confirmed by HiLo fluorescence microscopy. Erythromycin treatment resulted in less ribosome enrichment at the poles and the measured DL values increased significantly, but the relative increases were similar for the center of the cell and pole regions. Rifampicin treatment resulted in a complete loss of peripheral ribosome accumulation and the observed effects were even more pronounced than with erythromycin treatment. Thus, in the presence of rifampicin the DL increases, most likely as result of a decrease in viscosity due the loss of mRNA. However, the pole to middle DL ratios decreased only upon rifampicin treatment, while for all other conditions it remained the same. Based on our observations, the lower DL at the cell poles is not caused by the presence of (poly)ribosomes but most likely aggregated proteins, which are more abundant in old than new poles.

P-707
Structural basis for PI(4,5)P2-dependent FGF2 dimerization on membrane surfaces triggering FGF2 membrane translocation into the extracellular space
Fabio Lolicato[1,2], Julia Steringer[1], Roberto Saleppico[1], Daniel Beyer[1], Jaime Fernandez-Sobaberas[1], Sebastian Unger[1], Sabine Wegehingel[1], Hans- Michael Müller[1], Helge Ewers[3], Martin Hof[4], Radek Sachl[4], Petr Chlanda[5], Ilpo Vattulainen[2], Walter Nickel[1]
[1] Heidelberg University, Germany, [2]University of Helsinki, Finland, [3]Freie Universität Berlin, Germany , [4]J. Heyrovský Institute of Physical Chemistry, Czech Republic, [5]Heidelberg University, Germany

Fibroblast Growth Factor 2 (FGF2) promotes cell survival and is implicated in tumor-induced angiogenesis. FGF2 lacks a signal peptide, and it is one of the most prominent examples of extracellular proteins secreted by ER/Golgi-independent secretory pathways, processes collectively termed unconventional protein secretion. This process is initiated by PI(4,5)P2-dependent FGF2 recruitment at the inner plasma membrane leaflet. This results in the formation of membrane-spanning FGF2 oligomers within toroidal membrane pores. Our findings show that FGF2 forms ring-like structures on the surface of membranes through a process initiated by disulfide bridge dimers, which then leads to the formation of membrane- pore FGF2 oligomers. Here, using biochemical reconstitution experiments and live-cell imaging, we demonstrate that FGF2 variant forms lacking C95 could not oligomerize and form pores, preventing efficient translocation across the membrane. We also found that another cysteine residue, C77, plays a role in FGF2 secretion by facilitating interaction with the α1 subunit of Na, K-ATPase. However, C77 is not involved in oligomerization but rather in the early steps of the secretion process. Furthermore, using atomistic molecular dynamics simulations, we propose a mechanism for how FGF2 forms C95-C95 bridged dimers on membrane surfaces in a PI(4,5)P2-dependent manner. Combining our findings with cryo-electron tomography and AlphaFold2 multimer predictions, we provide the first structural understanding of how FGF2 organizes on the membrane as intermediates in unconventional secretion, which trigger membrane pores and translocate across plasma membranes in a GPC1-dependent way.

P-708
A magic-angle spinning solid-state NMR approach to decipher the evolution of cell surface architecture at atomic resolution
Antoine Loquet[1], Gaelle Lamon[1], Alons Lends[1], Isabel Valsecchi[2], Sarah Wong[2], James Tolchard[1], Axelle Grélard[1], Estelle Morvan[1], Erick Dufourc[1], Birgit Habenstein[1], Inaki Guijarro[2], Vishukumar Aimanianda[2]
[1] University of Bordeaux, [2] Institut Pasteur, Unité Mycologie Moléculaire, France

We report the development of an approach that employs magic-angle spinning nuclear magnetic resonance (MAS NMR) to decipher the evolution of the architecture of pathogenic cell-wall at atomic resolution. We exemplified our approach on Aspergillus fumigatus, an opportunistic airborne fungal pathogen. Understanding the cell-wall reorganization of this pathogen is important as it is the first fungal structural component to interact with the host immune system. We demonstrate that the A. fumigatus conidia at three crucial stages of their life cycle (dormant, swollen and germinating) can be characterized at atomic level by comparing MAS NMR data. Our data show a profound re-arrangement of the cell-wall during conidial germination process, at the level of polysaccharide composition, their molecular mobility and water-accessibility. Our approach provides an unprecedented molecular picture on how the conidial cell-wall is re-arranged and the constituent polysaccharides are re-shuffled during the process of germination. Our results depict complex compensation mechanisms that are taking place at the level of the atomic architecture of the cell-wall to re-balance the morphological changes associated with the germination. Our work opens an avenue to apply the methodology to other infectious micro-organisms to investigate their cell-wall architecture during their life cycles. Solid-state NMR molecular snapshots of Aspergillus fumigatus cell wall architecture during a conidial morphotype transition.

Poster Presentations
– Session 3 –

P-709
Structural Controllability of Unfolded Protein Response Network Models: Protein-Protein Interaction Databases Comparison

Nicole Luchetti[1,2], Alessandro Loppini[1], Margherita Anna Grazia Matarrese[1,3,4], Letizia Chiodo[1], Simonetta Filippi[1,5,6]
[1] Campus Bio-Medico University, Italy [2] IIT - Center for Life Nano Science, [3] Children Health Care System, [4]The University of Texas at Arlington , [5] National Institute of Optics, National Research Council, [6] International Center for Relativistic Astrophysics Network

The Unfolded Protein Response (UPR) is the cell mechanism for maintaining the balance of properly folded proteins in the endoplasmic reticulum (ER, the place in which the protein translation and folding take place). The activation of the UPR under ER stress conditions results in an adaptive response that prevents the increase of unfolded proteins (UPs) concentration over its physiological level, whose alterations may result in a critical accumulation of unfolded or misfolded proteins (MPs). If the UPR adaptive response to ER stress fails to restore cell homeostasis due to a persistent accumulation of UPs, the signaling program continues, and the ER stress sensors EIF2AK3 and ERN1 both drive multiple signaling outputs leading to apoptosis. It is possible to describe the UPR mechanism as a network, in which the proteins that take part in the stress response and consequent apoptosis are identified by the nodes, and the protein-protein interactions (PPI) are identified as the edges that connect proteins in pairs. As each database description of the interaction is intrinsically partial, it is possible to interpret the parameter value as the "degree of belief" in the occurrence of pairwise interaction. We underline that, for different analysis methods to be effective, sufficient information must be stored in PPI databases. Despite the general limitations intrinsic of the present PPI databases, our study demonstrates that the structural controllability can identify the ER stress sensors as the most important participants in the UPR adaptive response, even when a part of the biological information is missing.

P-710
Unravelling the folding of a large monomeric model protein at high resolution through HDX-MS

Romain Malempré[1], Julie Vandenameele[1], Sébastien Brier[2], Caroline Montagner[1], Christina Redfield[3], André Matagne[1]
[1] Université de Liège, Belgique, [2] Plateforme technologique de RMN biologique, C2RT, Institut Pasteur, France, [3] Department of Biochemistry, University of Oxford, United Kingdom

The detailed characterization of the folding of a large protein is conducted on a model enzyme, namely the BS3 class A β-lactamase. The principal goal is to determine the order of formation of its secondary structure elements using quenched-flow HDX pulse-labelling experiments, combined with both NMR and mass spectrometry (MS) measurements. Class A β-lactamases (Mr ca. 29000) are among the largest proteins studied in terms of their folding properties. Thus, the folding of β-lactamases has been characterized in some detail, and, as observed with most large proteins, folding is not kinetically two-state and intermediate partially folded species are observed. The highly conserved cis peptide bond between residues 166 and 167, in a long Ω-loop at the active site of these enzymes, controls important steps in the refolding reaction. The sequence of the two structural domains implies a substantial movement of the polypeptide chain during the folding process. Indeed, one of the two domains (composed of both α-helices and β-sheet) is made up of the N and C-terminal parts of the protein, whereas the other domain (all α) is formed by the central part of the polypeptide chain. HDX-MS experiments revealed the initial formation of molecules with native-like protection against exchange (EX1) in the secondary structural elements closest to the N- and C-terminal parts of the sequence, then propagating to the core of the protein. These results suggest the presence of an intermediate species with a folding nucleus that allows a productive process toward the formation of fully active enzyme molecules.

P-711
Using optical tweezers to observe RNA structural rearrangements under different physiological conditions.

Tycho Marinus[1], Kasia Tych[1]
[1] University of Groningen, Netherlands

The interest in RNA structure and its functions has grown steadily over the last two decades, followed by a rapid increase of interest caused by the vaccine for the recent pandemic. Classical methods to study biomolecules such as crystallography and nuclear magnetic resonance are possible but challenging to apply on RNA molecules. An alternative method often applied to study RNA is based on chemical modifications of unpaired nucleotides that are identified during sequencing (e.g. selective 2′-hydroxyl acylation detected by primer extension). However such methods rely on an assembly of molecules, making it difficult to identify structural intermediates. These intermediates are essential for the understanding of biological processes, and thus important for the development of safe and reliable therapeutics. In my research I am developing methods to investigate RNA molecules in a single molecule analysis using state of the art optical tweezers. While optical tweezers have been used in some studies for RNA molecules, the setup in our lab has a 5 channel chip, heat control and single molecule fluorescence detecting. This novel setup allows us to study a single molecule under a wide range of conditions, e.g. simulated cell crowding, heating and different buffer compositions. With this optical tweezers setup, we are able to investigate RNA molecules with unique spatial and temporal resolution, providing significant new insights into RNA structure and the many intermediates.

P-712
Traube's rule made quantitative: hydrophobic free energy (HFE) of linear alkylic molecules and their hydration features

Jorge Martins[1]
[1] CCMAR & DCBB-FCT, Universidade do Algarve, Portugal

The importance of aqueous solubility of alkylic molecules is unavoidable in physical biochemistry, as well as in environmental conservation, and biomedicine. The comprehension of hydrophobic hydration is still a matter of debate from its thermodynamic grounds to protein stability and ligand biding. Traube's rule (1891) launched a qualitative perception for the hydration of aliphatic acids, alcohols and amines based on the length of their methylenic chains. This work intends to quantitatively verify Traube´s rule defining HFE as a simple transfer of solutes from the gas phase to water, based on experimental values of Henry´s law constant. Homologous hydrocarbons are used to assess the differences in HFE between apolar and amphiphilic molecules. The dimensionless Henry's constants, for linear alkylic acids, alcohols, and amines (less than 10 carbons) were obtained from tabulated track and only consistent sources were considered. HFE values were plotted against the number of methylene groups. For hydrocarbons (propane to nonane), the plot is linear (R=0.997) and the intercept 7.22 kJ/mol, fairly compares with 7.57 kJ/mol for ethane. For the amphiphiles, the outcomes are in accordance with their respective ionic or polar features.

Poster Presentations
– Session 3 –

P-713
Integration of deep learning into a computational platform for the development and optimization of biopharmaceuticals

João Martins da Silva[1], Matheus Almeida[1], Jean Sampaio[1,2], Diego Almeida[1,2], Francisco Rabelo[3], Cesar Lincoln Mattos[3], Eduardo Gaieta[1,2], Geraldo Sartori[1,2]
[1] FIOCRUZ Ceará, Brazil, [2] Oswaldo Cruz Institute, [3] Federal University of Ceará

The use of antibodies (AB) as drugs has emerged as a promising approach for treating various diseases. The structural optimization of the 3D coordinates of AB is critical to improving therapeutic efficacy, and several deep learning approaches showed the potential to enhance this process. By training machine learning models on large datasets of AB-antigen (AG) complexes, one can develop algorithms to improve the CDR affinity to specific AG. Here we implement a computational method for modifying AB structures capable of considering the diversity of individual CDRs and the germline configuration, in addition to the mutations most likely to occur in nature. Initially, we built a structural database of AB containing descriptors based on their biochemical and physicochemical properties for use in machine learning routines to identify and optimize potential biopharmaceuticals against diseases of public health interest. Then, 3D structures of AB variable region complexed with AG were retrieved from the AbDb database and standardized in sequence length and numbering. Subsequently, ten AB-AG complexes were used to define and implement the extraction of the descriptors of interest with graph representation that will be used as input for the AI algorithms. Finally, an automation routine was created to implement the molecular descriptors elucidated by the smaller set in the set of all structures. A total of 13 structural descriptors have been implemented so far. As a result, more robust descriptors are being computed, and this database will be used for the future development of deep learning models.

P-714
Computationally designed gold nanoclusters with tuned properties for combination cancer therapy

María Francisca Matus[1], Sami Malola[1], Hannu Häkkinen[2]
[1] University of Jyväskylä, Department of Physics, Nanoscience Center (NSC), Finland., [2] University of Jyväskylä, Departments of Physics and Chemistry, Nanoscience Center (NSC), Finland.

Monolayer-protected gold nanoclusters (AuNCs) are a unique class of ultrasmall nanoparticles (<3 nm diameter) with definite chemical composition. Recently, they have shown outstanding potential as tunable materials for diagnostics and therapeutics and, owing to their atomically precise structure, are ideal candidates for building accurate computational models for experimental characterization and validation. Here, we described the interactions and binding energy of fourteen nanosystems based on Au144(p-MBA)60 (p-MBA=para-mercaptobenzoic acid) nanocluster with the AVB3 integrin using umbrella sampling simulations to obtain the best formulations for experimental studies. The targeted AuNCs were functionalized with arginine-glycine-aspartate (RGD) peptides and anti-cancer drugs, and we studied the role of ligand ratio in their optimal structural conformation using atomistic models. The results showed that the peptide:drug ratio is decisive for the potential targeting ability of the nanosystem, which depends on the intermolecular interactions established in the ligand layer. This computational approach provides vital information for ligand tuning and improved targeting at the atomic level, which is crucial for guiding the experimental stage.

P-715
Bacterial motion along regular polygon-shaped, zigzag-like, and chaotic trajectories

Andreas Menzel[1]
[1] Otto von Guericke University Magdeburg, Germany

In recent experiments, the motion of certain light-sensitive bacteria along regular polygon-shaped trajectories has been triggered by adjusted irradiation protocols. We address such types of kinked trajectories composed of discrete straight steps with persistent turns towards one side. Additionally, in our case, self-propulsion is biased towards a fixed direction, which can be triggered, for instance, by a nutrient gradient. It turns out that these three contributions to the motion in combination induce surprisingly rich nonlinear dynamics. With increasing directional bias, the regular polygons first are transformed into kinked cycloidal-like trajectories. Next, we observe straight motion. After that, zigzag-like trajectories emerge, where the regular zigzag is then transformed into double zigzags, quadruple zigzags, and so on. Moreover, for other strengths of drift, we find chaotic types of motion. As our analysis reveals, these results can be quantified in terms of a nonlinear dynamics theory. It suggests that our observations are due to bifurcational scenarios and period doubling. Additionally, we consider the collective motion of many such self-propelled individuals together. We find that the whole crowd can order orientationally so that all individuals step on average into the same direction. Moreover, the crowd can concentrate into one spot that then moves as a whole chaotically. As may be expected, disturbances and diversity among the individuals mostly reduce ordering. We hope that our results can stimulate corresponding interest, particularly in view of additional experimental realizations and especially concerning the collective behavior.

P-716
X-ray crystallography structure of the dysfunctional tau protein from Alzheimer's PHF

Klaudia Meskova[1], Michaela Skrabanova[1], Petra Majerova[2], Rostislav Skrabana[1]
[1] Institute of Neuroimmunology, Slovak Academy of Sciences, Dubravska cesta 9, 84510, Bratislava, [2] Axon Neuroscience CRM Services SE, Dvorakovo nabrezie 10, 81102, Bratislava, Slovakia

Alzheimer's disease (AD) is characterized by the accumulation of the amyloid beta peptide plaques and neurofibrillary tangles in the cerebral cortex. It was shown before that the neurofibrillary tangle is composed of two types of tau filaments: paired helical and straight filaments. Tau protein has an irreplaceable role in the microtubule assembly and regulation of the dynamics of microtubules. During Alzheimer's disease, the tau protein undergoes various post-translational modifications, which may influence the tau aggregation and structural changes during the AD progression, which results in the destabilization of microtubules and neuronal damage. First structural models of AD filaments at atomic levels were shown using cryo-electron microscopy from residues G274/304-V378. However, previous works demonstrated that the PHF core is made from the residues I297 – G391. Therefore, in our project, using X-ray crystallography, we try to supplement the knowledge of the tau filaments from AD PHF. Our strategy is based on the co-crystallization of specific PHF recombinant antibodies, which were generated in mice immunized with AD brain extracts, in complex with different recombinant tau peptides (tau dGAE and its short fragments) which represents the PHF core units. Until now, we have crystallized eight different binary and ternary complexes, however, diffraction data were obtained from five of them at 1.5 – 3Å at DESY Hamburg and PSI Switzerland synchrotron source of X-ray radiation. In the next steps, we will do molecular replacement and structure refinement of our crystals.

Poster Presentations
– Session 3 –

P-717
SAKe protein as scaffold for on-surface self-assembly
Andreu Mor Maldonado[1], Satf Wouters[1], Gangamallaia Velpula[1], Steven de Feyter[1], Arnout Voet[1]
[1] KU Leuven, Belgium

In our globalized world, controlling the spread of diseases is becoming increasingly challenging due to the ease of travel. This has brought attention to the need for quick, reliable, and affordable diagnostic tools. Point-of-care diagnostics currently rely on immunobiosensors, which use antibodies as ligands. However, this method has limitations such as low surface coverage and expensive production. To address this, there is a need for a small molecule that can specifically recognize an analyte and improve surface functionalization. Here we present a small pseudo-symmetric protein named SAKe, inspired by beta propellers, as a possible alternative to immunobiosensors. SAKe has been shown to self-assemble at the liquid-solid interface of highly-ordered pyrolytic graphite (HOPG) and of mica, forming large order hexagonal lattices. The symmetry of the protein as well as functional groups designed to directly interact with the surface are the driving force for on-surface self-assembly. Furthermore, we have demonstrated that the re-engineered SAKe protein can specifically bind to natural targets, with biolayer interferometry (BLI) experiments showing binding affinities on the micromolar range. Moreover, the robustness of the SAKe protein makes it a perfect model for the study of the effect of symmetry and protein functionalization on protein-metal frameworks. Overall, SAKe is a promising candidate for the development of quick, reliable, and affordable diagnostic technologies.

P-718
Nanogels from Guanosine Hydrogels: An innovative tool for drug delivery?
Paolo Moretti[1], Alessia Pepe[1], Martina Mariani[1], Paolo Mariani[1]
[1] Marche Polytechnic University, Italy

Nanostructured systems aimed at drug delivery represent a new frontier in the biotechnological field. In this scenario, Nanogels (NGs) have been considered excellent carriers. We developed a new type of NGs from the self-assembled guanine G-hydrogel, G-NG. Two guanine derivatives enter as gelators in the composition of the hydrogel: guanosine 5'- Monophosphate (GMP) and guanosine (Gua). The hydrophilicity of GMP allows solubilizing the hydrophobic Gua, through the formation of G-quartets by non-covalent Hoogsteen bonds; the stacking of G-quartets leads to the formation of columnar helicoidal structures, the G-quadruplexes, that constitute the strands of the 3D network. The final hydrogel can swell up to a water content of 99% v/v. To modulate G-NG size and stability, we developed different production protocols and tested the effects of different salts. Structural properties have been characterized using DLS, AFM and confocal microscopy: the results show the morphological characteristics and stability as a function of temperature and ageing. We performed SAXS to understand both the inner structure of the G-NGs and the interactions among the particles. These preliminary results open the door to the use of a new supramolecular, self-assembling system that can be a very useful drug delivery system for specific molecules.

P-719
Single molecule experiments highlight the role of NBS1 and Xrs2 in DNA tethering by MRN and MRX
Carl Möller[1], Rajhans Sharma, Robin Öz, Giordano Reginato[2,3], Elda Cannavo[2,3], Sriram Kesarimangalam[1], Petr Cejka[2,3], Fredrik Westerlund[1]
[1] Department of Life Sciences, Chalmers University of Technology, Gothenburg, SE 41296, Sweden, [2] Institute for Research in Biomedicine, Universitá della Svizzera Italiana, Bellinzona, CH 6500, Switzerland, [3] Department of Biology, Institute of Biochemistry, Eidgenössische Technische Hochschule (ETH) Zürich, Switzerland

DNA double stranded breaks (DSBs) can be detrimental to the cell and need to be correctly repaired as efficiently as possible. The initial steps in repair of DSBs involves bringing the free ends in close proximity and one of the first responders to a DSB is MRE11-RAD51-NBS1(MRN), with a homologue in yeast, MR-Xrs2 (MRX). Here we report how MRN(X) interacts with DNA to promote annealing of single-stranded overhangs on individual DNA molecules using nanofluidic channels. Nanofluidics is particularly suitable to study reactions involving DNA ends since, in contrast to most existing single molecule techniques, no tethering of the DNA to a substrate is required. We make several important observations on the differences in interactions with DNA between MRN and MRX. We show that the DNA end-joining activity is dependent on NBS1 and Xrs2, which is in contrast to several other important functions of both MRN and MRX. Furthermore, we report that Xrs2 possesses significant DNA end-joining capabilities on its own, but not NBS1. We believe our findings provide insights into the scaffolding functions of the MRN(X) complex in processes involving DNA repair and genome integrity.

P-720
Coarse-grained molecular dynamics simulations of parental histone H3/H4 recycling
Fritz NAGAE[1], Shoji TAKADA[1], Tsuyoshi TERAKAWA[1]
[1] Department of Biophysics, Graduate School of Science, Kyoto University, Japan

In eukaryotic DNA replication, a nucleosome is dismantled ahead of the replication fork, and histones are recycled to replicated DNA strands to preserve the epigenetic information. Previous studies have shown that a replicative helicase, Mcm2-7, associates with histone H3/H4, and this association plays a pivotal role in histone recycling. Also, strand bias, in which histones are mostly recycled to one of the two replicated strands, causes asymmetric epigenetic inheritance important for cell differentiation. However, the molecular pathway of the H3/H4 deposition to the replicated strands and the decisive protein factors for the strand bias have been elusive. Here, we performed coarse-grained molecular dynamics simulations of a Y-forked DNA engaged Mcm2-7 bound to H3/H4 and observed that the Mcm2 N-terminal tail deposited the H3/H4 onto the replicated strands in favor of the lagging strand over the leading strand. Next, we added Cdc45 and GINS to the simulations, unexpectedly observing the association between Cdc45 and H3/H4 and the leading strand bias promoted by this association. Also, we found that Pol ε or RPA modulated the strand bias. Together, these simulations provided the experimentally testable hypothesis on the molecular mechanism of histone recycling and its strand bias.

Poster Presentations
– Session 3 –

P-721
Liposome-based microfluidic platform for standardized analysis of antimicrobial peptides
Janina Nandy[1], Christian Nehls[1]
[1] Research Center Borstel - Leibniz Lung Center, Borstel, Germany

Antimicrobial resistance to antibiotics is increasing dramatically worldwide and threatens to become a major public health crisis in the coming years. A promising alternative to conventional antibiotics is antimicrobial peptides (AMPs). A set of synthetic AMPs was developed at the Research Center Borstel using a genetic algorithm and optimized against different bacterial strains. In this project, the membrane-lytic activity of the top- ranked AMPs is investigated in a high-throughput liposome-based microfluidic platform. Liposomes resembling the phospholipid bilayer of bacteria are prepared by octanol-assisted liposome assembly [1]. The platform enables the controlled production and immobilization of liposomes, as well as real-time observation of AMP-liposome interactions by a dye-release assay using fluorescence microscopy. A bacterial lipid composition of different complexity is used to advance the accuracy of the model system to closely mimic the natural lipid envelope. In addition, the microfluidic setup is optimized, for example, to separate residual octanol from the vesicles. Overall, the optimization of both the bacterial model system and microfluidic set up will help us in more accurate and reliable screening of antimicrobial peptides and improve our comprehension of the mechanism of action of AMPs. The comparison of the membrane-lytic activity with microbiological measurements will facilitate the prediction of antimicrobial activity of AMPs.

P-722
Peptide dynamics in membrane mimics resolved by combining NMR experiments and MD simulations
Ricky Nencini[1], Samuli Ollila[1], Morgan Regnier[1], Sofia Backlund[1], Efstathia Mantzari[1], Cory Dunn[1]
[1] Institute of Biotechnology, University of Helsinki, Helsinki, Finland

NMR spin relaxation times provide atomistic resolution information on the dynamics of peptides and other biomolecules, yet their interpretation is tedious for other than globular proteins. On the other hand, molecular dynamics (MD) simulations provide detailed models for biomolecules with a high spatiotemporal resolution, but their accuracy is often compromised by the accuracy of simulation parameters. Here we show how peptide dynamics in membrane mimics can be resolved by directly combining NMR experiments and MD simulations. We were able to reproduce experimental spin relaxation times for six different types of peptides (peripheral, transmembrane and mitochondria tail anchors) in membrane mimicking micelle environment using MD simulations. These MD simulations were then used to give a detailed interpretation of the complex dynamics of peptides in membrane mimicking environment. Our results pave the way for a combined approach of NMR experiments and MD simulations to understand complex peptide and protein dynamics in membrane environments.

P-723
Biophysical models elucidate neuronal dynamics in C. elegans.
Martina Nicoletti[1,2], Alessandro Loppini[1], Letizia Chiodo[1], Viola Folli[2], Giancarlo Ruocco[2], Simonetta Filippi[1]
[1] Campus Bio-Medico University, Rome, Italy, [2] Center for Life Nano & Neuro Science, Italian Institute of Technology, Rome, Italy

Elucidating the biological mechanisms underlying the functioning of a nervous system is an open challenge in neuroscience and requires the comprehension of the brain at different scales ranging from the single-cell to the network level. The C.elegans brain has been fully mapped in terms of neurons and connections. However, the physiological bases of single-neuron functioning are still poorly explored despite the recent progress of C. elegans electrophysiology. In this work, we propose a framework for biophysically accurate modeling of the C.elegans neurons that could help elucidate novel mechanisms in the neuronal dynamics of the nematode and provide a guide for electrophysiological recordings. Our models are based on the Hodgkin-Huxley model adapted to reproduce the characteristic neuronal responses of sensory, motor, and interneurons observed in the nematode. Overall, we modeled a set of 15 ionic currents and combined them based on the gene expression data to reproduce the physiological behavior of different classes of neurons. Moreover, for each neuron, we study in detail the role of the different ionic currents in membrane voltage dynamics. Among all the results, our analysis identified T-Type calcium channels as the best candidates to explain the bistability observed in RMD motor neurons. In conclusion, our work provides a framework to be integrated with experimental studies to elucidate the fundamental principles of C. elegans brain functioning.

P-724
Supported Lipid Bilayer Platform for Characterization of Lipid Nanoparticle-Based Endosomal Escape Mechanism
Simon Niederkofler[1], Petteri Parkkila[1], Elin Esbjörner Winters[1], Fredrik Höök[1]
[1] Chalmers University of Technology, Sweden

Major advancements regarding effective RNA therapeutics have been made in recent years, enabling cellular RNA delivery with sufficient efficacy to pass the criteria for therapeutic use. Nevertheless, the delivery efficiency of current RNA transport vehicles is low, a few percent at best, and further improvement is required for impact beyond vaccines. In particular, a better understanding of biophysical mechanisms affecting RNA delivery is crucial for the design of improved therapeutics. Using RNA-loaded lipid nanoparticles (LNPs), we are working on elucidating the escape mechanisms of the RNA from the endosomal compartment into the cytosol, commonly referred to as endosomal escape. To achieve this, we investigate the interaction of LNPs with a supported lipid bilayer (SLB) designed to mimic the endosomal lipid membrane. The results obtained suggest a pH-induced LNP-SLB fusion as the major mechanism for the endosomal escape event. Furthermore, fluorescent labeling of LNPs allows single-particle quantification of, e.g., the propensity to undergo such fusion, dependence on pH, lag-times between pH change and fusion, and change in fluorescence signal of labeled compounds upon fusion. Further, interaction between LNPs and serum-proteins is inevitable upon in-vivo administration and leads to the formation of a so-called protein corona, which is in turn expected to influence the nature of LNP uptake and endosomal escape. We therefore use the experimental setup to quantify effects of the protein coronation on the endosomal escape. We believe that this methodological approach could facilitate the development of nanoparticle-based drug delivery strategies by giving in depth mechanistic insights on biophysical mechanisms.

Poster Presentations
– Session 3 –

P-725
Molecular mechanism of lipid droplet biogenesis
Vincent Nieto[1], Jackson Crowley[1], Denys Santos[2], Luca Monticelli[1]
[1] University of Lyon, CNRS, Molecular Microbiology and Structural Biochemistry (MMSB, UMR 5086), F-69007, Lyon, France, [2] Federal University of Pernambuco, Department of Fundamental Chemistry, Recife, Brazil

Lipid droplets (LDs) are organelles that regulate energy and lipid metabolism within cells. LDs formation primarily occurs in the tubular areas of the endoplasmic reticulum (ER), where neutral lipids like triglycerides accumulate in lens-shaped blisters. Once the nascent LD grows beyond a specific threshold, it buds from the ER membrane, towards the cytosol. However, the budding mechanism has never been experimentally observed. Here, we developed a new, reliable computational methodology that simulates the initial steps in LD biogenesis, from LD nucleation to budding. Simulations revealed that LDs require a certain degree of asymmetry between the two membrane leaflets to bud, regardless of membrane morphology. Seipin, an essential protein for proper LD function, promotes an asymmetric shape of nascent LDs but is not sufficient to promote budding on its own. Instead, seipin increases the mechanical stability of the LD-tubule connection. Additionally, simulations highlighted the crucial role of the ER's oil/phospholipid synthesis ratio in preserving the mechanical stability of the network and ensuring a stable LD-tubule connection. This novel computational methodology will enable simulations of more complex membrane system transformations. This study sheds light on the early stages of LD biogenesis and provides insights into the factors that contribute to this process.

P-726
Harnessing Extremely Disordered Chromatin Remodeling Proteins with Single-Molecule Spectroscopy
Kateryna Nitsenko[1], Pétur Heiðarsson[1]
[1] University of Iceland, Iceland

High-mobility group (HMG) proteins are a superfamily of intrinsically disordered architectural transcription factors that are associated with type 2 diabetes, nucleotide excision repair inhibition and malignant tumors, making them attractive drug targets. HMGA proteins have been suggested to participate in differentiation processes and facilitate chromatin remodeling through the eviction of linker histone H1 from nucleosomes. Here we employed single-molecule Förster resonance energy transfer (smFRET) to study the kinetic mechanism of HMGA1 and H1 competitive nucleosome binding. We reconstituted 197 bp fluorescently labelled nucleosomes based on the strongly positioning 601 Widom sequence. We then measured transfer efficiency histograms for thousands of individual freely diffusing nucleosomes under various conditions. Histone H1 binding leads to a change in the interdye distance due to charge neutralization of the DNA backbone that leads to a high-FRET “closed” state. Addition of HMGA1 reverted the nucleosome back to a low-FRET “open” state in a concentration dependent manner. At near-physiological salt concentrations this competitive substitution occurred on very slow timescales, presumably due to the long residence times of H1 on the nucleosome. We are currently undertaking kinetic experiments to elucidate the full kinetic network which will ultimately build a strong platform for understanding HMGA1 functions in molecular detail.

P-727
The Filamentous Growth of Fungal Killers: A Combined Mathematics and Lab Approach
Sophie Nye[1], Callum Parkin[1], Alexandra Brand[1], David Richards[1]
[1] University of Exeter, United Kingdom

A combination of in vitro experiments, image and data analysis, and mathematical modelling, has been used to better understand the dynamics governing calcium signalling and the adaptation of Candida albicans (an invasive filamentous fungus). Despite filamentous fungi causing over 1.5 million life-threatening infections in humans per year, their growth mechanisms are poorly understood, and treatment options are limited, with these fungi being highly adaptable and resistant to drugs. Calcium signalling regulates many different processes in C. albicans, including filamentous growth, virulence, and is vital for drug resistance. Experimental work has included observing calcium spiking in C. albicans mutants after exposure to various treatments. The analysis of these signals has revealed information about the calcium channels and how well the different mutants adapt. The Hodgkin Huxley model (or equivalent circuit model) is a well established model that was originally used to describe action potential propagation in neurons, by equating cell components to electric circuit components, and has been applied to pancreatic beta cells, plant cells, and has been applied here to describe calcium signalling in C. albicans. It has been fitted to experimental data, and used to predict behaviours of calcium signalling following stressors.

P-728
NMX Macromolecular Diffractometer at the European Spallation Source
Esko Oksanen[1,2], Giuesppe Aprigliano[1], Rosa Camilleri Lledó[1], Dorothea Pfeiffer[1,3], Jerome Samarati[1,3], Gergely Nagy[1,4], Marton Marko[4], Justin Bergmann[1,2]
[1] European Spallation Source ESS ERIC, Lund, Sweden [2] Lund University, Lund, [3] European Organisation for Nuclear Research (CERN), Geneva, [4] Centre for Energy Research (EK-CER), Budapest

The NMX Macromolecular Diffractometer is optimised for small samples and large unit cells dedicated to the structure determination of biological macromolecules by crystallography. Neutron macromolecular crystallography is mainly driven by its ability to locate hydrogen atoms in biological macromolecules. NMX is a macromolecular diffractometer that uses the time-of-flight (TOF) quasi-Laue technique. The typical wavelength band is 1.8-3.55 Å, but wavelengths up to 10 Å are available. The collimation system tailors the beam size and divergence to the needs of the experiment. The sample is mounted on a six-axis robotic arm that allows the orientation to be optimised. The detectors are also mounted on robotic arms, which allows their positions to be optimised for the experiment. This allows data collection from crystals with unit cells up to 300 Å or more. The detector technology is based on gas-electron multipliers (GEMs) with Gd as a neutron converter. This combines large detector area with high spatial resolution and time-of-flight capability.

Poster Presentations
– Session 3 –

P-729
Mechanism of Hsp70 activation: how J-proteins push for hydrolysis
Michał Olewniczak[1], Marcin Pitek[2], Łukasz Nierzwicki[1], Jacek Czub[1], Bartłomiej Tomiczek[2]
[1] Gdańsk University of Technology, Poland, [2] Intercollegiate Faculty of Biotechnology, University of Gdansk and Medical University of Gdansk, Poland

Hsp70 are chaperones that play a central role in maintaining protein homeostasis. The catalytic cycle of Hsp70 consists of subsequent opening and closing of its substrate binding domain that is allosterically coupled to the occupancy of the nucleotide binding domain. This cycle is strictly controlled by co-chaperone J-domain proteins which stimulate the ATPase activity of Hsp70. Although Hsp70 are one of the most extensively studied allosteric proteins, the exact mechanism of their activation by JDPs is still missing. Here, we used MD simulations combined with dynamic network analysis and free energy methods to clarify the mechanism of Hsp70 activation by JDPs on the example of DnaK and DnaJ, an Hsp70/JDP pair from E. coli. We show that the binding of the DnaJ J-domain results in the rearrangement of the nucleotide binding pocket of DnaK into a stimulated state. With network analysis, we further reveal that the allosteric signal for this rearrangement is initiated by the DnaJ helix II and is transmitted to the binding pocket by the specific beta-strand of NBD. Our results also show that the conserved HPD motif of JDPs ensures the tight binding of J-domain, crucial to the stimulation of the ATPase activity.

P-730
Evaluating druggability by means of coarse-grained molecular dynamics simulations
Philipp Orekhov[1]
[1] Shenzhen MSU-BIT University, China

Both protein-protein and protein-ligand complexes are characterized by the presence of so-called hotspots, i.e., interaction sites of binding partners that make the greatest contribution to the binding free energy and play a critical role in the formation of tight interaction. Accurate and efficient assessment of binding hotspots at protein-protein and protein-ligand interfaces is an extremely important step at the early stages of drug design campaigns particularly those striving for new PPI inhibitors. Here, we present an efficient computational approach for the identification of interaction hotspots, prediction of binding sites for PPI inhibitors, and estimation of druggability by means of coarse-grained molecular dynamics simulations using the popular MARTINI force field. The approach is aimed to overcome some limitations of existing methodologies, including significant speed up (in comparison to all-atom simulations) and more efficient exploration of the chemical space of potential ligands. Moreover, since the ELNEDIN protein model in MARTINI does not account for protein flexibility limiting thorough exploration of potential binding sites, we also propose a scheme for simulations of multiple protein conformations within the MARTINI framework. The project is supported by the National Natural Science Foundation of China, grant #32250410316.

P-731
Design and development of functional amyloid-based hydrogels as a versatile drug-delivering depot
Komal Patel[1], Namrata Singh[1], Pradeep Kadu[1], Radni Deshpande[2], Manisha Poudyal[1], Debdeep Chatterjee[1], Surabhi Mehra[1], Ambuja Navalkar[1], Sadhana Sathaye[2], Samir K. Maji[1]
[1] Indian Institute of Technology Bombay, [2] Institute of Chemical Technology Mumbai, India

Small molecular drugs and drugs of peptide/protein origin have low oral or transdermal bioavailability with a short in vivo half-life. Such drugs require delivery by infusion, frequent injections, or subcutaneous administrations, demanding a need to develop robust, tunable, scalable, and biocompatible scaffolds for drug delivery applications. In this regard self-assembly of designed peptides can be a versatile strategy to generate functional materials for drug delivery. Since amyloid fibrils of proteins/peptides are highly stable against extreme physical and biological conditions such as extreme pH, proteases, and temperature, we propose that non-toxic amyloid fibrils can be used for developing hydrogel for drug delivery applications. We designed amyloid hydrogels based on functional amyloid sequences, which can encapsulate several hydrophobic and hydrophilic protein/drug molecules and release them in a controlled manner exhibiting their biological activity. For example, when the insulin- encapsulated hydrogel was injected subcutaneously in the diabetic rat model, the released insulin showed a drop in the blood glucose level. Owing to the properties of the hydrogel that render tuneable physical properties, controlled degradability, and capability to protect the labile payload from degradation, amyloid hydrogels can serve as a versatile drug-delivering depot agent for various protein/peptide/drugs and small molecules.

P-732
Backmapping Coarse-grained Molecules to Atomistic Resolution via Chemistry-informed Machine Learning
Christian Pfaendner[1,2], Viktoria Korn[1], Benjamin Unger[1,2], Kristyna Pluhackova[1,2]
[1] Stuttgart Center for Simulation Science - University of Stuttgart, Germany [2] Artificial Intelligence Software Academy - University of Stuttgart

Molecular dynamics (MD) simulations offer invaluable insights to complement experimental methods. MD not only provides unmatched spatial resolution, but also allows us to study the dynamics of systems in small time steps. However, due to the high computational demand of atomistic MD simulations the system sizes and simulation times are still limited to the nanometer and microsecond time scales. Sequential multiscale MD simulations offer a promising alternative by combining the increased sampling of coarse-grained simulations with the accuracy of atomistic simulations. This is achieved by seamlessly switching between the two resolutions. While coarse-graining is straightforward, the reverse transformation from low to high resolution, also termed backmapping, is a non-trivial task. State-of-the-art algorithms may yield incorrect stereochemistry, fail to recover specific protein secondary structure elements like beta sheets, and neglect the Boltzmann distribution. To address these limitations, we currently developing a novel chemistry-informed machine learning framework for backmapping that is based on the Boltzmann distributions of structural features and learns the correct stereochemistry from atomistic data. In addition, its models are pre-trained to cover the most common biological systems and its generic structure minimizes the need for extensive user input.

Poster Presentations
– Session 3 –

P-733
OPTOCAPACITIVE CURRENTS ELICITED BY PHOTOLIPID EXCITATION

Juergen Pfeffermann[1], Rohit Yadav[1], Carlos A. Z. Bassetto Jr[2], Toma Glasnov[3], Francisco Bezanilla[2,4], Peter Pohl[1]
[1] Institute of Biophysics, Johannes Kepler University Linz, Linz, Austria, [2] Dept. of Biochemistry and Molecular Biology, University of Chicago, Chicago, USA, [3] Karl-Franzens-University, Graz, Austria, [4] Centro Interdisciplinario de Neurociencias, Universidad de Valparaiso, Chile

Sudden changes in membrane cation permeability elicit action potentials in excitable cells. Light exposure may trigger them if the plasma membrane harbors light-sensitive ion channels (channelrhodopsins). Yet, fast jumps in membrane capacitance may render such channels superfluous, as experiments with photothermally active membrane-bound nanoparticles have shown. We now propose a further simplification of the approach. It does not require channelrhodopsins or light-absorbing nanoparticles. Instead, we use photolipids with acyl chains that contain azobenzene moieties. Their light-triggered reversible photoisomerization is responsible for the change in membrane capacitance. As shown by our experiments on planar bilayers, these capacitance changes are the source of optocapacitive current. The magnitude of the current spikes depends on the light intensity, photolipid concentration, and membrane compressibility. We observe similar optocapacitive currents in cells containing the photolipids in their plasma membranes. These cells exhibit light-induced action potentials in the absence of channelrhodopsins. Our observation paves the way for the use of photolipids for neuronal stimulation.

P-734
Viscosity prediction of dense monoclonal antibody solutions from atomistic simulations

Tobias Prass[1], Michaela Blech[2], Patrick Garidel[2], Lars Schäfer[1]
[1] Center for Theoretical Chemistry, Faculty of Chemistry and Biochemistry, Ruhr-University Bochum, Bochum, Germany, [2] Boehringer Ingelheim Pharma GmbH & Co. KG, Corporate Division Biopharmaceuticals, Process Science, Protein Science, Biberach an der Riss, Germany

The viscosity of highly concentrated protein solutions depends on molecular interactions that are challenging to probe and fine-tune experimentally. We use multi-microsecond atomistic molecular dynamics (MD) simulations with explicit solvent to predict the dynamic viscosity of high-concentration solutions of a monoclonal antibody from the pressure fluctuations. The simulations yield lower viscosity values than those obtained from rheology experiments, but they capture the relative increase in viscosity from 200 mg/ml to 250 mg/ml protein concentration. This work demonstrates the potential and the limitations of atomistic simulations for predicting the rheological properties of dense solutions of large and flexible multi-domain proteins.

P-735
Uncovering the Mechanical Properties and Folding of Single-Stranded RNA using Optical Tweezers: A Study on Influenza A Viral ssRNA Genomes

Carlos R. Pulido[1], Xavier Viader-Godoy[2], María Manosas[3], Jaime-Martín Benito[4], Felix Ritort[3], Rebeca Bocanegra[1], Borja Ibarra[1]
[1] Instituto Madrileño de Estudios Avanzados (IMDEA) Nanociencia, [2] Universitá degli Studi di Padova, [3] Universitat de Barcelona (UB), [4] Centro Nacional de Biotecnología (CNB-CSIC), Spain

The structures and functions of nucleic acids are closely related to their mechanical properties. Despite the multiple biological roles of single- stranded RNA (ssRNA), the mechanical features and the factors that determine the folding of naturally occurring ssRNA molecules are poorly understood. We aimed to fill this knowledge gap by pulling mechanically on individual, biologically active ssRNA molecules using optical tweezers. We measured the average extensions of Influenza A viral ssRNA genomes as a function of the applied force and the ionic composition to extract information about their elastic properties and quantify the amount of secondary structure. We quantified the basic parameters that define the mechanical properties of ssRNA and introduced a theoretical model that sheds light on the interplay between base pairing, base stacking and electrostatic interactions in regulating the folding of ssRNAs.

P-736
How to use advanced microscopy to study peroxisomes – a multimodal approach from FCS, photoactivation and STED to Minflux

Katharina Reglinski[1,2], Maurice Faletta[1], Dorottya-Zsofia Koppenhagen[1], Delgir Zakinova[1], Silvia Galiani[3], Pablo Carravilla[1,2], Wolfgang Schliebs[4], Ralf Erdmann[4], Christian Eggeling[1,2,3]
[1] Institute of Applied Optic and Biophysics, Friedrich-Schiller University Jena, Max-Wien-Platz 1, 07743 Jena, Germany, [2] Leibniz-Institute of Photonic Technologies, Albert-Einstein Strasse 9, 07745 Jena, Germany, [3] MRC Human Immunology Unit and Wolfson Imaging Centre, MRC Weatherall Institute of Molecular Medicine, University of Oxford, Headley Way, Oxford, OX3 9DS, United Kingdom , [4] Institute of Physiological Chemistry, Systemic Biochemistry, Ruhr-University Bochum, Universitätsstraße 150, 44801 Bochum, Germany

Peroxisomes are small (100-300 nm) intracellular organelles that fulfil many anabolic and catabolic functions. Mistargeting of peroxisomal proteins, which are imported from the cytosol, therefore leads to severe diseases making this organelle an object of research of utmost importance for medical applications. The import of proteins is mediated in a by now unknown manner through a transient translocation pore which assembles either in a way similar to pore forming toxins or through a liquid/liquid phase separated matrix. We here aim at adding knowledge about peroxisomes using a toolbox of advanced (super-resolution) microscopy methods. We use STED and Minflux super-resolution microscopy and spectroscopy to study the distribution of peroxisomal proteins involved in protein translocation on the peroxisomal membrane as well as we studied the diffusion dynamics of the peroxisomal import receptor and its cargo proteins in the cytosol of cells. Further, we employ a photoactivatable protein to induce and quantify peroxisomal import as well as we study peroxisomal movements in living cells through fast time-lapse imaging and sophisticated image analysis.

Poster Presentations
– Session 3 –

P-737
Edible alginate-based films as natural anti-SARS-CoV-2 barriers

Anna Reichel[1], Miguel A. Cerqueira[2], Ana Catarina C.O. Leite[2], Ana L. Tomás[1], Patrícia M. Silva[1], Nuno C. Santos[1], Michele Michelin[3,4], Pablo Fuciños[2], Lorenzo M. Pastrana[2]
[1] Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, 1649-028, Lisbon, Portugal, [2] International Iberian Nanotechnology Laboratory, Av. Mestre José Veiga, 4715-330, Braga, Portugal, [3] Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057, Braga, Portugal, [4] LABBELS – Associate Laboratory, Braga, Guimarães, Portugal

SARS-CoV-2 viability on food surfaces and its potential propagation through the food chain can pose a significant public health threat and bring new challenges to the food system. This study presents evidence that edible films can effectively combat SARS-CoV-2. We evaluated sodium alginate-based films containing gallic acid (GA), geraniol (G), and green tea extract (GTE) for their antiviral activity against a SARS-CoV-2 clinical isolate by quantifying the viral load with plaque assays. Our results demonstrate a strong in vitro antiviral activity against SARS-CoV-2 showing more than 3-log reductions in viral titers with all tested films. Dose-response analyses showed that for GA films, a higher concentration of the active compound (1.25 %) was required to achieve similar results compared to the lower concentrations of G and GTE (0.313 %). Moreover, we evaluated the critical concentration of active compounds in the films for their stability during storage by performing shelf-life experiments. These revealed that GA-loaded films lost their activity after 2 weeks of storage, whereas films with G and GTE showed a decrease in activity only after 4 weeks. Our results indicate that these edible films and coatings can be used as antiviral materials on food surfaces or on materials in contact with food, thereby reducing the spread of viruses through the food chain.

P-738
A transverse flow imaging approach to study torsional dynamics during DNA replication

Thomas Retzer[1,2], Karl Duderstadt[1,2]
[1] Max Planck Institute of Biochemistry, Martinsried, Germany, [2] Physik Department, Technische Universität München, Garching, Germany

The double helical structure of DNA poses a topological challenge during DNA replication. Parental DNA must be unwound to synthesize the daughter strands, creating overwind DNA ahead that acts as a barrier if left unresolved. Topoisomerases, a class of enzymes that manage DNA topology, can only resolve the accumulating twist to some extent which suggests the replisome has intrinsic mechanisms for coping with topological stress. We developed a modular toolkit of DNA building blocks to mimic different topological scenarios in reconstituted DNA replication assays imaged with a single-molecule fluorescence microscope. We find that topological barriers ahead of the replication fork lead to pausing and a reduction in replication rate. To further evaluate these dynamics, we developed a transverse flow imaging approach in which the parental, leading, and lagging strands are spatially resolved during ongoing synthesis. Our observations hint at a dynamic exchange of topological tension by rotation when topological challenges are encountered. Our experimental approach can easily be adapted for different replication systems to decipher the influence of topological stress.

P-739
TDP-43 structure and interactions

Caterina Ricci[1], Valentina Bonetto[2], Alfredo Cagnotto[2], Mario Salmona[2]
[1] BioMeTra, UNIMI, Milan, Italy, [2] Mario Negri Institute for Pharmacological Research, Milan, Italy

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease affecting motor neurons located in the frontal cortex, brainstem and spinal cord, with a wide genetic and clinical heterogeneity. However, up to 97% of ALS cases present the cytoplasmic aggregation of TAR DNA-binding protein 43 (TDP-43) in degenerating motor neurons, suggesting that TDP-43 is an hallmark of the disease with a pivotal role in ALS. TDP-43 is a 43 kDa protein that plays a crucial role in RNA processing by regulating various aspects of mRNA stabilization, production, and splicing. While it is normally located in the nucleus, a hyperphosphorylated, ubiquitinated, and cleaved form of TDP-43 can aggregate in the cytoplasm, impairing cell physiology at different levels. The debate still exists as to whether the neurodegeneration results from a loss of function of nuclear TDP-43, a gain of toxic properties of aggregated TDP-43, or a combination of both. Despite its relatively small size, determining the structure of the full-length TDP -43 protein has proven to be a challenge, given the large portion of the protein with intrinsically disordered features. Therefore, it is crucial to determine the structure of TDP-43 in solution to understand its behaviour, both alone and in interaction with different partners that can influence its folding and localization. In this study, we investigated the structure of full-length TDP-43 proteins in an aqueous environment using Small Angle Scattering techniques and explored their interactions with various potential partners.

P-740
An Alternative to FRET: DNA-Protein Interactions with Ångström Resolution on a Confocal Microscope

Lars Richter[1], Alan M. Szalai[1], Giovanni Ferrari[1], Izabela Kamińska[1,2], Bosong Ji[1], Andrés Vera Goméz[1], Ingrid Tessmer[3], Philip Tinnefeld[1]
[1] Department Chemie and Center of Nanoscience (CeNS), Ludwig-Maximilians-Universität München, Butenandtstraße 5-13, 81377 München, Germany, [2] Institute of Physical Chemistry of the Polish Academy of Sciences, Kasprzaka 44/52, 01-224 Warsaw, Poland, [3] Rudolf Virchow Center, University of Würzburg, 97080 Würzburg, Germany

Single-molecule FRET is a powerful technique that has been pushing dynamic structural biology. Its application has been limited by its requirement for double labeling, a small dynamic range, as well as photobleaching and complex acceptor photophysics. We expanded graphene energy transfer, where the two-dimensional material acts as an unbleachable broadband energy transfer acceptor for studying DNA bending by bulges and enzymes, e.g. endonuclease IV. Furthermore, we visualized the dynamic behavior of the tumor-relevant MGMT alkyltransferase with Ångström spatial resolution. Compared to FRET, graphene energy transfer combined with an elegant immobilization strategy offers a longer distance range and a smoother d^(-4) distance dependence of the energy transfer. As no acceptor labeling is required, the method is simpler and yields more photon-efficient precision compared to FRET in several applications.

Poster Presentations
– Session 3 –

P-741
Dynamic second-harmonic imaging of divalent cation translocation through giant vesicle membranes
David Roesel[1,2], Maksim Eremchev[2], Chetan S. Poojari[3], Jochen S. Hub[3], Sylvie Roke[2]
[1] J. Heyrovsky Institute of Physical Chemistry, Czech Republic, [2] École Polytechnique Fédérale de Lausanne, [3] Saarland University

The interaction of divalent cations with lipid membranes is crucial for the structure and proper function of cell membranes. In order to understand molecular level mechanisms behind those interactions, the role of interfacial water cannot be omitted. A recent improvement in imaging throughput has resulted in the construction of a second harmonic (SH) imaging device that can non-resonantly and dynamically image interfacial water molecules. Here, we use this label-free approach to investigate the mechanism of divalent cation transport through lipid membranes using giant unilamellar vesicles (GUVs) in aqueous solution. By SH imaging interfacial water, we create spatiotemporal images from which structural heterogeneity, binding constants and translocation times can be retrieved. We observe binding heterogeneity, which implies that local chemical structure is relevant for ion transport. The comparison of surface potential values and translocation rates for several divalent ions (Cu2+, Ca2+, Ba2 +, and Mg2+) points towards a new ion permeation mechanism.

P-742
Conformational heterogeneity and structural dynamics of biomolecules in vivo with cell injection and smFRET
Florian Rost[1,2], Andreas Hartmann[1], Michael Schlierf[1,2,3]
[1] B CUBE - Center for Molecular Bioengineering, TU Dresden, Germany [2] Fakultät Physik TU Dresden, [3] Cluster of Excellence, Physics of Life, TU Dresden

Single-molecule Förster Resonance Energy Transfer (smFRET) has revolutionized the investigation of the conformational heterogeneity and structural dynamics of biomolecules in vitro. However, all in vitro experiments a lack highly crowded, heterogeneous environment as found in a cellular context. To address this challenge, while maintaining the ability to sample conformational dynamics on the singlemolecule level, microinjection of a limited number of fluorescent molecules in cells has been developed. In particular, using microinjection allows to deliver FRET labeled molecules to the cytosol, or even organelles, e.g. the nucleus, and monitor conformational states and dynamics. In combination with single- molecule tracking individual molecules can be tracked over seconds. We developed a highly automated injection setup with differential interference contrast (DIC) imaging and determine conformational heterogeneity and dynamics of biomolecules using HILO illumination smFRET microscopy. We anticipate that a thorough comparison of conformational dynamics in vivo and in vitro will yield new insights into biological complexity.

P-743
HIV-1 Capsid Elasticity and Nuclear Entry: A Novel Perspective on Infection
Itay Rousso[1], Akshay Deshpande[1], Christopher Aiken[2]
[1] Ben-Gurion University of the Negev, Department of Physiology and Cell Biology, Beer Sheva, Israel, [2] Vanderbilt University Medical Center, Department of Pathology, Microbiology and Immunology, Nashville, TN, USA

HIV-1 infection requires passage of the reverse transcribing viral core through the nuclear pore of the cell, a process that depends on properties of the viral capsid. Recent studies have suggested that HIV-1 cores can pass through the nuclear pore without prior disassembly of the viral capsid. Interactions with the nuclear pore complex are apparently necessary but not sufficient for nuclear entry, as substitutions in regions of the CA protein that are distinct from known protein binding sites can result in impaired nuclear entry. Here we show that the HIV 1 core is highly elastic and that this feature is linked to nuclear entry and infectivity. Using a novel atomic force microscopy-based approach, we found that purified wild type cores return to their normal conical morphology following a severe forced compression. By contrast, cores from two nuclear entry-defective CA mutants underwent breakage upon compression. A suppressor mutation restored elasticity and infectivity to one of these mutants. Core elasticity was reduced by addition of concentrations of the capsid-binding antiviral drugs Lenacapavir and PF74 that inhibit nuclear entry. Our results indicate that capsid elasticity is a fundamental property of the HIV-1 core that enables its passage through the nuclear pore complex, thereby facilitating infection. These results provide new insights into the mechanisms of HIV-1 nuclear entry and the antiviral mechanisms of HIV-1 capsid inhibitors.

P-744
Zn acts as a switch for the regulation of phase separation of SOD1
Sumangal Roychowdhury[1], Bidisha Das[1], Priyesh Mohanty[2], Azamat Rizuan[2], Joy Chakraborty[1], Jeetain Mittal[2], Krishnananda Chattopadhyay[1]
[1] CSIR-Indian Institute of Chemical Biology, India [2] Texas A&M University

Misfolding and/or aggregation of Cu/Zn superoxide dismutase (SOD1) is associated with the neurodegenerative disease - Amyotrophic lateral sclerosis (ALS). SOD1 is also associated with stress granules (SGs) which are a type of membraneless organelle believed to form via liquid-liquid phase separation (LLPS) of several proteins containing low-complexity, disordered regions. Using a combination of biophysical experiments and computational methods we report here that structural disorder in two loop regions of SOD1 induced by the absence of metal cofactor - Zn, triggers its LLPS. The phase-separated droplets give rise to aggregates which eventually form toxic amyloids upon prolonged incubation. The addition of exogenous Zn to immature, metal-free SOD1 and the severe ALS mutant - I113T, stabilized the loops and restored the folded structure, resulting inhibition of LLPS and subsequent aggregation. In contrast, the Zn-induced inhibition of LLPS and aggregation was found to be partial in the case of another severe ALS-associated mutant - G85R which has reduced Zn binding propensity. Moreover, a less-severe ALS mutant - G37R with perturbed Cu binding does not undergo LLPS. In conclusion, our work establishes a role for Zn-dependent modulation of SOD1 disorder and LLPS as a precursor phenomenon that may lead to the formation of toxic amyloids associated with ALS.

Poster Presentations
– Session 3 –

P-745
Pilot standardized database for biophysical data
Juan Sabin[1], Jan Stransky[2], Agerschou Emil Dandanell[2], Mark Williams[3], Jan Dohnalek[2]
[1] Software 4 Science Developments S.L.- AFFINImeter Santiago de Compostela, Spain, [2] Institute of Biotechnology of the Czech Academy of Sciences, Vestec near Prague, Czech Republic, [3] ISMB-BIOSPHYSX Centre, Institute of Molecular and Structural Biology, Birkbeck, University of London, London, UK

The field of molecular-scale biophysics suffers from the lack of standardized data formats for the many different experimental techniques that may be applied to study biological systems. MOSBRI (MOlecular-Scale Biophysics Research Infrastructure) project has launched an international survey to understand in detail the needs of the biophysics community in terms of data standardization in molecular biophysics. This knowledge is guiding the efforts for the provision of new data standards for biophysical data exchange and archiving, to ensure interoperability of information obtained by different biophysical methods and with other bioinformatics research infrastructures producing and processing biological/biomolecular data. The final goal of MOSBRI in the area of data management is the implementation of a pilot standardized database of biophysical data under the FAIR principles (Findability, Accessibility, Interoperability and Reusability). For this purpose, Surface Plasmon Resonance (SPR), Bio-Layer Interferometry (BLI), and Micro-Scale Thermophoresis (MST) have been prioritized for their high need for data standards and repository, and for the current high number of users among the biophysical community.

P-746
With of without disorder, why investigate the dynamics at protein-protein interfaces?
Sophie Sacquin-Mora[1]
[1] CNRS, France

The modeling of protein assemblies at the atomic level remains a central issue in structural biology, as protein interactions play a key role in numerous cellular processes. For globular proteins, this problem is traditionally addressed using docking tools, where the quality of the models is based on their similarity to a single reference experimental structure. However, using a static reference does not take into account the dynamic quality of the protein interface. Using all-atom classical Molecular Dynamics simulations, we investigate the stability of the reference interface, and of models of various accuracies, for three complexes that previously served as targets in the CAPRI competition. To assess the quality of the models from a dynamic perspective, we set up new criteria which take into account the stability of the reference experimental protein interface. We show that, when the protein interfaces are allowed to evolve along time, the original ranking based on the static CAPRI criteria no longer holds. For IDPs and proteins comprising disordered fragment the issue of dynamics is even more crucial as the protein-protein interface is likely to undergo important conformational changes along time. Here we present the case of the fuzzy complex formed by tubulin and the tau IDP. In particular we highlight the part play by the disordered tubulin C-terminal tails in stabilizing the assembly.

P-747
Structural dynamics of inner-ear cadherin-23 and protocadherin-15 ectodomains visualized by high-speed atomic force microscopy
Prithwidip Saha[1], Claire Valotteau[1], Elakkiya Tamilselvan[2], Daisy Alvarado[2], Collin Nisler[2], Vincent Lynch[3], Marcos Sotomayor[2], Felix Rico[1]
[1] Aix-Marseille Université, CNRS, INSERM, LAI, Turing Centre for Living Systems, 13009 Marseille, France, [2] Department of Chemistry and Biochemistry, The Ohio State University, Columbus, OH, 43210, USA, [3] Department of Biological Sciences, University at Buffalo, SUNY, Buffalo, USA

A fundamental biological function in vertebrates is hearing, which relies on mechanotransduction, a process in which mechanical signals from sound are converted into electrochemical signals. Cadherin-23 (CDH23) and protocadherin-15 (PCDH15), non-classical members of the cadherin protein family, are directly involved in this process that occurs in the sensory hair cells of the inner ear. A pair of CDH23 binds a pair of PCDH15 to form a fine "tip-link" filament that initiates sensory perception by conveying tension to inner-ear mechanotransduction channels. High-resolution structural models of monomeric ectodomains of both proteins have been established using x-ray crystallography and molecular dynamics simulations. However, real-time observation of the structural dynamics of these individual proteins and the complex at the single molecule level is yet to be accomplished. Here, we use high-speed atomic force microscopy imaging to visualize the full length ectodomains of mouse CDH23 and PCDH15 on mica at nanometer spatial and sub-second temporal resolutions. In addition, their conformational dynamics are studied at different Ca2+ concentrations and in the presence of a chelating agent to better understand the role of Ca2+ in maintaining the ectodomains linker regions' integrity. Under physiologically relevant conditions, CDH23 shows a relatively flexible and curved ectodomain structure. Occurrences of parallel cis and antiparallel trans homo-dimerization are also evident in a few cases. In contrast, PCDH15 exhibits a stable and rigid structure with transient splitting into two strands, supporting the hypothesis of parallel cis homo-dimerization.

P-748
A Multi-Scale Modelling of an Enzymatic PET Degradation
Mehdi Sahihi[1], Pierre Fayon[1], Lionel Nauton[1], Patrice Malfreyt[1]
[1] Université Clermont Auvergne, CNRS, Clermont Auvergne INP, Institut de Chimie de Clermont-Ferrand, F-63000 Clermont-Ferrand, France

Poly(ethylene terephthalate), PET, is one of the most produced plastics worldwide. Computational modeling could be a valuable tool to investigate the complicated process of PET biodegradation by PETase enzyme. Here, we investigate the enzyme adsorption onto the PET surface using a comprehensive computational approach. The adsorption process is discussed by calculating the adsorption free energy and the enzyme structural changes using molecular simulations. Then, the interaction between a PET oligomer and the enzyme residues of the active pocket is investigated. Finally, we complete by a QM/MM calculation of the enzymatic mechanism. The MD simulation results indicate that PET-enzyme interaction energy is about -1102 kJ.mol-1. Analyzing the Rg, tilt angle, contact area, etc., revealed a slight loss of enzyme compactness during its adsorption. QM/MM calculations show that a reaction intermediate is obtained, which corresponds to the formation of the bond between the oxygen atom of the SER165 with the Carbon atom of PET. Moreover, an eABF calculation was performed, and the free energy curves along the reaction coordinates were obtained.

Poster Presentations
– Session 3 –

P-749
How sensitive and site-specific hydrogen peroxide signaling can be achieved in human cells: spatially localized redox relays

Armindo Salvador[1,2,3], Matthew Griffith[1,4], Adérito Araújo[5], Rui Travasso[6]
[1] Center for Neuroscience and Cell Biology - The University of Coimbra, Portugal [2] Coimbra Chemistry Center □ Institute of Molecular Sciences (CQC□ IMS), University of Coimbra, [3] Institute for Interdisciplinary Research, University of Coimbra, [4] Department of Mathematical Sciences, University of Bath, [5] CMUC, Department of Mathematics, University of Coimbra, [6] CFisUC, Department of Physics, University of Coimbra

H2O2 is a key signaling agent in important physiological processes such as cell proliferation, inflammation, and apoptosis. However, it achieves sensitive and specific regulation remains unclear. At the cytosol of human cells, H2O2 signaling is spatially localized due to high activity of the peroxiredoxin (Prdx)/thioredoxin (Trx) system. And it is partly mediated by redox relays whereby Prdx act as H2O2 receptors, being oxidized, and in turn oxidize other protein targets. We used reaction-diffusion models of the cytosolic Prdx1/2-Trx1 system to analyze how spatial localization contributes for signaling specificity and sensitivity. The results show: (i) Prdxs' disulfide and sulfenic forms and Trx1 disulfide are spatially localized, Prdx1 species being more so than Prdx2's. (ii) Scaffold proteins (ScP) recruiting both Prdx and regulation targets to H2O2 supply sites can greatly improve redox relays' H2O2 detection limits. (iii) The input dynamic range is mainly determined by the association constant between redox target and ScP, rather than by Prdxs' kinetic properties, allowing substantial target oxidation with little overall Prdx oxidation. (iv) Site- specific ScP allow H2O2 released to the cytosol at distinct sites to independently regulate distinct genes and processes.

P-750
Controlling the assembly of protein nanofibril hydrogels

Rodrigo Sanches Pires[1], Christofer Lendel[1]
[1] Division of Applied Physical Chemistry, Department of Chemistry, KTH Royal Institute of Technology, Sweden

Whey protein nanofibrils (PNFs) are non-pathogenic amyloid-like material building blocks originating from side-stream protein-rich isolates from the milk industry. At low pH and high temperature, whey protein isolate (WPI) forms PNFs with two distinctive morphologies: straight and curly. Acid-based hydrolysis is the rate-limiting step in WPI fibrillation. As the proteins get hydrolyzed, peptide-building-blocks are released into solution and assemble into PNFs. The protein-to-proton ratio determines hydrolysis' extension and what peptide populations are formed, explaining the morphological switch. Recently, we observed that seeding of WPI mixtures has a profound effect on PNF morphology, with seeds from straight PNFs being able to propagate their morphology even in conditions where the WPI/proton ratio should yield curly PNFs (high WPI concentration). Contrarily, seeds from curly PNFs are unable to propagate their morphology. Additionally, seeding with straight PNF seeds is accompanied by hydrogel formation, which can be processed into a variety of materials. We explore the effects of seeding in the propagation of morphology and gel formation. By combining micro equilibrium dialysis and extensive AFM statistical analysis, we determine that morphology propagation and hydrogel formation is explained by an increase in fibrillation efficiency and the formation of PNF populations with longer contour lengths. We demonstrate that morphology propagation is related to seed elongation, and that changing seed length affects PNF size polydispersity. This provides both PNF and material tunability for different applications.

P-751
Probing physical properties of single amyloid fibrils using nanofluidic channels

Nima Sasanian[1], Rajhans Sharma, Quentin Lubart, Sriram KK[1], Marziyeh Ghaeidamini[1], Kevin Dorfman[2], Elin Esbjörner[1], Fredrik Westerlund[1] [1] Chalmers University of Technology, Sweden[2] University of Minnesota-Twin Cities

Amyloid fibrils formation via protein misfolding and aggregation is associated with many diseases, including neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease. The presence of polymorphism phenomenon in a single protein sample makes it important to analyze amyloid fibrils on the single fibril level. We here introduce the concept of nanofluidic channel analysis to the study of single, fluorescently labeled amyloid fibrils. The confinement of amyloid fibrils in nanochannels makes it possible to measure their extension at each width as well as their emission intensity. We used Odijk's theory for strongly confined polymers to determine the persistence length of each fibril. A majority of the persistence lengths were in the 1-10 m regime and for both the Alzheimer's protein amyloid- (1-42) and the Parkinson's protein-synuclein we find at least two populations of fibrils with different persistence lengths, indicating the co-existence of polymorphs with different physical properties. We foresee that the nanofluidics methodology that we have established here will be a useful future tool to study amyloid fibrils on the single fibril level to inform on heterogeneity in their physical properties and interactions.

P-752
All atom molecular dynamics simulations of the interfacial behaviour of mAbs

Suman Saurabh[1], Cavan Kalonia[2], Zongyi Li[3], Peter Hollowell[3], Thomas Waigh[3,4], Peixun Li[5], John Webster[5], John Seddon[1], Jian Lu[3], Fernando Bresme[1]
[1] Department of Chemistry, Molecular Sciences Research Hub Imperial College, W12 0BZ,London, United Kingdom, [2] Dosage Form Design and Development, BioPharmaceutical Development, BioPharmaceuticals R&D, AstraZeneca, Gaithersburg, Maryland 20878, United States, [3] Biological P hysics Group, School of Physics and Astronomy, Faculty of Science and Engineering, Oxford Road, The University of Manchester, Manchester M13 9PL, UK, [4] Photon Science Institute, The University of Manchester, M13 9PL, UK, [5] STFC ISIS Facility, Rutherford Appleton Laboratory, Didcot, OX11 0QX, UK

Adsorption of mAb at interfaces might lead to protein structural deformation, and therefore it is an undesirable phenomenon in therapeutic formulations, as it leads to loss in therapeutic efficacy. We have performed all atom molecular dynamics simulations of mAbs to identify the mechanisms responsible for mAb interfacial adsorption at the water surface, water-oil interfaces, and mineral surfaces. We demonstrate that slight disruptions of the mAb natively folded structure can drive the interfacial adsorption at the water surface, and we identify aggregation prone regions at the mAb surface. These all atom models do also provide a molecular understanding of the experimentally observed structural behaviour of adsorbed mAb monolayers as a function of pH and salt concentration. Finally, the molecular insight can be used to extend the computational studies to coarse-grained simulations of Fab and Fc domains at the water-oil interface, which can be used to investigate the structure of interfacial protein assemblies, The information obtained from the above mentioned simulations open the route to build models to interpret neutron reflectivity measurements and potentially to rationally design non adsorbing mAbs.

Poster Presentations
– Session 3 –

P-753
Elucidating the conformational transitions of large proteins and protein complexes via an improved coarse-grained simulation algorithm
Domenico Scaramozzino[1], Laura Orellana[1]
[1] Protein Dynamics and Mutation Lab, Department of Oncology-Pathology, Karolinska Institutet, Sweden

Proteins are dynamical macromolecules that often adopt different conformations to perform targeted biological tasks. Trapping functionally relevant conformational states is of paramount importance to get mechanistic insights into protein biological behavior. Current experimental techniques are rarely able to elucidate the conformational paths that connect these functional states and that are associated with complex mechanisms such as protein-substrate binding and channel opening-closing. Computational methods can thus help to unravel the nature of these conformational changes. One of such methods is eBDIMS, a technique based on coarse-grained elastic network models and Langevin simulations that generates smooth protein transitions between end-states and predicts the structures of known intermediates along the paths. We recently developed an improved version of eBDIMS that allows to simulate conformational changes of huge proteins in various oligomeric states (> 450 kDa and 4000 amino acids) in feasible computational times. Here we show the transitions of some of these large proteins, e.g. the tumor-suppressor neurofibromin, the human transient receptor potential melastatin 2, etc. Interpreting our simulation results in light of the structural information available in the Protein Data Bank, we can shed new light on the important molecular mechanisms at the single-protein level.

P-754
The conformation of pyroglutamated amyloid β (3-40) and (11-40) fibrils – extended or hairpin?
Holger A. Scheidt[1], Alexander Korn[1], Daniel Huster[1]
[1] Institute for Medical Physics and Biophysics, Leipzig University, Germany

Amyloid β (Aβ) peptides of varying lengths and with different posttranslational modifications form senile plaques in the brains of Alzheimer's patients. These different variants vary in fibrillation kinetics and cell toxicity. In the core of the amyloid plaques, especially N-terminally truncated Aβ peptides with a cyclic pyroglutamate in the final position 3 or 11 are found in high concentrations. Therefore, the monomer structure in fibrils formed by these two Aβ variants is of high interest to understand their differing behaviour. In a previous solid-state NMR study of pGlu11-Aβ (11-40) fibrils we revealed an intermolecular contact Gly25/Ile31, which did not fit to any of the hairpin or U-shaped structural models of fibrils of wildtype Aβ (1-40) available at this time. Using solid-state NMR we observed additional intermolecular contacts for Phe19/Leu34, Ala21/Leu34 as well as Gly25/Ile31 and were able to confirm a newly suggested model form the Tycko lab with an extended structure for the monomers also for the fibrils of both pyroglutamated variants. Especially, the contact Gly25/Ile31 is a distinctive indicator for the extended structure compared to the other known structure models. Additionally, using a construct, where the turn region of Aβ is replace by an azobenzene photoswitch, which is tuneable between hairpin and extended structure, it is shown that under the used experimental conditions only extended trans monomers form fibrils.

P-755
One fits all: A universal laser illumination source that enables new biophysical methods
Daniel Schröder[1,2], Philipp Müller[3], Oleksii Nevskyi[4], Francisco Paez Larios[1,2], Anindita Dasgupta[1,2], John Wigg[2], Philipp Hoess[5], Jonas Ries[5], Jerome Wenger[6], Emmanuel Margeat[7], Jörg Enderlein[4], Christian Eggeling[1,2]
[1] Leibniz Institute of Photonic Technology Jena, Germany, [2] Institute of Applied Optics and Biophysics, Friedrich Schiller University, Jena, Germany, [3] Physics and Astronomy, Friedrich Schiller University, Jena, Germany, [4] Physics, Third Institute of Physics Biophysics, Georg-August- Universtät, Göttingen, Germany, [5] Cell Biology and Biophysics, European Molecular Biology Laboratory, Heidelberg, Germany, [6] Aix Marseille Univ, CNRS, Centrale Marseille, Institut Fresnel, AMUTech, Marseille, France, [7] Centre National de la Recherche Scientifique, Institut National de la Santé et de la Recherche Médicale, Université Montpellier, Centre de Biologie Structurale, Montpellier, France

P-756
Ellipsometric detection of bacteria using dielectrophoresis
Fenja Schroeder[1], Soraya Zangenehzadeh
[1] Technische Universität Braunschweig, Germany

The aim of this research is to detect bacteria by ellipsometry and evaluate if dielectrophoresis (DEP) can be useful to improve the limit of detection of optical biosensors. Ellipsometry is based on the investigation of the polarization state of reflected light. Ellipsometry allows non-destructive and contactless analysis of thin films in seconds. Our measurement setup includes a flowcell which guides bacteria in liquid environment to a sensing surface, that consists of a glass substrate coated with thin film gold electrodes. The bacteria studied in these measurements were E. coli K12. By irradiating the electrode structures under a certain angle of incidence surface plasmon resonance (SPR) occurs. The excitation of SPR is highly dependent on the refractive index at the metal-liquid-interface and can be monitored by ellipsometry. The presence of bacteria changes the refractive index of the liquid and therefore changes the excitation of SPR. Sensors based on SPR can only provide information on the refractive index very close to the metal interface. To increase the number of bacteria at the metal interface DEP was applied. For positive DEP, the bacteria are accelerated to regions of high field inhomogeneity, which occur at the electrodes edges. Hence bacteria are collected directly on the electrodes, the change of SPR is magnified. To explore the efficiency of bacteria collection by DEP and the behavior of different electrode geometries in ellipsometry, a variety of electrode structures were designed. In this work we were able to detect bacteria and monitor the effect of DEP by ellipsometry.

Poster Presentations
– Session 3 –

P-757
Characterization of the Physicochemical Properties of Fluorescent Filovirus Pseudotypes
Kerstin Seier[1,2], Malgorzata Graul[1,2], Dario-Valter Conca[1,2], Marta Bally[1,2]
[1] Department of Clinical Microbiology, Umeå University, Sweden, [2] Wallenberg Centre for Molecular Medicine, Umeå University, Sweden

Virus pseudotypes, i.e. virus particles combining the viral core of one virus type and the membrane glycoprotein (GP) of another one, have long been used to study virus attachment and entry of highly pathogenic viruses. However, their implementation in single particle tracking studies crucially relies on pure and homogeneous particle preparations. Here, we produced lentivirus-based pseudotypes of the deadly filoviruses Ebola and Marburg, with an mCherry-tagged viral core. Fluorescence-based colocalization experiments via GP immunostaining reveal that conventional production protocols based on transient transfection result in samples containing large fractions of particles lacking GP, with high batch-to-batch variability. We show that this can be significantly improved using stable cell lines for pseudotyping. We also quantified the GP content of the particles on a batch basis, using Western blots, and on a single particle level by cryotomography. Next, we investigated the influence of the GP density on the virus binding kinetics and avidity to heparan sulfate, using single particle visualization by TIRF microscopy . Our work illustrates the importance of characterizing and controlling the properties of virus pseudotypes, an aspect which is often overlooked. Our improvements for a more homogeneous sample production will strengthen the interpretation of experimental results.

P-758
Polymerization Ratchet by DNA Origami and RecA: A Novel Single-Molecule Force Spectroscopy Tool
Boxuan Shen[1,2], Marco Lolaico[2], Björn Högberg[2]
[1] Biohybrid Materials, School of Chemical Engineering, Aalto University, Finland, [2] Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Sweden

Polymerization ratchets are ubiquitous force-generating motors, with actin and microtubules being common examples in nature. In this study, we aim to develop a novel polymerization ratchet using DNA origami and RecA protein for single-molecule force spectroscopy. We designed a dynamic DNA origami structure as a mechanical framework, while RecA polymerization along a ssDNA track generates force. Total internal reflection fluorescence (TIRF) microscopy and DNA-PAINT pipeline were used to monitor and visualize the ratchet's performance. Our results show successful construction and functionality of the polymerization ratchet, providing detailed insights into polymerization dynamics. The polymerization ratchet demonstrates potential as a single-molecule force spectrometer, enabling the study of various biomolecular interactions. This novel platform offers potential in nanotechnology and synthetic biology, enhancing our knowledge of biomolecular forces and advancing molecular tool development.

P-759
Improving flexible protein fitting into cryo-EM maps using multiple conformers generated by AlphaFold 2
Tatiana Shugaeva[1], Nandan Haloi[1], Rebecca J Howard[2], Erik Lindahl[1,2]
[1] Department of Applied Physics, Science for Life Laboratory, KTH Royal Institute of Technology, Sweden, [2] Department of Biochemistry and Biophysics, Science for Life Laboratory, Stockholm University, Sweden

Recent advances in cryo-electron microscopy have enabled us to obtain near atomic resolution structures of challenging protein targets. Refining an initial atomistic model into a target density map is one of the key reconstruction steps, where molecular dynamics simulations can be used to achieve a good balance between physical plausibility and map fitting scores. However, proteins with multiple functional states often remain a challenge for simulations-based refinement methods, in particular when map resolution is not high enough to build de-novo models. Here, we introduce a new refinement approach in which 1) numerous initial models are generated by stochastic subsampling the multiple sequence alignments space in AlphaFold2, 2) resulting models are subjected to structural based clustering, and 3) swarms of density-guided molecular dynamics simulations are performed from the centroid structures. We show the improved fitting accuracy compared to single starting point scenarios for the epsilon subunit of ATP synthase and CGPCR, which undergo dramatic conformational switches. We are also currently testing our approach on a variety of other proteins that visit multiple functional states and are of biomedical interest.

P-760
Model-based evaluation of biophysical properties of gap junction channels from electrophysiological recordings at a single- channel level
Mindaugas Snipas[1,2], Lukas Gudaitis[1], Lina Kraujaliene[1], Tadas Kraujalis[1,3], Vytas Verselis[4]
[1] Institute of Cardiology at Lithuanian University of Health Sciences, Kaunas, Lithuania, [2] Department of Mathematical Modelling, Kaunas University of Technology, Kaunas, Lithuania, [3] Department of Applied Informatics, Kaunas University of Technology, Kaunas, Lithuania, [4] Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine, New York, USA

Single-channel level recording via the patch clamp technique allows for assessing the biophysical properties of various types of ion channels. However, electrophysiological recordings of single gap junction (GJ) channels are hindered by their unusual intercellular configuration and natural clustering into plaques. In addition, until recently, there were no mathematical models that could adequately explain both steady-state and kinetic properties of GJ channel gating, which is a necessary first step for model-based evaluation of single-channel level characteristics. For these reasons, the methods for reliable cross-correlation of data recorded at macroscopic and single-channel levels are still lacking in studies of gap junctional electrophysiology. To address this issue, we combined our previously published four-state model (4SM) of GJ channel gating with a maximum likelihood estimation (MLE)-based analysis of electrophysiological recordings. First, we consider the evaluation of single-channel characteristics and the methods for efficient stochastic simulation of single GJ channels from the kinetic scheme described by 4SM. We then present an MLE- based methodology for extraction of information about gating parameters of GJ channels from electrophysiological recordings which exhibit visible unitary events. The validity of the proposed methodology is first illustrated using stochastic simulations and then extended to real electrophysiological data.

Poster Presentations
– Session 3 –

P-761
Characterization of Ligand Unbinding Mechanisms and Kinetics for NiFe Hydrogenase Mutants Using τRAMD
Fartin Sohraby[1], Ariane Nunes-Alves[1]
[1] Technische Universität Berlin, Germany

Hydrogenases are important enzymes due to their ability to act as efficient catalysts for hydrogen oxidation and biofuel production. However, some NiFe hydrogenases are inhibited by O2 and CO. A possible strategy to obtain resistant enzymes is to block access to the catalytic site by mutation. Focusing on NiFe hydrogenases from Desulfovibrio fructosivorans and 10 different mutants, we used molecular dynamics (MD) simulations and the enhanced sampling method τRAMD (τ-Random Accelerated Molecular Dynamics) to study substrate (H2) and inhibitor (O2 and CO) unbinding. In τRAMD a force is applied to the ligand to increase the chances of observation of unbinding events, which usually happen beyond the timescale of conventional MD simulations. So far, the relative residence times from simulations reproduce the experimental ones. Understanding how these mutations affect the unbinding events can help us to design new mutants to achieve O2- or CO-tolerant NiFe hydrogenases.

P-762
The EuPRAXIA photon beams: ultra-bright light pulses for imaging and spectroscopy
Francesco Stellato[1,2], EuPRAXIA Collaboration
[1] University of Rome Tor Vergata,Italy [2] INFN

EuPRAXIA is a leading European project aimed at the development of a dedicated, groundbreaking, ultra-compact accelerator research infrastructure based on novel plasma acceleration concepts. EuPRAXIA is an important intermediate step between proof-of-principle experiments and ground-breaking, ultra-compact accelerators for photon science, structural biology, particle physics detector development, materials science, medical imaging, radiation therapy, and industrial applications. The INFN Laboratori Nazionali di Frascati will host one of the EuPRAXIA research infrastructures and will be equipped with an X-band LINAC followed by a plasma wakefield acceleration stage driving the first plasma-based free electron laser (FEL). The FEL will be characterized by a small footprint and will deliver ultra-bright photon pulses for experiments in the water window (3-5 nm) to the user community. The facility will host a second photon beamline with seeded FEL pulses in the range between 50 and 180 nm. In addition, EuAPS, the EuPRAXIA Advanced Photon Source, will exploit the ultra-short X-ray pulses emitted by the electrons accelerated in plasma wakefields, the so-called betatron radiation, in a dedicated beamline. Here we describe the foreseen applications of the FEL and betatron photon sources, which include imaging and spectroscopy (absorption, emission and Raman) measurements on biological samples, providing information about their structure and dynamical behaviour.

P-763
Single viral particle analysis by digital influenza assay
Kazuhito Tabata[1]
[1] The University of Tokyo, Japan

We have successfully developed a digital influenza assay that detects individual influenza virus particles by probabilistically entrapping them in a microchamber using a device with an array of tens of fL microchambers. By counting the number of signals detected, an absolute quantification of the number of virus particles is possible. A dilution series was performed against the previously measured titre of infection (PFU/ml) and the concentration of viral particles (particles/ml) was determined from the number of signals obtained in the digital assay and the results were plotted on a graph. The very linear results obtained indicated that the digital assay was highly quantitative. On the other hand, the concentration of viral particles was high in relation to the value of the infected titre. The ratio, particles / PFU rate (PPP rate), reached 189, indicating that the number of viral particles was much higher. This indicates that most of the virus particles are DI particles (virus particles that cannot be infected). When infection experiments were performed with this virus at different MOIs, a rapid increase in the infection rate was observed after 20 viral particles were infected per cell, and the infection rate reached 100% after about 50 viral particles. This suggests that multiple DI particles enter the same cell to establish infection.

P-764
Functional conversion of the hemolytic/hemagglutinating lectin by addition of 19 amino acids to C-terminal of the protein.
Yuki Takahashi[1], Shuichiro Goda[1]
[1] Soka University, Japan

Lectin AML-I from the coral Acropora millepora shows amino acid sequence homology to the hemolytic lectin CEL-III from the sea cucumber (Cucumaria echinata) that lyses erythrocytes. AML-I exhibits agglutinating activity against rabbit erythrocytes, but it exhibits hemolytic activity when the C-terminal 19 residues, which are not conserved in CEL-III, are removed. Therefore, we aimed to insert a TEV protease recognition sequence at the boundary with the C-terminal 19 residues of AML-I and to regulate both activities by proteases. Escherichia coli BL21(DE3) was used to produce the target protein, and the resulting inclusion bodies were solubilized with a denaturant and refolding was done by dilution. Purification was performed by Ni-NTA affinity chromatography. Using the obtained protein, the activity against rabbit erythrocytes was measured. The full-length AML-I that has a protease site showed the highest aggregation activity at pH 6.0. After TEV protease treatment, it aggregated and precipitated. Therefore, it was purified by adding arginine to prevent aggregation, and showed hemolytic activity at pH 8.0. These results indicate that we succeeded in creating a protein that regulates the hemolytic and agglutinating activities of erythrocytes using TEV protease.

Poster Presentations
– Session 3 –

P-765
FRET Systems with RNA Fluorescent Aptamers
Sofie Thomsen[1], Maria Illum Andersen[1], Nestor Sampedro Vallina[1], Ebbe Sloth Andersen[1,2], Victoria Birkedal[1,3]
[1] Interdisciplinary Nanoscience Center (iNANO), Aarhus University, Denmark [2] Department of Molecular Biology and Genetics, Aarhus University, [3] Department of Chemistry, Aarhus University

Fluorescent RNA aptamers are short, synthetic oligonucleotide sequences that fold up into well-defined tertiary structures and selectively bind cognate fluorogens. Upon binding, the fluorescence of otherwise weakly emitting fluorogens is enhanced many-fold. Fluorescent aptamers can be expressed co-transcriptionally and thereby serve as markers for probing RNA folding processes. Aptamer-based Förster Resonance Energy Transfer (FRET) systems would allow probing RNA conformational changes. RNA origami allows the design of co-transcriptionally folded RNA nanostructures of different sizes and shapes. We use this approach to incorporate two different fluorescent aptamers at predefined positions in the RNA structure and quantify the FRET effect. Our work aims at developing aptamer-based FRET systems capable of reporting on RNA structural dynamics.

P-766
Multi-surface disinfection based on UV-C irradiation to mitigate SARS-CoV-2 transmission
Ana Tomás[1], Anna Reichel[1], Patrícia Silva[1], Nabiha Sedrine[2,3], Pedro Silva[2,3], João Pinto[4], Inês Calado[4], Joana Campos[4], Ilídio Silva[4], Vasco Machado[4], Roberto Laranjeira[4], Paulo Abreu[4], Paulo Mendes[2,3], Nuno Santos[1]
[1] Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal, [2] Castros S.A., São Félix da Marinha, Portugal,
[3] MATGLOW, Espinho, Portugal, [4] CeNTI - Centre for Nanotechnology and Smart Materials, Vila Nova de Famalicão, Portugal

Rapid widespread of COVID-19 emphasized disinfection of common spaces as an essential tool to mitigate viral transmission. In this study, our aim was to generate coherent and translational datasets of effective UV-C-based SARS-CoV-2 inactivation protocols for different surfaces. Virus infectivity after UV-C exposure of several porous (bed linen, upholstery, synthetic leather, clothing) and non-porous (plastic, stainless steel, glass, ceramics, wood, vinyl) materials was assessed through plaque assay, using a SARS-CoV-2 clinical isolate. Studies were conducted under controlled environmental conditions, with an ozone-free 254-nm UV-C lamp and irradiance values quantified using a 254 nm-calibrated sensor. Reference porous and non-porous materials were selected to assess the decrease of infectious virus particles as a function of UV-C dose. Remaining surfaces were tested with selected critical doses. Results show that UV-C irradiation is effectively inactivating SARS-CoV-2 on the reference surfaces. However, an efficient reduction in the number of infectious viral particles (LD90) was achieved much faster and at lower doses in the non-porous reference material (0.85 mJ/cm2) than in the porous reference material (119.2 mJ/cm2). Moreover, the treatment effectiveness of porous materials was demonstrated to be highly variable and composition dependent. These results support the optimization of UV-C-based technologies, enabling the adoption of effective customizable protocols that will help to ensure high antiviral efficiencies.

P-767
A SERS aptasensor for a highly sensitive and selective detection of biomarkers
Ioan Turcu[1], Simion Astilean[2], Monica Focsan[2], Ana-Maria Craciun[2], Alina Vasilescu[3], Daniel Marconi[1], Alia Colnita[1], Monica Potara[2]
[1] Molecular and Biomolecular Physics Department, National Institute for Research and Development of Isotopic and Molecular Technologies, Cluj- Napoca, Romania, [2] Nanobiophotonics and Laser Microspectroscopy Center, Interdisciplinary Research Institute in Bio-Nano-Sciences, Babes- Bolyai University, Cluj-Napoca, Romania, [3] International Centre of Biodynamics, Bucharest, Romania

The identification and quantification with high specificity and sensitivity of biomarkers represents one of the "holy grails" of modern medical diagnostics. The present work is focused on designing an aptamer-modified plasmonic nanoplatform able to offer relevant solutions for medical diagnosis. While the aptamers attached onto the metallic surface specifically recognize the target biomarkers, the signal transduction is based on ultrasensitive Surface Enhanced Raman Scattering (SERS). The fabricated plasmonic nanoplatform is characterized by scanning electron microscopy (SEM), optical reflectivity spectra, SERS measurements and finite-difference time-domain (FDTD) simulations. The designed SERS- based bioassay enables reproducible, highly sensitive and selective detection of relevant biomarkers. Due to the simplicity of the assay and robustness of molecular bioreceptors, the proposed strategy offers an attractive perspective in designing portable nanosensors for rapid detection of various diseases biomarkers. Acknowledgements: The authors acknowledge the financial support from UEFISCDI, Project PN-III-P2-2.1-PED-2021 -1998.

P-768
Tau protein induces membrane damage
Vicky Ury-Thiery[1], Cécile Feuillie, Yann Fichou, Michael Molinari, Sophie Lecomte
[1] CBMN University of Bordeaux, France

Tau, a microtubule associated protein, is an intrinsically disordered protein involved in various diseases called tauopathies, such as frontotemporal dementia and Alzheimer's disease. Indeed, in pathological conditions, tau can disassemble and accumulate in the cytosol of neuronal cells, leading the formation of amyloid fibers. The pathogenic mechanism of tau remains poorly understood. Previous studies have shown (i) the ability of tau to form fibers upon binding with negatively charged cofactors such as heparin, nucleic acids or lipids, and (ii) the preferential disruption of membrane composed of negatively charged lipids after incubation with a tau construct called K18. Here, we first focused on the disease-associated mutation P301L of the full-length Tau and its impact on POPC and POPS, lipids present in the inner membrane of neurons. To address this, we have combined polarized ATR-FTIR (Fourier-transform infrared in attenuated total reflection) and AFM (atomic force microscopy) to characterize tau-membrane interactions. Our results reveal that Tau protein can induce damage to both lipid bilayers, independently of the lipid nature, but following a seemingly different mechanism. A detergent like effect is observed on POPC membranes. On POPS membranes, protein accumulation is observed at the bilayer surface, possibly promoting tau aggregation.

Poster Presentations
– Session 3 –

P-769
Stabilization of mRNA lipid nano particles
Sibel Uzuncayir[1], Paula Muresan[2], Sylvain Prévost[3], Martine Moulin[3], Harald Pichler[4], Michael Frieß[4], Gemma Navarro[2], Trevor Forsyth[1]
[1] Lund University, Sweden, [2] Curevac, Germany, [3] ILL, France, [4] Graz University, Austria

The Covid-19 pandemic resulted in the release of the new class of vaccine products- the mRNA vaccines. These consist of synthetic mRNA strands packed in lipid nano particle (LNP) that deliver the mRNA to the cells, initiate antigen production and lastly result in immune protection. The function of mRNA in vivo depends on effective, safe and stable delivery to allow cellular uptake and RNA release. One of the main disadvantages of mRNA vaccines is that they need to be stored at ultra-low temperatures, hampering the world-wide distribution during the pandemic. To overcome this hurdle the characterization of the structure-activity relationship of mRNA LNPs formation is crucial for fully understanding their mechanism and in order to improve their stability. LNPs are multicomponent systems consisting of a shell and a core. The shell consists of PEG, a helper lipid such as DSPC and Cholesterol, whereas the core mainly consists of an ionizable lipid and mRNA. In particular, the interaction of mRNA, water and ionized lipids in the core of LNP seems to play a major role in degradation and understanding these processes will aid to optimize mRNA LNP design in the future. Here, we determine structural features such as changes in morphology and degradation processes of mRNA LNP in different settings and with different lipid compositions by combining cryo-electron microscopy, SAXS, CV-SANS with LNP standard assays (DLS, Ribogreen, RNA integrity and protein expression) to gain insight in their formation kinetics and investigate the cause of their instability during storage.

P-770
OPTICAL BIOSENSING AND SINGLE-MOLECULE DETECTION ENHANCED WITH AEROTAXY LIGHTGUIDING NANOWIRES
Julia Valderas Gutiérrez[1,2], Rubina Davtyan[1,2], Sudhakar Sivakumar[1,2], Nicklas Anttu[3], Yuyu Li[4], Patrick Flatt[4], Jae Yen Shin[1,2], Christelle N. Prinz[1,2], Fredrik Höök[1,5], Thoas Fioretos[6], Martin Magnusson[1,2], Heiner Linke[1,2]
[1] NanoLund, Lund University, Sweden [2] Division of Solid State Physics, Lund University, [3] Physics, Faculty of Science and Engineering, Åbo Akademi University, [4] AlignedBio AB, Lund, [5] Department of Physics, Chalmers University of Technology, [6] Division of Clinical Genetics, Lund University

Surface-based fluorescence sensors are powerful tools for the detection of diagnostic biomolecules at low concentrations. In this context, the sensitivity of conventional planar substrates can be improved at least 10 times using lightguiding semiconductor nanowires which, due to their optical properties, can collect, enhance and re-emit directionally the signal from close-bound fluorescent biomolecules. However, so far vertical arrays of crystalline nanowires are required, grown by low-throughput batch-based method epitaxy have been required. Alternatively, aerotaxy has been proposed as a continuous, high-throughput technique compatible with the mass production of high-quality nanowires. Hence, we compared the signal intensity of GaP aerotaxy and epitaxy nanowire platforms to flat surfaces using conventional epifluorescence microscopy. Both aerotaxy and epitaxy nanowires presented a total signal intensity proportional to their density, one and two orders of magnitude higher, respectively, than planar surfaces; and a comparable average signal enhancement per nanowire for both samples, also verified by numerical modeling. Interestingly, both types of nanowires lit up when individual molecules are bound, getting brighter as the number of bound molecules increases, until saturation. Thus, nanowires reveal their potential for sensitive biosensing, including the detection of single-molecules, compatible with low-cost scalable aerotaxy production.

P-771
3D super-resolution microscopy and tracking using supercritical angle fluorescence and defocused imaging
Lukas Velas[1], Anna Gaugutz[1], Philipp Zelger[2], Alexander Jesacher[2], Stefan Howorka[3], Gerhard Schütz[1]
[1] Institute of Applied Physics, TU Wien, Austria [2] Institute of Biomedical Physics, Medical University Innsbruck, [3] Department of Chemistry, University College London

Single molecule localization microscopy (SMLM) is an increasingly popular technique used in imaging of biological samples that allows for the observation and analysis of individual molecules at high spatial resolution. While localizing along the lateral directions is usually straightforward, estimating axial positions at a comparable precision is known to be more difficult. Whenever a molecule is sufficiently close to the coverslip, it becomes feasible to draw additional information from near field coupling effects: super-critical angle fluorescence (SAF) can be exploited to boost the axial localization precision. Here, we used defocused imaging to study the 3D spatial distribution of the T cell receptor within an immunological synapse formed between T cells and functionalized supported lipid bilayers. The results show a tighter contact formed in the case of activated T cells with an average cleft size of 18 nm in comparison with 31 nm measured for resting T cells. Moreover, we used the technique for 3D single molecule tracking of DNA origami nanostructures, successfully resolving 10 nm differences in z direction. In conclusion, our technique is easy to implement and offers access to 10 nm isotropic localization precision for nanoscopy and tracking in samples which are in a close vicinity to a coverslip.

P-772
Excitation-emission matrices of exosomes isolated from urine
Martina Velísková[1], Milan Zvarík[1], Daniela Džubinská[1], Iveta Waczulíková[1]
[1] Faculty of Mathematics, Physics and Informatics, Comenius University, Bratislava, Slovakia

Extracellular vesicles are present in all body fluids and their main role is intercellular communication. They are considered to be a source of potential biomarkers as they contain molecular signatures similar to the cells from which they were excluded. Among other things, bioactive molecules exhibit endogenous fluorescence. This property could be used in the diagnosis of various diseases based on the different fluorescent characteristics of extracellular vesicles. In our work, we focused on changes in the fluorescence characteristics of extracellular vesicles isolated from the urine of healthy controls (n = 9) and patients with bladder cancer (n = 12). Extracellular vesicles were isolated by the ultrafiltration method in conjunction with size exclusion chromatography. Using a fluorescence spectrophotometer and the appropriate software, we measured and visualized the excitation-emission matrices in the wavelength range of 200-800 nm. We identified five areas that were significantly different between the healthy group and the group of patients with bladder cancer. These areas could provide the basis for the development of a predictive test for use in clinical diagnostics in the future. So far, however, only pilot measurements have been carried out, but after the addition of more samples, we expect an increase in statistical power to distinguish differences in the investigated areas.

Poster Presentations
– Session 3 –

P-773
Analysis of proton exchange and photo-induced isomerization kinetics of the pH-sensitive cyanine dye CypHer5E

Chinmaya Venugopal Srambickal[1], Zhixue Du[1], Jerker Widengren[1]
[1] 1Experimental Biomolecular Physics, Department of Applied Physics, Royal Institute of Technology (KTH), Stockholm, Sweden

CypHer5E is a pH-sensitive cyanine fluorophore used as an intracellular pH indicator and for live-cell phagocytosis studies in lysosomes and other organelles with acidic pH. Although CypHer5E shows clear pH sensitivity, with roughly six times higher fluorescence emission in acidic pH compared to physiological neutral pH, a comprehensive study of its photophysical properties has not yet been reported. We used fluorescence correlation spectroscopy (FCS) to study the photophysical properties of cypHer5E including its protonation kinetics. FCS measurements were performed on CypHer5E, free in solution or bound to small unilamellar vesicles (SUVs), at different pH, buffer and excitation conditions. We found prominent fluorescence fluctuations of CypHer5E attributed to photo-induced isomerization and proton exchange. Our FCS data indicate that CypHer5E has different molecular brightness depending on the pH, as expected, but also coupled to the isomerization kinetics. With CypHer5E on SUVs, we furthermore observe membrane-coupled proton collecting antennae effects in the proton exchange kinetics of the fluorophore. Based on the FCS data, we established a simple electronic state model for CypHer5E, including protonation and photo-induced isomerization kinetics. This model can serve as a basis for a more quantitative interpretation of CypHer5E-based imaging and spectroscopic data from cells and extracellular vesicles.

P-774
Effect of RSV-infection on Hep-2 cell mechanics

Sarah Verbeek[1], Lifeng Liu[1], Yara Abidine[1], Edward Trybala[2], Tomas Bergström[2], Marta Bally[1]
[1] Department of Clinical Microbiology, Umeå University, Sweden, [2] Department for Infectious Diseases, Sahlgrenska University Hospital, Gothenburg, Sweden

Respiratory syncytial virus (RSV) is responsible for most cases of pediatric lower respiratory tract infections worldwide and can result in fatal complications in infants or elderly. No vaccine or effective antiviral treatment is available. RSV has been proposed to spread through formation of long filopodia and lamellopodia stretching to uninfected cells and by forming multinucleate cells (syncytia) through cell fusions. Preventing these processes could be a promising antiviral strategy. Lacking, however, is a time-resolved understanding of underlying mechanisms, as well as the interplay between cellular and viral factors therein. We hypothesize that RSV infection results in overall stiffening of cells, due to the extensive actin production necessary to form filopodia. On the other hand, in confluent infected cell layers, we expect syncytia to be softer than non-syncytial and non-infected cells. In this work, we use atomic force microscopy and force spectroscopy to (i) investigate the changes in overall stiffness and viscoelasticity of Hep-2 cells over time during infection, (ii) obtain detailed mechanical and morphological insights into podia formed by infected and non-infected cells and (iii) compare mechanics of syncytia and surrounding cells during infection. Subsequently, we will investigate how promising antiviral candidate drugs alter those properties.

P-775
Decoding the scanning parameters of iterative MINFLUX through Single Particle Tracking

Bela Vogler[1,2], Francesco Reina[1], Ziliang Zhao[1,2], Christian Eggeling[1,2]
[1] Leibniz Institute of Photonic Technologies e.V., Jena, Germany, [2] Friedrich Schiller University Jena, Jena, Germany

Detecting the lateral dynamics of molecules on living and model membranes is a challenging field of study, requiring microscopy techniques that can track molecules with single particle level of detail, nanoscopic localization precisions, and with high temporal resolution. MINFLUX microscopy is a recently developed technique that combines concepts of structured illumination and single molecule localization microscopy to achieve unprecedented levels of localization precision. “MINFLUX 3D”, its commercial implementation, relies on several scanning iterations to progressively improve the localization precision of single fluorophores. With this approach, it has been shown how it is possible to obtain single particle trajectories with kHz sampling rates. The iterative scanning procedure, however, raises concerns on whether the technique has any limitation. In particular, we sought to investigate the impact that specific scanning parameters had on the recorded single particle trajectories. We thus investigated the diffusion of single quantum-dot labelled phospholipid analogues on a homogeneous model membrane. The single particle trajectories thus obtained, revealed potential limitations of using iterative MINFLUX scanning for single particle tracking and systematic, unfavorable effects introduced by the scanning parameters. In an effort to theoretically understand possible origins of reported issues, we propose several thought experiments based on fundamental physical and mathematical principles. In conclusion, we highlight possible limiting factors present in the iterative scanning MINFLUX implementation that may advise the growing community in identifying appropriate fields of application.

P-776
AI enables nanotextural multiplexing of sub-cellular structures

Bela Vogler[1,2], Gregor Gentsch[2], Christian Franke[2,3,4]
[1] Leibniz Institute of Photonic Technologies e.V., Albert-Einstein Strasse 9, 07745 Jena, Germany, [2] Institute of Applied Optics and Biophysics, Friedrich Schiller University Jena, Germany , [3] Abbe Center of Photonics, Friedrich Schiller University Jena, Germany, [4] Jena Center for Soft Matter, Friedrich-Schiller-University Jena, Helmholtzweg 4, 07743 Jena, Germany

With the rise of optical super-resolution microscopy (SRM), intra-cellular structures can be analyzed with nanometer resolution. Here, specific fluorescent markers enable multi-color imaging of several sub-cellular structures, by multiplexing the labels based on their emission wavelengths and complementary spectroscopic readouts. However, the best resolving SRM approaches, i.e. STED, SMLM and MINFLUX, rely on special dyes with delicate photo-physical properties, which often limits the applicability of multi-color acquisitions, especially in complex biological contexts. To that end, we explore the analysis of nanoscopic textures inherent to intra-cellular organelles that can be utilized for computational multiplexing. Introducing textural demixing following Unet feature generation, trained on publicly available data, we demonstrate its potential to regressively extract at least three complex structures at a time from a single-channel grayscale SMLM image. We investigate the capacity of this context agnostic texture recognition and prove that AI is capable of identifying individual structures even when heavily overlaying each other. We farther demonstrate that AI trained on single organelles imaged by SMLM is able to multiplex single-color, multi-organelle SMLM data and show that our method is also applicable to MINFLUX data without the need for additional training. Texture-sensitive nanoscopy significantly broadens the scope of multi-color SRM by straight-forwardly enabling the multiplexed use of the best performing dyes in complex biological contexts.

Poster Presentations
– Session 3 –

P-777
Dynamic binding interactions of the Sars-CoV-2 protein nsp3 with viral and host cell partner proteins characterized by different biophysical methods including NMR spectroscopy

Katharina Vormann[1,2], Tobias Stief[1,2], Ralf Bartenschlager[3], Nils-Alexander Lakomek[1,2]

[1] Forschungszentrum Jülich, Institute of Biological Information Processing, Jülich, Germany , [2] Heinrich Heine University Düsseldorf, Institute of Physical Biology, Düsseldorf, Germany, [3] Deutsches Krebsforschungszentrum, Division Virus-Associated Carcinogenesis, Heidelberg, Germany

In response to the Severe acute respiratory syndrome coronavirus 2 (Sars-CoV-2) pandemic, novel anti-viral agents are needed. One potential drug target is the multi-domain non-structural protein 3 (nsp3). Nsp3 is involved in the formation of the Sars-Cov-2 replication organelle (RO), which drives the replication of the coronavirus in the host cell. Nsp3 is one major constituent of molecular pores embedded in the bilayer of Double Membrane Vesicles (DMVs). These DMV pores allow the import and export of viral RNA. As the largest protein encoded by the coronavirus, nsp3 comprises 16 domains, including the N-terminal Ubiquitin-like domain 1 (Ubl1), forming the cytosolic-faced prongs of the crown-like pore. Considering that ubiquitin-like domains are involved in the regulation of diverse biological processes, the list of potential viral and host cell interaction partners is substantial. Currently, information on the structure, dynamics, and binding partners of nsp3 is limited. Thus, the aim of these in vitro studies is to obtain novel insights into the structure and dynamics of nsp3-Ubl1 by different biophysical methods including circular dichroism (CD) and nuclear magnetic resonance (NMR) spectroscopy. Further, we investigate the dynamic binding interactions of nsp3-Ubl1 using bio-layer interferometry (BLI) and microscale thermophoresis (MST).

P-778
Assessing the Accessibility of Ligands to Prolyl Oligopeptidase: Implications for Neurodegenerative Disease Treatment

Katarzyna Walczewska-Szewc[1], Jakub Rydzewski[1]

[1] Nicolaus Copernicus University in Torun, Poland

Prolyl oligopeptidase (PREP) is an enzyme with diverse functions regulating neuropeptides and neurotransmitter release. One aspect of PREP's function that has garnered recent attention is its protein-protein interactions. Specifically, PREP has been shown to interact with intrinsically disordered proteins, like Tau and alpha-synuclein, which are implicated in the pathogenesis of several neurodegenerative diseases. Hampering such interactions could lead to effective therapies for these diseases. In this study, we focused on understanding the structure and function of PREP. Specifically, we investigated the entrance pathways for ligands to PREP using molecular dynamics simulations and enhanced sampling methods. While the binding sites of PREP inhibitors are relatively well-characterised, the pathways for ligand accession to the enzyme are not fully understood. Our results showed that some pathways required significantly less energy for the ligand to access the binding site than others. This information is critical for designing more effective PREP inhibitors that can enter the enzyme more easily, leading to more efficient inhibition of PREP and reducing the risk of neurodegenerative diseases. In summary, our study offers valuable insights into the structure and function of PREP and its potential as a therapeutic target. By identifying potential entrance pathways for ligands and evaluating their energetic costs, we provide a framework for designing more effective PREP inhibitors with implications for developing new treatments for neurodegenerative diseases.

P-779
Towards a wireframe DNA nanorobot for immunotherapy and its stability in vivo

Yang Wang[1], Janine Waldvogel[2], Björn Högberg[1]

[1] Karolinska Institutet, Sweden [2] Eidgenössische Technische Hochschule Zürich

Bridging cytotoxic T cells to cancer cells, exemplified by bispecific or even tri-specific T cell engagers, has emerged as a new and efficacious approach among current strategies of cancer immunotherapy. Although a few T cell engagers have been successfully developed in clinical assessment or already with clinical approvals, major concerns are corresponding potentially fatal adverse effects, including most notably the 'on- target, off-tumor' side effects. These lead to immune activation and cytokine release syndrome, due to linking T cells to normal cells and earlier T cell stimulation before their interaction with cancer cells. To overcome these problems, we are developing a wireframe-style, barrel-like DNA nanorobot that, when opened, will cross-link T cells to the desired target cells, triggering T cell cytotoxicity and killing of the target cancer cells. By spatiotemporally controlling the opening of the DNA nanorobot, we aim to selectively redirect the cytotoxicity of T cells to solid cancers and substantially mitigate corresponding adverse effects. We will also present a new method to obtain accurate information on DNA origami stability in vivo. To monitor the stability of the DNA origami nanorobot in vivo, we have developed a ligationsequencing method. This is a dye-free method that enables us to trace the structural integrity of DNA origami by ligating two adjacent staple strands (ligation-pair). The pairs can ligate only as long as the structure is intact, as sustained proximity in intact structures allows for ligation, whereas loss prevents it. Only 1 microliter of blood sample is needed for the analysis.

P-780
Impact of adhesion on the assembly and mechanics of a minimal actin cortex attached to the membrane via ezrin

Tobias Weege[1], Claudia Steinem[1,2]

[1] Georg-August-Universität, Institute of Organic and Biomolecular Chemistry, 37077, Göttingen, Germany, [2] Max Planck Institute for Dynamics and Self-Organization, 37077, Göttingen, Germany

The actomyosin cortex is a thin and contractile network within cells that is coupled to the plasma membrane. It is part of the cytoskeleton and plays an important role in e.g. cell shape, motility, and division. This project aims to shed light on the mechanical properties of the actomyosin network under the impact of adhesion. Therefore, a minimal actin cortex (MAC) will be constituted in giant unilamellar vesicles (GUVs) using microfluidic techniques. The attachment of the cortex to the membrane will be realized using the linkage of ezrin and the phospholipid PIP2, that is implemented in the GUVs membrane. To apply force on these GUVs, the improved light-induced dimer (iLID) protein pair will be introduced to this system. One part of the protein dimer is immobilized on a planar substrate, while the corresponding part is attached to the outside of the GUVs. Under illumination with blue light, the adhesion between these proteins can drastically be increased, inducing stress on the GUVs. Dynamic shape changes can then be observed using confocal microscopy, which allows for conclusions to be drawn about the mechanical properties of the MAC under adhesion.

Poster Presentations
– Session 3 –

P-781
Investigating the R-loop formation by CRISPR-dCas9 with ultrafast single-molecule twist measurements
Fabian Welzel[1], Julene Madariaga-Marcos[1], Dominik J. Kauert[1], Ralf Seidel[1]
[1] Peter Debye Institute for Soft Matter Physics, Universität Leipzig, Leipzig 04103, Germany

The Type II CRISPR nuclease Cas9 is a revolutionary tool in genomic engineering due to its small size and its ability to cleave both DNA strands. dCas9, the cleavage-deficient variant of Cas9, can bind to dsDNA without cutting the strands after successful R-loop formation. This has the potential to increase the specificity of binding and offers a new range of applications in genome editing. However, the process of R-loop formation has a considerable tolerance for mismatches. To prevent off-targeting, quantitative modelling of the recognition process would be desirable, which requires knowledge about the energy landscapes of the R-loop formation. Our approach to determine such energy landscapes is to measure DNA unwinding during R-loop formation using ultrafast twist measurements. Using DNA origami nanostructures as rotor arms, directly reporting about twist changes on a millisecond time scale, we can resolve real-time R-loop dynamics of CRISPR-Cas effector complexes at the single base-pair level and construct corresponding energy landscapes. With this, we achieve unprecedented insight in the R-loop formation by dCas9 and the impact of mismatches on this process.

P-782
A single-molecule view on non-homologous end-joining in bacteria
Fredrik Westerlund[1], Evgeniya Pavlova[1], Anusha Budida[1], Elin Persson[1], Robin Öz[1], Sriram KK[1], Raphael Guerois[2]
[1] Chalmers University of Technology, Sweden[2] Université Paris-Sud

Efficient repair of double-stranded DNA breaks (DSBs) is detrimental for keeping genomic information intact. A major DSBs repair pathway is non-homologous end-joining (NHEJ), where two free DNA ends are joined in the absence of a DNA template. NHEJ was for long considered to exist only in eukaryotes, but has recently also been discovered and characterized in bacteria. The minimal bacterial NHEJ machinery includes only the Ku protein that brings DNA ends together, and a ligase, typically Ligase D, that covalently joins them. Studying the molecular mechanism of bacterial LigD/Ku/DNA complex formation is important to understand the bacterial life cycle and adaptivity, in particular during sporulation (e.g. for B. subtilis), and in turn in the development of drugs that target bacterial NHEJ. In this work, we combine single molecule experiments, in particular nanofluidics where all DNA ends are free, and biochemical assays, to explore the DNA-Ku-LigD interactions in B. subtilis and M. tuberculosis. Ku from both bacteria bridges DNA with 12 nt complementary 5'-overhangs, but the synapsis formed by M. tuberculosis Ku is weaker. On the other hand, M. tuberculosis Ku supports synapsis of blunt DNA ends while B. subtilis Ku does not promote a long-lived synapsis on blunt ends. Furthermore, single-molecule and bulk experiments with Ku and LigD variants highlight DNA-protein interactions crucial for efficient DNA end-ligation.

P-783
Signaling by cluster formation investigated with single-molecule fluorescence in living cells
Sophie Weyrauch[1], Chenyang Lan[1,2], Juheyong Kim[3], Svenja Ulferts[4], Fernando Aprile-Garcia[5], Abhinaya Anandamurugan[3], Robert Grosse[4], Ritwick Sawarkar[6], Aleks Reinhardt[7], Thorsten Hugel[1]
[1] Institute of Physical Chemistry and CIBSS, University of Freiburg, Germany, [2] PicoQuant GmbH, Berlin, Germany, [3] Institute of Physical Chemistry, University of Freiburg, Germany, [4] Institute of Experimental and Clinical Pharmacology and Toxicology, University of Freiburg, Germany, [5] Max Planck Institute of Immunobiology and Epigenetics, Germany, [6] Medical Research Council (MRC), University of Cambridge, Cambridge, United Kingdom, [7] Yusuf Hamied Department of Chemistry, University of Cambridge, Cambridge

Molecules in cells may self-organize into membraneless assemblies at nanometre to micrometre scale to tune diverse signaling activities. However, our current understanding of condensate formation within cells is largely based on observing the final near-equilibrium condensate state. So far less understood is the formation and dynamics of small assemblies, called clusters, because clusters are difficult to observe in real time in living cells. We fill this gap by using fluorescence microscopy in a home-built HILO (highly inclined and laminated optical sheet) microscope. This is combined with photobleaching step analysis and tracking of cluster size changes over time. Altogether, this integrated approach allows us to quantify cluster formation in living cells. The protein of interest is a transcription factor, namely negative elongation factor (NELF). Under stress conditions, clusters of NELF are formed to ensure its rapid availability for the downregulation of housekeeping genes. We reveal heterogeneous clusters that dynamically grow and shrink. These clusters are still observable in the presence of p38 kinase inhibitor although large NELF condensates do not form. Therefore, we anticipate that small clusters are precursors of large condensates and play an important role in cellular signaling.

P-784
Refining Nucleic Acid Force Fields with Machine Learning: A Roadmap for Improving Predictive Capacity and Universality
Miłosz Wieczór[1], Diego Gallego[1], Israel Serrano[1], Alireza Ketabdari[1], Juan Aranda[1], Subhamoy Deb[1], Modesto Orozco[1]
[1] IRB Barcelona, Spain

Atomistic force fields (FFs) are empirical energy functions widely used in computational chemistry to describe molecular interactions at the atomic level. While FFs have significantly advanced in recent years, particularly in modeling proteins, nucleic acid FFs have lagged behind due to the complexity of their conformational space and dynamics. Additionally, the non-bonded properties in the parametrization of nucleic acid FFs have shown significant deviations from experimental values, especially in estimations of association free energy. In this study, we propose a roadmap towards accurate and universal nucleic acid FFs by exploring three major approaches: reinforcement learning, machine-learned interatomic potentials augmented with active learning, and classical bottom-up physics-based methods. Specifically, we focus on improving the non-bonded component of existing FFs to better reproduce experimental affinities between nucleosides, enhance the description of stacking interactions, and achieve good agreement with NMR reference data. Importantly, the resulting FFs maintain stability in standard validation systems and offer automated approaches to address challenging cases. The proposed approaches represent a significant step towards developing nucleic acid FFs with improved predictive capacity and universality. By refining the non-bonded properties of these FFs, we demonstrate promising results in bridging the gap between computational predictions and experimental data for nucleic acid systems.

Poster Presentations
– Session 3 –

P-785
Fully Automated Screening of Compound Libraries in Drug Discovery Using Docking and Molecular Dynamics
Sebastian Wingbermühle[1], Erik Lindahl[1]
[1] KTH Royal Institute of Technology, Sweden

In drug discovery, initial large-scale experimental screenings often identify only a small number of hits. Therefore, we develop a computational workflow ranking drug candidates by their binding affinity to the target protein. First, all compounds in a library are docked to the protein. The compounds with the poses scoring best are grouped into pairs, and their relative free energy of binding to the protein is calculated using the Accelerated Weight Histogram method (AWH) for alchemical transformations. Randomly moving between λ-windows, one AWH simulation samples the complete alchemical path connecting two compounds. The probability to visit a λ-window depends on its estimated free energy such that the sampling of the alchemical transformation and the free energy estimate improve each other in a positive feedback loop. Our workflow converts docking poses into input for molecular dynamics (MD) simulations without human intervention. In particular, it assigns force field parameters (AMBER) to the protein-compound complex and determines the smallest set of atoms to be alchemically transformed in a compound pair. We extensively test AWH's robustness to (sub-optimal) initial configurations from docking on a benchmark with 13 proteins and approximately 500 ligand pairs for which experimental relative binding free energies are available. Moreover, absolute binding free energies from MD for up to 20,000 protein-ligand complexes from PDBbind (version 2020) are used to train two machine-learning models: one selecting the best compound for the target, the other improving pose selection during docking.

P-786
Engineered Multi-Nodal Neuronal Networks with Predefined Afferent-Efferent Connectivity for Advanced Modelling of Network Function and Dysfunction
Nicolai Winter-Hjelm[1], Pawel Sikorski[2], Axel Sandvig[1,3,4,5], Ioanna Sandvig[1]
[1]University of Science and Technology (NTNU), Norway, [2]University of Science and Technology (NTNU), Norway, [3]St. Olav´s University Hospital, Trondheim, Norway, [4]Umeå University Hospital, Sweden, [5]Umeå University Hospital, Sweden

Neuronal networks in the brain self-organize and establish complex assemblies in which topology influences function and vice versa, through tight control of afferent-efferent connectivity. Microfluidic technologies can be used to engineer neuronal networks that recapitulate key aspects of this topological organization in vitro. However, approaches facilitating structuring and retainment of a controlled, directional network topology over time are still limited. Furthermore, the effects of the underlying topological features of such engineered neuronal networks on their functional profile are still largely underexplored. In this study, we utilized microfluidic devices featuring axon guiding channels with geometrical constraints inspired by a Tesla valve to promote unidirectional axonal outgrowth between neuronal populations. Viral tools were used to fluorescently visualize the networks' structure, and surface embedded nanoporous microelectrodes to study the networks´ functional dynamics. We demonstrate that these microdevices effectively promote establishment of predefined unidirectional connectivity between neuronal populations, shown as induced feedforward signal propagation in response to electrical stimulations. Our results furthermore indicate that such structured networks exhibit a more efficient network organization with segregated, yet integrated dynamics compared to single population unstructured networks. A key advantage with our device is the ability to selectively and longitudinally study and manipulate the structure and function of neuronal networks with high accuracy. As such, this approach has the potential to provide novel insights into the development, topological organization, and neuroplasticity mechanisms of neuronal assemblies at the micro- and mesoscale in healthy and perturbed conditions.

P-787
Computational Approaches to Liquid-Liquid Phase Separation of Partially Disordered RS-Proteins
Yannick Witzky[1], Stephan Hobe[1], Andreas Wachter[1], Arash Nikoubashman[1], Friederike Schmid[1]
[1] Johannes Gutenberg University, Germany

RS-proteins are a class of proteins that contribute to light-activated gene regulation (via an alternative splicing mechanism) in plant morphogenesis. It has been hypothesized that liquid-liquid phase separation (LLPS) plays an important role for the regulation mechanism. Studying these proteins is challenging because they contain both intrinsically disordered regions (IDRs) – which presumably control the LLPS – as well as folded domains that contain the functionally important RNA binding sites. Here we use and compare different coarse-grained models to study the condensation and phase behavior of RS proteins: Commonly used IDP models as well as the structure predictive UNRES model. We specifically focus on the on the single-chain conformations, phase behavior and the accessibility of RNA binding site.

P-788
Single Particle Tracking PALM Provides Insight into Nucleoid Remodeling in Deinococcus radiodurans
Jip Wulffele[1], Pierre Vauclare[1], Francoise Lacroix[1], Jean-Philippe Kleman[1], Pascale Servant[2], Fabrice Confalonieri[2], Dominique Bourgeois[1], Joanna Timmins[1]
[1] Institut de Biologie Structurale, Univ. Grenoble Alpes, CEA, CNRS, F-38044 Grenoble, France, [2] Insitute for Integrative Biology of the Cell, 91190 Gif-sur-Yvette, France

Single molecule fluorescence imaging has become an indispensable tool for biological research, enabling investigation of molecular structures and dynamics at the nanoscale. Single molecule imaging adds a particular advantage to the study of bacteria, which due to their small size present a challenge for conventional fluorescence microscopy methods. Here, we employed photoactivated localization microscopy (PALM) and single particle tracking PALM (sptPALM) to visualize stress-induced nucleoid remodeling in Deinococcus radiodurans. Nucleoid remodeling has emerged as a generic stress response in bacteria and is thought to facilitate DNA repair and protect the genomic DNA against further damage. The molecular mechanisms driving this remodeling, however, are not well understood. We addressed this question by studying the stress induced changes in nucleoid morphology and in diffusion dynamics of histone-like protein HU, the most conserved and one of the most abundant nucleoid associated proteins in bacteria. PALM imaging revealed that the nucleoid of D. radiodurans adopts a rounded and compacted morphology during stationary phase and during recovery from UVC induced DNA damage as compared to during exponential phase. However, despite the morphological similarities, sptPALM showed that the diffusion dynamics of HU are drastically different in stationary phase cells and cells recovering from DNA damage: while HU diffusion is decreased during stationary phase, it is increased after exposure to UVC irradiation. We discuss how to interpret these changes in HU diffusion with respect to the altered nucleoid morphology.

Poster Presentations
– Session 3 –

P-789
Investigating the mechanisms of enzyme diffusion using mass photometry
Yulia Yancheva[1], Saniye Kaya[1], Marco Fraaije[1], Matthias Heinemann[1], Katarzyna Tych[1]
[1] University of Groningen, Netherlands

In recent studies it has been suggested that there is an upper limit of the Gibbs free energy that cells can dissipate, which might be posing a fundamental thermodynamic limit on cell growth. One of the proposed reasons for the existence of this limit is that catalytically induced enzyme movement causes stirring of the cell cytoplasm, perturbing biomolecular functions: some enzymes have been observed to diffuse faster than what is expected from Brownian motion in the presence of their substrates. However, the existence of this phenomenon, named enhanced enzyme diffusion (EED), has been a topic of debate in recent literature, having been confirmed by some studies and denied by others, with concerns regarding an experimental artefact. One possible explanation for the observed EED is that oligomeric enzymes dissociate to their subunits during catalysis: according to Stokes-Einstein equation the diffusion coefficient of a spherical particle is inversely proportional to its radius. In our study we use mass photometry, a label-free single molecule interferometric light scattering technique, to investigate the dependence of the oligomeric states of several enzymes of their substrate concentration. We compare the ratios of oligomeric states in the presence and absence of substrate as well as different substrate and inhibitor concentrations. We observed the dissociation of catalase and aldolase in the presence of their substrates, but not in the presence of an inhibitor, leading us to the conclusion that enzyme dissociation occurs due to catalysis as a regulatory mechanism.

P-790
Surface plasmon resonance biosensors analysis with different functionalized layers for bacteria detection
Soraya Zangenehzadeh[1,2], Svenja Herdan[1], Emil Agócs [1,2], Laurie Neumann [1], Rebekka Biedendieck [3], Dieter Jahn [3], Bernhard W.Roth[2,4], Hans- Hermann Johannes [1,2], Wolfgang Kowalsky [1,2]
[1] Institute for High Frequency Technology, [2] Cluster of Excellence PhoenixD, [3] Institute of Microbiology and Braunschweig Integrated Centre of Systems Biology, [4] Hannover Centre for Optical Technologies, Germany

Surface plasmon resonance biosensors are widely used as a real-time and label-free method for detection of microorganisms. While these biosensors are usually performed based on intensity measurement, phase change analysis improves their sensitivity due to its sharper response. In this project, the spectroscopic ellipsometry technique is used to measure changes in the phase and amplitude ratio of reflected light in the Kretschmann-Raether configuration to detect Gram-negative Escherichia coli (E. coli). This technique determines the optical properties and thickness of thin films by analyzing the polarization changes of the light after reflection or transmission from the layer of interest. In our recent research, modified porphyrin structures were synthesized for self-assembled monolayer formation on a gold surface as an antenna to monitor E. coli bacteria. A modified porphyrin was attached to the gold surface by thioacetyl groups, and an additional peptide ligand was attached to its structure for the purpose of capturing Gram-negative bacteria. In this work, we compare the performance of SPR biosensors using modified porphyrin and antibodies. Furthermore, for both intensity and phase changes, the optical response of the sensor structure is analyzed with respect to the ellipsometric measurement. The results show that, the resonance wavelength shifts are 2.5 nm for phase changes measurement and 0.6 nm for intensity ratio measurement for a 39 nm functionalized gold layer with modified porphyrin structure. In conclusion, Gram-negative E. coli bacteria can be linked through the porphyrin on the gold surface and detect by spectroscopic ellipsometry technique for biosensing applications.

P-791
Deciphering the effect of RNA on alpha-synuclein phase separation with fluorescence fluctuation spectroscopy
Sabrina Zappone[1,2], Eleonora Perego[1], Jakob Rupert[1,3], Elsa Zacco[1], Eli Slenders[1], Gian Gaetano Tartaglia[1,3], Giuseppe Vicidomini[1]
[1] Istituto Italiano di Tecnologia, [2] University of Genoa, [3] Sapienza University of Rome, Italy

Recruiting biomolecules inside liquid-like condensates, which result from a liquid-liquid phase separation process, is one of the strategies that cells implement to organize reactions in space and time. Enhancing the local concentration and promoting conformational changes, phase separation of amyloidogenic proteins may affect their assembly in aggregates associated with cytotoxicity. Recently, it has been shown that the aggregation of the amyloidogenic protein alpha-synuclein can be preceded by phase separation in liquid-like condensates. Interactions with other partners, such as RNA molecules, might influence alpha-synuclein phase separation and conversion of condensates to aggregates. How RNA modulates the process and the aggregation propensity of the protein, especially at the early stages, is still unclear. A highly sensitive and easily approachable methodology to study phase separation at the molecular scale in terms of dynamics is also missing. To overcome this limitation, we apply a comprehensive fluorescence lifetime and correlation analysis to investigate the effect of RNA molecules on alpha-synuclein phase separation. In particular, we used a new single-photon detector array integrated into a confocal microscope to implement fluorescence lifetime fluctuation spectroscopy (FLFS) and study the time evolution of alpha-synuclein phase transitions focusing on the early time points. Combining qualitative (diffusive modes, structural changes) and quantitative (condensate size) information provided by FLFS, we show how RNA modulates the kinetics behind the formation of nanoscale alpha-synuclein condensates. Deciphering the impact of RNA on alpha-synuclein phase separation is crucial for obtaining an extensive view of the key determinants of protein aggregation propensity.

P-792
Collective Variables based enhanced MD promotes the sampling of non-canonical DNA dynamics
Zhengyue Zhang[1,2,3], Miroslav Krepl[1,4], Vojtěch Mlýnský[1], Petr Stadlbauer[1], Giovanni Bussi[5], Petr Šulc[6], Chad Simmons[6], Nicholas Stephanopoulos[6], Jiří Šponer[1]
[1] Institute of Biophysics of the Czech Academy of Sciences, , Czech Republic, [2] Masaryk University, Czech Republic, [3] Masaryk University, Czech Republic, [4]Palacky University Olomouc, Czech Republic, [5] Scuola Internazionale Superiore di Studi Avanzati (SISSA), Italy, [6]Arizona State University, USA

The understanding of DNA structure is a cornerstone of investigations on biological processes, structure-based drug discovery, and DNA nanomaterial designs. The diversity of DNA structure and dynamics, however, has brought major obstacles towards further studies and applications. Despite various technical/experimental developments in Structural Biology, reaching high time- and scale-resolution details of DNA structures remains challenging. Therefore, with the potent computational capability, Molecular Dynamics (MD) is a powerful tool to fill the information gap. By focusing on two types of non-canonical DNA structures, Holliday Junction (HJ) and Guanine-Quadruplex (G4s), which represent the complicated DNA structural dynamics, as examples, and combining MD simulations with Collective Variables (CVs) based enhanced sampling methods, we observed abundant conformation changes of HJ and G4s at atomistic level and suggested potential intermediates on profiles of DNA structural transitions. During the explorations, we also raised attention to the inaccurate Lennard-Jones parameters and suggested system- specific modifications on the OL15 DNA force field. Overall, our studies demonstrated the power and perspective of MD simulations and CVs-based enhanced sampling methods, which could inspire and cooperate with the experiments for future breakthroughs in the studies of DNA structure and dynamics.

Index of presenting authors

For affiliation, please see corresponding Abstract.