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LATE ABSTRACTS

Poster Presentations

– Session 1 –

P-195

Redox regulation of Kv7 channels through EF3 hand of calmodulin

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Neuronal KV7 channels, important regulators of cell excitability, are among the most sensitive proteins to reactive oxygen species. The S2S3 linker of the voltage sensor was reported as a site mediating redox modulation of the channels. Recent structural insights reveal potential interactions between this linker and the Ca2+-binding loop of the third EF-hand of calmodulin (CaM), which embraces an antiparallel fork formed by the C-terminal helices A and B. We found that precluding Ca2+ binding to the EF3 hand, but not to EF1, EF2 or EF4 hands, abolishes oxidation-induced enhancement of Kv7.4 currents. Monitoring FRET between helices A and B tagged with fluorescent proteins, we observed that S2S3 peptides cause a reversal of the signal in the presence of Ca2+, but have no effect in the absence of this cation or if the peptide is oxidized. The capacity of loading EF3 with Ca2+ is essential for this reversal of the FRET signal, whereas the consequences of obliterating Ca2+ binding to EF1, EF2 or EF4 are negligible. Furthermore, we show that EF3 is necessary and sufficient to translate Ca2+ signals to reorient the AB fork. Our data is consistent with the proposal that oxidation of cysteine residues in the S2S3 loop relieves Kv7 channels from a constitutive inhibition imposed by interactions between the EF3 hand of CaM which is necessary and sufficient for this signaling.

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Candidalysin uses an unexpected mechanism to form membrane pores.

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C. albicans infection requires the action of the virulence factor candidalysin (CL), which damages the plasma membrane of epithelial human cells. However, the molecular mechanism that CL uses to permeabilize membranes was unknown. We used biophysical and cell biology methods to reveal that CL forms membrane pores using a unique molecular mechanism (Russell & Schaefer, et al. 2022 eLife). We observed that CL readily assembles into linear polymers in solution. Our data indicate that the basic structural unit in polymer formation is a CL oligomer, which is probably an octamer. Polymers grow by addition of CL oligomers, and long polymers can close into a loop. CL loops spontaneously insert into the membrane to become membrane pores. We identified the G4W mutation, which inhibited the formation of CL polymers in solution and prevented formation of pores in synthetic lipid membranes. Studies in oral epithelial cells (TR146) showed that G4W CL failed to activate the danger response signaling pathway, a hallmark of the pathogenic effect of CL. These results indicate that CL polymerization in solution is a necessary step for the damage of cellular membranes. Atomic force microscopy revealed co-existence of simple pores with complex pores decorated with protrusions. We propose that this structural rearrangement represents a maturation mechanism that stabilizes pore formation. Our investigation not only unravels a new paradigm for the formation of membrane pores, but additionally identifies CL polymerization as a therapeutic target to treat candidiasis.

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Optimal Resolution to Model Macromolecular Crowding Effects: Lessons from GB1 Dimerization

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Biochemical reactions occur in a heterogeneous and crowded environment. The crowder molecules exclude the reactant molecules from occupying certain regions of the cell and form soft nonspecific interactions (like hydrogen bonding and hydrophobic interactions), resulting in changes in the reaction thermodynamics and kinetics. Computer simulations are useful tools to obtain mechanistic insights into crowder induced changes to the reaction. However, from a simulation perspective, it is important to decipher the level of structural resolution in a protein-crowder model that can faithfully capture the influences of crowding on protein association with limited computational resources. Here, we investigate the dimerization of model system GB1 in the presence of lysozyme crowders at two structural resolutions. The lower resolution model assumes both protein and crowder species as spherical beads, similar to the analytical scaled particle theory model, whereas the higher resolution model retains residue specific structural details for protein and crowder species. From the higher resolution model, it is found that GB1 dimer formation is destabilized in the presence of lysozyme crowders, and the destabilization is more for the side-by-side dimer compared to the domain-swapped dimer, in qualitative agreement with experimental findings. However, the low resolution CG model predicts stabilization of the dimers in the presence of the lysozyme crowder, similar to the SPT model. Our results indicate a nontrivial role of the choice of model resolution in computer simulation studies investigating crowder induced effects.

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Mutations in tau protein influence aggregation propensity through conformation modulation

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Aggregation of the intrinsically disordered protein tau is a hallmark of several diseases, called tauopathies, including Alzheimer's disease. Mutations in the gene encoding tau are responsible for a class of inherited tauopathies called frontotemporal dementia and parkinsonism linked to chromosome 17Q (FTDP-17). These mutations are thought to trigger FTDP-17 by modulating the formation of tau amyloid fibrils. We have explored the mechanisms through which the diseases-associated single point mutations promote amyloid formation. We combined biochemical characterization and small angle X-ray scattering (SAXS) with advanced data treatment to study six different tau mutations. We found that the mutations promote aggregation to different degrees and can modulate tau conformational ensembles, intermolecular interactions and liquid-liquid phase separation propensity. In particular, we found a direct correlation between the aggregation lag time of the mutants and their radius of gyration. We show that mutations disfavor intramolecular protein-protein interactions which in turn favor extended conformations and promote amyloid aggregation. This work proposes a new connection between the structural features of an IDP monomer and their propensity to aggregate, providing a novel assay to evaluate aggregation propensity of tau variants.

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Membrane-Induced Conformational Switching and Activation of Apoptotic Inhibitor Bcl-xL

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Permeabilization of the mitochondrial outer membrane–a point of no return in apoptotic regulation–is tightly controlled by proteins of the Bcl-2 family. Apoptotic inhibitor Bcl-xL is an important member of this family, responsible for blocking the permeabilization, and is also a promising target for anti-cancer drugs. Bcl-xL exists in the following conformations, each believed to play a role in the inhibition of apoptosis: (i) a soluble folded conformation, (ii) a membrane-anchored (by its C-terminal α8 helix) form, which retains the same fold as in solution and (iii) refolded membrane-inserted conformations, for which no structural data are available. In this study, we present the summary of the application of various methods of fluorescence spectroscopy for studying membrane interaction of Bcl-xL, and specifically the formation of the refolded inserted conformation. We discuss the application of environment-sensitive probes, Förster resonance energy transfer, fluorescence correlation spectroscopy, and fluorescent quenching for structural, thermodynamic, and functional characterization of protein-lipid interactions, which can benefit studies of other members of Bcl-2 (e.g., Bax, BAK, Bid). The conformational switching between various conformations of Bcl-xL depends on the presence of divalent cations, pH and lipid composition. This insertion-refolding transition also results in the release of the BH4 regulatory domain from the folded structure of Bcl-xL, which is relevant to the lipid-regulated conversion between canonical and non-canonical modes of apoptotic inhibition. Supported by Grant 0122U001388 from the Ministry of Education and Science of Ukraine and by NIH grant R01GM126778.

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Phospholipid biomembrane treated with saponins – how to increase the effectiveness of antibiotics?

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Saponins are a large group of compounds characterized by their amphiphilic nature and natural origin. Their surfactant-like structure is responsible for their interaction with biomembranes; this includes pores formation, permeabilization, insertion, effects on the lateral organization, and dynamic properties of biomembranes [1]. Such properties make them a potential promising adjuvant not only for vaccines but also for antifungal, antibacterial, and anticancer pharmaceuticals. Most previous research concern the interaction of saponins with sterols, mostly cholesterol present in mammal cells [2], [3]. On the other hand, there is still a lack of research regarding the interaction of phospholipids and sterols present in fungal and bacterial cells with saponins. Thus, in this research, we decided to evaluate the interaction of different types of saponins with model phospholipid bilayers, as well as, in-vitro against microorganisms. This includes spherical bilayers structural studies such as Fourier Transform Infrared Spectroscopy, Differential Scanning Calorimetry, Atomic Force Microscopy, and Small Angle X-ray Scattering measurements. Moreover, zeta potential evaluation, assessment of hydrodynamic diameter of vesicles, and fluorescent leakage assays were also performed. As a bilayer model membrane, liposomes with different compositions were used. Obtained results suggest the changes in bilayer properties. Moreover, the leakage assays suggest higher digitonin affinity to ergosterol rather than cholesterol. A higher value of fluorescent intensity was obtained for POPC/Ergosterol treated with 0.03mM digitonin than for POPC/Cholesterol (both 70:30 %mol) - intensity 0.50 to 0.37 (a.u.) respectively. However, the interaction of saponins with phospholipid chains is strongly connected to the phospholipid headgroup rather than the chain. This can be concluded for zeta potential results – for the ethanolamine group, there has been a change for - 18mV, while for the choline headgroup for -12.5mV, while exposed to Sapindus mukorossi extract. This proves the potential for saponin use not only as a drug adjuvant but also as an additional agent in liposomal Drug Delivery Systems.

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A One-Bead-Per-Saccharide (1BPS) Model for Multiscale Modelling of the Brain ECM

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Glioblastoma is one of the most common and malignant brain tumors with a five-year survival rate of only 6%. The progression of this tumor is associated with an increase in its stiffness and remodeling of the extracellular matrix (ECM). The long-term aim of our work is to understand the relationship between this remodeling and the increased stiffness of the brain. For this we are currently developing a multiscale computational model of the brain ECM. The ECM of many tissues is based on collagen, whereas the brain ECM is mainly composed of hyaluronic acid, chondroitin sulfate proteoglycans and tenascins. We used iterative Boltzmann inversion (IBI) to develop a one-bead-per-saccharide (1BPS) model for hyaluronic acid and chondroitin sulfates based on an existing 3-bead-per-saccharide model. The coarse graining of the 1BPS model allows to make predictions that match experimental observations in length scales relevant to the brain ECM without any fitting parameters.To model proteoglycans, we have combined the 1BPS model with a one-bead-per-aminoacid (1BPA) model previously developed in our group and an elastic network model for the folded sections. This model has been validated for aggrecan (a well-studied proteoglycan) in terms of radius of gyration, and is being used now for predicting structure–property relationship of brain-specific proteoglycans, such as brevican, neurocan and versican. At a next stage, the model for hyaluronic acid and brain-specific proteoglycans will be combined with a model of tenascins in an ECM network model.

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Raman spectroscopy for noninvasive tissue-specific study of carotenoids in live insects

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Conventional study of carotenoids in small insects is challenging since it requires their extraction from biomass. We developed a noninvasive label-free approach to study the content and composition of carotenoids in living insects with resonance Raman spectroscopy applying 532 nm laser irradiation. This approach allows for avoiding insect sacrificing and carotenoid isolation. We verified the non-invasiveness of Raman spectroscopy to study living insects and estimated the carotenoid concentration in different segments of living insects at different stages of the same insect. The wings contain more carotenoids than the rest of the body, and the length of isoprenoid chains reduces as the larva matures. To investigate the food chain in insect community, we estimated carotenoid content and concentration in a plant gall of a hawkweed Hieracium × robustum, larva, and pupa of herbivorous gall wasp Aulacidea hieracii (Hymenoptera: Cynipidae) that induces a gall on the hawkweed, and a parasitoid wasp Eurytoma cynipsea (Hymenoptera: Eurytomidae) that attacks the gall wasp. The parasitoid larvae consume the gall wasp larvae that consume the gall tissues. We showed that carotenoid composition in the parasitoid closely corresponds to that of its diet—the gall-former. On the contrary, carotenoid composition in the gall-former was independent of that in the gall tissues. To conclude, Raman spectroscopy was shown as a powerful technique for a noninvasive tissue-specific study of small insects and the food chains in insect communities. Nikelshparg et al. Chemoecology 32, 31–40 (2022).

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AFM-based nanomechanical biomarkers: from cancer to pulmonary fibrosis

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A range of pathological conditions, including cancer and pulmonary fibrosis, are closely related to alterations in the content, structure, and mechanical properties of biological tissues. Fibrosis and desmoplasia (a specific type of cancer fibrosis), lead to tissue stiffening that poses a major barrier to effective drug delivery due to blood vessel inefficiency and hypo-perfusion. Also, the efficacy of standard chemotherapies and pulmonary fibrosis therapies vary due to intra- and inter- patient variability and due the absence of specific biomarkers that can characterize the state of a specific fibrotic tissue. Consequently, there is an urgent need for the development of novel biomarkers that characterize the mechanical state of a particular pathological tissue to support the development of novel therapeutic strategies. Atomic Force Microscopy (AFM) arises as a unique tool for assessing the nanomechanical properties of tissues. In this work we present our research results on using AFM and optical microscopy techniques for assessing the nanomechanical profile of highly desmoplastic tumors and fibrotic lungs, during cancer and pulmonary fibrosis progression. Also, AFM techniques were used for assessing specific treatment outcomes. Our results highlight that AFM is sensitive enough to assess small nanomechanical alteration during pathological conditions progression and during or after treatment. The identification of unique AFM-based nanomechanical fingerprints can lead to the development of novel mechanical biomarkers for treatment perdition and monitoring.

P-204

Rapid Conformational Switching of Single DNA Hairpins

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DNA hairpins play an essential role in replication initiation and are commonly used across a number of synthetic bionanotechnological applications. The thermodynamic details of hairpin formation have been extensively investigated; however, the kinetic aspects remain largely underexplored. Here we use single-molecule Förster Resonance Energy Transfer (smFRET), as a powerful approach to quantify the opening and closing dynamics of both freely diffusing and surface immobilised adenosine-rich hairpins, conjugated with the fluorophores Cy3 and Cy5. By modelling FRET efficiency trajectories with Hidden Markov based algorithms, relative populations and time-dependent kinetic switching rates were extracted. Two environment-sensitive states corresponding to “open” and “closed” forms of the hairpin were thus identified. The folding reaction was observed to be controllable and strongly dependent on a variety of factors including pH, and ionic strength as well as the presence of a range of molecular weights of macromolecular crowders. A label-free sensing strategy based on quartz crystal microbalance with dissipation (QCM-D) monitoring is also implemented to quantify the conformation of surface-immobilised hairpins via measurement of the acoustic frequency and dissipation. These results also distinguish between open and closed forms of the molecule, confirming that transitions between states are reversible and controllable. The acquisition of single-molecule FRET trajectories from both freely-diffusing and surface-tethered hairpins, coupled with QCM-D measurements, serves to enhance our fundamental mechanistic understanding of DNA hybridization and may have important implications for applications involving biosensing and bioswitches.

P-205

Anti-amyloid activities of BRICHOS are regulated by a pH-sensitive switch – implications for protein misfolding diseases

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Proteins can form amyloid fibrils or amorphous aggregates, which can lead to disease. Molecular chaperones can prevent both types of protein aggregation, but the mechanisms are not fully understood. The BRICHOS domain is a small heat shock protein-like chaperone, with multiple activities against amyloid fibril formation, amyloid-induced neurotoxicity, and amorphous protein aggregation. Malfunction of BRICHOS, even with enhanced chaperoning capacity, can lead to human amyloid diseases, while the underlying pathogenic mechanism is to be elucidated. Using interdisciplinary methods, we show that BRICHOS effects against fibril formation and amyloid-induced toxicity are differently regulated by a conserved aspartate residue. At neutral pH, BRICHOS efficiently prevents amyloid-induced neurotoxicity, but between pH 6.0–7.0 this Asp gets protonated, which selectively abolishes the effects against amyloid-induced neurotoxicity, whereas the efficiency against amyloid fibril formation is oppositely enhanced. The BRICHOS ability to suppress amorphous protein aggregation is unchanged by Asp to Asn mutations but this activity is mediated by a loop region comprising three hydrophobic motifs. Furthermore, the Asp is evolutionarily highly conserved in >3000 analysed BRICHOS domains but is replaced by Asn in some BRICHOS families and animal species, suggesting diverse BRICHOS activities. These results reveal that BRICHOS activity can be affected by environment and shed light on how chaperone dysregulation causes disease.

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Ultrasensitive Fluorescence Detection of Amyloid-Induced Vesicle Damage

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Membrane-protein interactions are critical for many biological processes, with membrane perturbation strongly linked to neurodegenerative pathways in dementia. To gain mechanistic insights into these interactions, we have developed high-throughput fluorescence assays based on Förster resonance energy transfer (FRET) to measure interactions between lipid vesicles and pre-formed neurotoxic amyloid-β (Aβ) oligomers. Our approach involves the immobilisation of highly-curved vesicles containing the membrane probes DiI and DiD, and single vesicle imaging to quantify changes in FRET upon Aβ perturbation. Our study reveals that pre-fibrillar Aβ oligomers induce vesicle perturbation comprising permeabilization, swelling, and fusion events, which may have implications for critical transport pathways. Our FRET-based approaches offer high sensitivity to nanoscale morphological changes within single vesicles, reveal otherwise hidden conformational states, and are likely to be applicable beyond the sub- micron sized vesicles studied here for contributing new clues to membrane disruption induced by a wide variety of perturbative molecules and proteins.

P-207

Spatiotemporal variation of macromolecular crowding in cells

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The interior of the cells is rich in different types of biomacromolecules, creating a crowded milieu. This highly crowded environment and the resulting high excluded volume influence processes such as diffusion, folding, conformation, and aggregation or association. Macromolecular crowding effects should be highly dependent on the distribution and organization of biomacromolecules and increases with, for instance, a cellular volume decrease induced by osmotic stress or mechanical pressure. It is, however, not clear how the macromolecular crowders are arranged in the cell and how this depends on stress conditions. Here, we probe macromolecular crowding at different locations in the cytoplasm of mammalian cells and how this changes after cell stress. We used a genetically encoded Förster resonance energy transfer FRET-based sensor that measures macromolecular crowding in the cytoplasm of living cells. Local variation in macromolecular crowding was measured by targeting the sensors to specific regions of the cell, by fusing it with specific localization domains. Next, we determined that the differences between local crowding changes in response to cell stress and subsequent cell recovery. Such changes in local crowding could be hotspots for pathogenic protein behavior.

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Modulating thermostability of bacterial EF-Tu through subtle and minimalist engineering

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Molecular basis of thermostability in proteins is diverse and not fully resolved. To better understand it, we have solved high-resolution crystal structures of four 0.5 – 3.5-billion-year old ancestral bacterial Elongation Factor-Thermounstable (EF-Tu) proteins. Comparing the modern and ancient EF-Tu structures, we could identify only two key interactions in the thermophilic EF-Tus that are absent in the mesophilic variants. One interaction involves a Tyrosine located between the P-loop and Switch-I of the G-domain that forms a H-bond with the α- phosphate of GTP/GDP. The other interaction involves a salt-bridge between a conserved Arginine from Domain II and a Glutamic acid from Domain III. Mesophilic EF-Tus lack these stabilizing interactions as a Phenylalanine replaces the Tyrosine in the G domain and an Aspartic acid substitutes the Glutamic acid in domain III. We could switch thermostability and thermoactivity of the modern thermophilic and mesophilic EF-Tus by swapping these amino acids, and could clarify the molecular mechanisms by conducting molecular dynamics simulations. Our analysis suggests that nature has evolved thermostability in EF-Tu through subtle and minimalistic engineering.

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In vitro and In-cell characterization of Quadruplex-duplex hybrids: conformation, folding, and recognition by drug-like ligand molecule

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G-quadruplexes (G4) represent one of the crucial non-canonical structures in the genome's non-coding part, such as promoters of (onco-)genes and telomeres having significant biological functions. A quadruplex-duplex hybrid (QDH hereafter) is designed upon the juxtaposition of a duplex stemloop onto a G4 across the multiple edges of a quadruplex core. QDH shows diverse functionalities ranging from (patho)biological processes to nanotechnology. Under in vitro conditions, the individual conformations can be separated and characterized at the atomic level. But, the identity of the conformation, which is responsible for biological function in vivo, remains obscured due to the lack of suitable technology allowing highresolution structural studies under physiological conditions in vivo. The biggest challenge is to develop a ligand specific to a bioactive genomic QDH sequence with minimal off-target binding. This work showed the selective recognition and structural elucidation of distinct QDH conformation from the PIM1 gene by two small-molecule ligands with state-of-the-art NMR in conjugation with low-resolution spectroscopic techniques. The structural insights will help to design sequence and scaffold-specific ligands. On the contrary, we customized "in-cell" NMR strategies to monitor de-novo QDH DNA folding and ligand binding in Xenopus laevis Oocytes (eukaryotic cell model) by using selectively labeled 19F modified nucleotides containing 2-deoxy-2-fluoro-arabinonucleic acid (FANA). In conclusion, we aim to address ligand interactions of non-canonical nucleicacids in vitro and living cells by integrating low- and high-resolution spectroscopy.

P-210

Protonuclei in silico: The impact of crowding and confinement on the condensation of intrinsically disordered nuclear proteins

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The liquid-liquid phase separation (LLPS) of intrinsically disordered proteins plays an integral part for the formation of membraneless organelles in cells, which in turn have key functional and regulatory roles. The majority of ongoing studies on LLPS focus on bulk solutions of purified proteins, but living cells are highly influenced by macromolecular crowding and confinement exerted by the cell membrane. To mimic more closely conditions prevalent in cellular environments, we perform coarse-grained molecular simulations [1] of the low-complexity domains of heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1) and fused in sarcoma (FUS) in spherical confinement, where we systematically vary the fraction of the crowding agent polyethylene glycol (PEG). We further introduce cross-links between the PEG chains to create a viscoelastic network, finding that the network dramatically limits the size and mobility of the protein condensates.

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Hydrogel-based biosensors for non-contact monitoring of glucose and pH levels

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Hydrogels are water insoluble hydrophilic polymers capable of taking in water while maintaining their structure. By incorporating functional co-monomers in the polymer matrix one can design hydrogels sensitive to different environmental parameters. Biocompatible hydrogels that are responsive to specific molecules such as glucose and undergo reversible volumetric changes under variable glucose concentration can be used as implantable glucose sensors. Monitoring glucose levels is of major importance for diabetic patients. Diabetes is a chronic disease characterized by disorder of glucose metabolism which results in elevated concentration of blood glucose. A major issue in diabetic patients is the self-monitoring of glucose levels with conventional glucose-meters that are invasive causing pain and possible infections. Here we present the properties of implantable hydrogel-based biosensors for non-contact subcutaneous monitoring of glucose and pH-levels measured by scanning using optical coherence tomography (OCT). Both types of biosensors undergo significant reversible volumetric changes with an average response time of 10 min. The glucose sensitive hydrogel undergoes 12% expansion when placed in a 20 mM glucose solution while the pH sensitive hydrogel shrinks by 21%when the solution pH changes from 7 to 9. Optical coherence tomography is a non-invasive imaging technique that utilizes infrared light and provides depth-resolved cross-sectional images. Combined with the implantable hydrogel sensors OCT can have high potential for continuous in-vivo monitoring of glucose or pH levels.

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Exploring the structural and biochemical characterization of membrane proteins in near-native conditions

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Integral membrane proteins perform key cellular functions. While they represent about a third of all proteins encoded in the human genome, they are the target of roughly 70% of all approved drugs. Unfortunately, their structural and biochemical characterization has often required their removal from the membrane using mild detergents. This brings the protein out of its native environment, which, in many cases, compromises function. Alternatively, synthetic polymers made of alternating acidic and hydrophobic groups can directly extract membrane proteins into a nanodisc made by its native lipids. However, they usually show lesser solubilization capacity, and their general applicability for membrane protein biochemistry is still poorly understood. Here, we directly solubilized the membrane proteome of HEK293 cells using different nanodisc-forming polymers as well as commonly used detergents, and used mass spectrometry to compare them. Our results show that different classes of polymers show distinct solubilization profiles, which are themselves different from those observed with detergents. We observed no correlation between number of transmembrane domains and solubilization ability. Interestingly, polymers with cycloalkane side chains show solubilization capabilities which rival those observed with commonly used detergents such as DDM and LMNG. Finally, we used electron cryo-microscopy to explore the structural characterization of selected membrane proteins embedded into nanodiscs in a detergent free manner. We discuss challenges and potential pitfalls of native nanodiscs for Cryo-EM.

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Structural and energetic determinants for substrate transport by the serotonin transporter

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The serotonin transporter (SERT) is a sodium driven secondary active transporter expressed on pre-synaptic serotonergic neurons. Its role is to retrieve previously released serotonin (5HT) neurotransmitters back into the synapse, thereby ensuring neurotransmitter homeostasis and shaping the signal of synaptic signal transmission. Clinically approved prescription drugs, but also illicit drugs of abuse interfere with the transport function of SERT by blocking the transport cycle or by inducing reverse transport. Both alterations result in a rise of extracellular neurotransmitter concentrations. We combined in-silico studies, chemical synthesis and in-vitro experiments to investigate the molecular determinants that trigger substrate occlusion and cellular uptake. We find that the length of the aliphatic chain of 5HT is essential for energy transduction leading to SERT occlusion. A slight variation of the chain length by a congeneric series of 5HT analogues that changes to position of the positively charged nitrogen had a strong impact of binding, transporter occlusion, substrate uptake, and the free energy surface of substrate induced transporter occlusion. Interrupting the conformational coupling leads to a reduction or a blockage of substrate uptake by impaired energy coupling. We found that the cognate substrate 5HT shows the highest uptake rate and is optimised in size to fit the occluded state, but too small for the open state, thereby efficiently triggering SERT occlusion and initiating substrate uptake.

P-214

Recombinant anti-amyloid BRICHOS domain for Alzheimer's disease treatment in mouse models

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Alzheimer’s disease (AD) is the most common form of dementia and a progressive neuro-degenerative disorder. The pathological features of AD mainly include senile plaques formed by the deposition of β-amyloid (Aβ) peptide, neurofibrillary tangles of tau, and neuroinflammation as evidenced by astrocyte and microglial activation and increased levels of proinflammatory cytokines. Many attempts have been made to target Aβ pathology to treat AD but without much breakthrough, therefore, there is an urgent need for effective strategies. The molecular chaperone BRICHOS domain is a potent inhibitor of Aβ amyloid fibril formation in vitro, attenuates Aβ neurotoxicity in hippocampal slice preparations ex vivo and in Drosophila and mouse models in vivo. The aim of this study is to treat 9-month-old APPNL-G-F knock-in AD mouse models, which have fully developed AD pathologies, with the blood-brain barrier-permeable recombinant human (rh) Bri2 BRICHOS R221E. The App NL-G-F and age-matched wildtype mice were treated with rh Bri2 BRICHOS R221E or vehicle for two months via intravenous tail injection, and the neurological improvement and anti-inflammatory responses were assessed. The results showed that the treatment with rh Bri2 BRICHOS R221E significantly reduced neuroinflammation in APP NL-G-F knock-in AD mouse models. Further, rh Bri2 BRICHOS R221E treatment reduced the level of some proinflammatory cytokines in both hippocampus and cerebral cortex. Taken together, these observations indicate that the BRICHOS domain holds a translational potential in the fight against AD.

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Strategies and applications for rotational diffusion measurements of large biomolecular complexes using selective time-resolved anisotropy with reversibly switchable states

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Time-resolved fluorescent anisotropy (TR-FA) is a valuable tool to probe the rotational diffusion of small molecules, peptides and proteins. TR-FA is limited to fast diffusion of sufficiently small target structures via the fluorescent lifetime of the labeling fluorophore. To extend the range of probable biomolecular complexes, we utilize long-lived molecular transitions of reversibly switchable fluorescent proteins (rsFPs). Using visible light of different wavelengths rsFPs can be selectively switched between a fluorescent ON and a non-fluorescent OFF state. When these transitions are induced with polarized light, they can be used to measure rotational diffusions over a time domain orders of magnitude longer than possible with TR-FA. We call this new method selective time-resolved anisotropy with reversibly switchable states (STARSS) (1). The use of genetically encoded rsFPs makes STARSS particularely suitable for live cell applications, but also requires special labeling strategies to achieve a tight correlation between the rotational movement of the rsFP probe and the labeled target structure over the full observation time. I will discuss important considerations for STARSS measurements in vitro and in living cells that allow us to observe rotational diffusion dynamics of biomolecular complex in the MDa size range that have previously been inaccessible. (1) Volpato, A., Ollech, D., Alvelid, J. et al. Extending fluorescence anisotropy to large complexes using reversibly switchable proteins. Nat Biotechnol (2022). https://doi.org/10.1038/s41587-022
-01489-7

P-216

Cooperative change in internal dynamics during streptavidin-biotin binding

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Molecular dynamics are vital for the function of proteins. Upon protein ligand binding, changes of conformational entropy occur in the protein and its hydration layer, as well as its internal dynamics. The binding of biotin to streptavidin was investigated using QENS, as well as Thermal Diffusion Forced Rayleigh Scattering (TDFRS) and ITC. This interaction is enthalpy driven, with an opposing entropic component. An experimental investigation of the components of the entropy change, specifically the change in conformational entropy, indicates it is strongly opposed to the binding. This adverse change in entropy has to be compensated, the strongest candidate is a supportive entropy change of the surrounding hydration layer. It is also interesting that the change in conformational entropy upon saturation with biotin is on the same order of magnitude as that of protein folding, without an associated significant structural change. On the ps timescale the surface residues of free streptavidin show a jump diffusive dynamic, while the fully saturated less flexible streptavidin biotin complex does not exhibit jump diffusive motions. This is indicative of the dynamic of streptavidin being changed for the whole protein upon biotin saturation, instead of only in the binding pocket. Experiments focusing on the internal dynamics at different degrees of biotin saturation were performed. It was found that the internal dynamics of streptavidin are already drastically altered for only one biotin binding, which corresponds to 25% saturation. Such a suppression of dynamics over the whole protein upon partial saturation is best explained by a cooperative process.

P-217

GTP hydrolysis by eIF2 and binding of eIF5B rearrange the human 48S initiation complex for ribosomal subunit joining

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GTP hydrolysis by eIF2 and eIF5B-mediated joining of the 60S subunit are major checkpoints in translation initiation by the 48S initiation complex (48S IC). At present, it is unclear how GTP hydrolysis by eIF2 and eIF5B binding remodel the 48S IC and clear the anti-association activity of eIF3 to promote subunit joining. Here, we use cryo-EM to visualize key intermediates of the human 48S IC reconstituted on mRNA with a cognate AUG codon in the presence of eIF1, eIF1A, eIF2–GTP–Met-tRNAiMet, eIF3, eIF5 and eIF5B–GTP. In the initial state with eIF1, the initiator tRNA basepairs only partially with the mRNA codon and the small ribosomal subunit (SSU) adopts an open conformation. Start codon recognition triggers closure of the SSU, replacement of eIF1 by eIF5, and a large-scale rearrangement of eIF2 after GTP hydrolysis, resulting in tRNA release by eIF2 and making way for eIF5B. Importantly, these changes also initiate dissociation of the N-terminal domain (NTD) of eIF3c from the SSU intersubunit side, thereby abolishing the ribosomal anti-association activity of eIF3c. eIF5B initially binds to the 48S IC in presence of eIF2 and eIF5, and reorients the tRNA. Subsequent dissociation of eIF5 and eIF2 results in almost complete dissociation of the eIF3c-NTD and a gradual change in tRNA orientation, which would allow 60S subunit joining. In summary, our findings reveal how GTP hydrolysis by eIF2 enables handover of initiator tRNA to eIF5B and initiates eIF3 rearrangements to facilitate 60S subunit joining.

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Small-angle X-ray scattering study distinguishing PEG crowder's effects on hemoglobin solution structure

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Polyethylene glycols (PEGs) are widely used as precipitants for protein crystallization, crowding agents, or stabilizers in pharmaceutical formulations. Different UV, circular dichroism, IR and NMR spectroscopy studies were dedicated to the PEG effect on the protein structures. We report small-angle X-ray scattering (SAXS) results on the effect of PEGs on human hemoglobin (Hb) solution structure depending on the PEG molecular weight and concentration. A significant difference in the Hb molecule shape and flexibility was found in the presence of PEG4000 and PEG600. The comparison of Hb gyration radii at varying PEG600 concentrations showed an altered macromolecule solution size at high PEG600 concentrations. We hypothesize that the changes in the structural parameters are not only related to monomer-dimers-oligomers crowding effects, but also to differences in the protein folding caused by PEGs. This is suggested by comparing the data for two PEG sizes at equimolar concentrations of PEG monomers per protein molecule. The results provided a better understanding of the PEG interaction with Hb and complemented previous knowledge.

P-219

Effect of lipid membrane compartmentalization on bacterial cell division

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Cell division is a central process to life as it ensures that newer cells can be formed from older ones. Over the course of evolution, living cells have developed a sophisticated machinery to divide their compartments in a highly regulated manner. Developing minimal cell division models have long been the goal of bottom-up synthetic biology as these would help elucidate the transition from a chemical to a biological system at the origins of life. The presence of membrane compartments with coexisting liquid phases have distinct compositions, which help in the reorganization of both lipids and proteins. Conformational changes leading to bacterial division are achieved through different biophysical strategies, including local variations in the chemical composition of the membrane, the reorganization of the bacterial proteins and the expression of curvature-generating lipids and proteins. Here, we present a simple model that mimics the bacterial cell division process in the light of membrane properties and to understand how the membrane compartments affect the interaction of bacterial proteins (specifically MinCDE and FtsZ) during this process. This would be a beneficial in-vitro system to provide a basic framework for understanding cellular division mechanism.

P-220

Single-molecule FRET study of conformational dynamics of CRABP1 under denaturing conditions in crowded environments

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In this work, the conformational dynamics of the cellular retinoic acid binding protein 1 (CRABP1) under denaturing conditions in the absence and presence of crowding agents are studied by single-molecule FRET. In order to adequately probe the conformational dynamics, a FRET pair (AL488/AL647) is chosen that offers a high FRET efficiency contrast in denaturing environment. Urea is used to ensure chemical denaturation of CRABP1. To mimic the dense cellular environment around CRABP1, PEG2000 and PEG4000 are used as crowding agents. CRABP1 is first studied under native conditions in the absence of crowding agents to investigate the folded state, which is characterized by a single static population in the corresponding FRET histogram. Upon stepwise increase in urea concentration, conformational dynamics between the folded and the denatured states are observed, indicated by a dynamic shift from the static FRET line to longer fluorescence lifetimes. At the transition mid-concentration Cm, the influence of crowding agents on the conformational exchange is studied. The kinetics of the underlying dynamics at Cm in presence and absence of crowding agents are revealed by photon distribution analysis, suggesting a two-state dynamic exchange on the time scale of hundreds of microseconds.

P-221

Deep learning protein conformational space

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Determining the different conformational states of a protein and the transition paths between them is key to fully understanding the relationship between biomolecular structure and function. This can be accomplished by sampling protein conformational space with molecular simulation methodologies. Despite advances in computing hardware and sampling techniques, simulations always yield a discretized representation of this space, with transition states undersampled proportionally to their associated energy barrier. We present a convolutional neural network that learns a continuous conformational space representation from example structures, and loss functions that ensure intermediates between examples are physically plausible. We show that this network, trained with simulations of distinct protein states, can correctly predict a biologically relevant transition path, without any example on the path provided. We also show we can transfer features learned from one protein to others, which results in superior performances, and requires a surprisingly small number of training examples.

P-222

Resonance Light Scattering Correlation Spectroscopy and Its Bionanophotonics

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Resonance light scattering correlation spectroscopy (RLSCS) is a new single particle detection technique. RLSCS curve is measured by autocorrelating on the obtained RLS photon fluctuation with time occurred within a laser-illumination volume (less than 1 femtoliter). RLSCS can provide with the concentration, diffusion coefficient (translation and rotation) of nanoparticles and RLS brightness per particle, etc. We proposed its theoretical model and studied the diffusion behaviors of nanoparticles in the solution including its translational diffusion and rotational diffusion, and established the measurement methods about their concentration, size, aspect ratios and size distribution of nanoparticles based on the constructed confocal RLSCS optical detection system. And, based on these measurement methods with RLSCS, we developed some detection strategies for homogeneous DNA detection, homogeneous immunoassay, apoptosis assay, and protein corona formed in the NP's surface. Further, using the EMCCD as optical detector instead of SPCM, we developed tempo-spatially resolved RLSCS methods and their theoretical models based on the total internal reflection (TIR) and dark-field illumination optical configurations. These methods extended the optical excitation configuration of RLSCS from single spot excitation (confocal configuration) to wide-field excitation (light-sheet configuration) and makes RLSCS have the ability for multiple channels detection with tempo-spatial resolution. Finally, we successfully used these methods for investigating dynamic behaviors of gold nanoparticles (GNPs) within living cells and determined the concentration of protein or enzyme in single living cells.

P-223

Scrutinizing the protein hydration shell from MD simulations against consensus small-angle scattering data from a worldwide round-robin benchmark

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The proteins hydration shell plays key roles in protein stability, folding, recognition, enzyme activity and several others. However, it remained unclear whether the hydration shell predicted by explicit-solvent MD simulations matches experimental conditions, while accurate experimental probes of the hydration shell structure remained limited. Small-angle scattering (SAS) in solution using X-rays (SAXS) or neutrons (SANS) in principle provides information on the hydration shell, since both the radius of gyration (Rg) and the zero-angle scattering (I0) depend on the hydration shell contrast relative to bulk solvent. Using MD simulations and explicit-solvent SAXS/SANS calculations, we computed Rg and I0 for five different proteins and a set of 18 different combinations of protein force fields and water models, and we validated the simulation results against consensus data from a recent worldwide round robin benchmark (Trewhella, Vachette et al., doi:10.1107/S2059798322009184). Overall, we find remarkable agreement between MD simulations and consensus SAS data, however the agreement is protein and force field-dependent. Beside the influence of the shape, geometry, surface composition and size of the protein under investigation, certain force field/water model combinations are underestimating the hydration layer contrast significantly, while some water models with increased dispersion interactions may, for some proteins, overestimate the hydration layer contrast. Our study shows that explicit-solvent SAS calculation and consensus SAS data provide a novel route for scrutinizing the hydration layer of proteins.

P-224

Towards a Minimal Markov Model for Rotary Catalysis of F1-ATPase

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F1-ATPase, the catalytic domain of the F-ATP synthase, is an ATP-driven rotary molecular motor. Despite wide acceptance of the rotary catalysis concept, a consensus on the precise mechanism of F1-ATPase remains elusive. Here we aim at a minimal, unified and thermodynamically consistent model that reproduces all essential experimental results on F1-ATPase, including a tight coupling between catalysis and rotation, nucleotide titration curves, and rotation kinetics. We formulated a generic Markov model incorporating only a few essential degrees of freedom (DOF) such as discrete orientations of the γ-subunit and several β-subunit conformations. Several alternative assumptions about the DOFs were explored, resulting in a set of model variants. Each variant was trained using a Bayes approach and cross-validated. Only one variant agrees with all experimental data, which we propose as a minimal model for F1-ATPase. This model reconciles the decade-long bi- site vs. tri-site controversy – both pathways contribute, dominating at different ATP concentrations – and assigns crystal structures to defined stages in the catalytic cycle. Unexpectedly, to explain all the experimental data, at least four conformations of the β-subunits are required. Additionally, proper modelling of the interactions between the γ- and β-subunits is essential.

P-225

Study on Phase Separation of Fused in Sarcoma (FUS) by Fluorescence Correlation Spectroscopy

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Liquid-liquid phase separation (LLPS) of Fused in Sarcoma (FUS) has emerged as a fundamental principle underpinning cellular function and malfunction. However, we know little about the FUS phase transition process from individual molecules to nanoscale condensates, which is likely to play important roles in various cellular processes. Here we propose the FCS method to systematically study the phase separation process of FUS protein from single molecule to nanoscale condensates with fluorescent tag enhanced green fluorescent protein (EGFP) in different solution environments and incubation conditions. The characteristic diffusion time **(τD)** of the protein complexes can be obtained from the FCS curve, which increases with the growth of the protein hydration radius at the same environmental viscosity. The bigger τ**D** of the protein complex, the larger the aggregates formed by the phase separation of FUS. We constructed the FUS phase separation platform, then made the biomolecules incubate with FUS-EGFP at the concentrations from 5 nM to 200 nM. By this method, we have plotted FUS phase diagrams based on τ**D**, evaluated the inhibition efficiency of various biomolecules on FUS phase separation, and identified single-stranded DNA sequences that can interact with FUS and thus inhibit its phase separation. The FCS-based method for protein phase separation research has great potential for discovering interactions related to phase separation in organisms and for screening drugs for neurodegenerative diseases by targeting phase separation.

P-226

Investigating the Feasibility of Mid-range Infrared Spectroscopic Analyzer (MIRA) for Quantification of Poly-sorbate 20

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The accurate quantification of polysorbate 20 in drug formulations is of critical importance in the pharmaceutical industry. In this study, the behavior of PS20 with different placebos and a drug substance using mid-infrared spectroscopy was investigat-ed. Infrared spectroscopy was applied to determine the correlation between the absorption values of the ν(C=O) and the ν(C-O) band of PS20 and the nominal PS20 content. A linear regression analysis showed a strong correlation between the absorption values and the nominal PS20 content. To validate the accuracy of our method, a cross-validation model was applied to deter-mine the effectiveness of MIRA for the quantification of PS20 in different samples. The study showed that the method is capa-ble of quantifying PS20 as low as 0.04 g/L in the placebos and 0.12g/L in drug substance with high accuracy and precision, demonstrating the high sensitivity and reproducibility of the MIRA analyzer. The results demonstrate the potential of this method as a useful tool for the pharmaceutical industry, given its ability to accurately quantify PS20 in drug substances.

P-227

Role of cholesterol and membrane dynamics in pore-formation mechanism of Listeriolysin O

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Listeriolysin O (LLO) is a pore-forming toxin belonging to the family of cholesterol-dependent cytolysins (CDCs). It is major virulence factor secreted by Listeria monocytogenes; a causative agent of listeriosis which is a serious food-borne disease that severely affects pregnant women and immunocompromised individuals. Cholesterol is known to play an indispensable role in pore-formation mechanism of CDCs, however, the implication of cholesterol in action of LLO is not well understood. In our study, we have investigated role of cholesterol in the pore-formation mechanism of LLO. We utilized a multitude of biophysical and biochemical assays with artificial membranes, erythrocytes, and epithelial cells serving as the primary test subjects. We observed that LLO displays a unique mechanism of cholesterol interaction during pore-formation as compared to the other CDCs. Cholesterol enhances the saturation threshold of the membrane-LLO association, and it is obligatory for further oligomerization, unlike other CDCs. The molecular basis of LLO-cholesterol interaction has been traced to a conserved cholesterol recognition motif (CRM). We unravel role of CRM in overall membrane association of LLO apart from recognition of cholesterol. We have also observed that in absence of functional CRM, LLO utilizes membrane line tension as an alternative means for LLO-membrane association. In summary, our study offers new insights regarding pore-formation mechanism of LLO, and it underscore the significance of interplay between membrane dynamics and pore-formation that can be extrapolated to the biology of pore-forming toxins.

P-228

Biophysical investigations of antimicrobial peptide mimics for mechanistic studies

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Antimicrobial resistance has emerged as a rising issue plaguing the world health today. With this, the focus on the development of Antimicrobial peptide and its various mimics too has been intensified. However, we still need a detailed analysis on the driving force of the membrane perturbing activities of the AMP and their various mimicking agents. Here, we shall be discussing the two branches of AMP mimics, peptoids and small molecules. Further, we explore the mode of action behind the antimicrobial properties displayed by the library of mimics. To address this big challenge of understanding how the membrane active compound behaves, each of the compounds were introduced to the liposomes, mimicking the bacterial model membranes, and their interactions was recorded using solid state nuclear magnetic resonance spectroscopy. We observe that each of the mimics interact with the liposome as the global shape of the vesicles gets deformed. Also we observed a definite interaction with the fatty acyl chain as changes in order parameters were observed with respect to that of pure lipids. Also, to develop a thorough understanding of the different membrane interactions of these compounds, several fluorescence studies were executed using both bacterial and mammalian model membranes. One of the studies include, the dye release studies which provides information on the abilities of the membrane active compounds to form pores. The calcein dye release test revealed that the small molecule mimics undergo a cooperative effect. For the peptoids investigated, the extent of release could be correlated with their antimicrobial activity as well as the toxicity data.

P-229

Effects of Choline bicarbonate: maleic acid-based Deep Eutectic Solvents on the Microrheology of Mucus

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Mucus is a slippery viscoelastic layer that is majorly found above the epithelial cell layers of the gastrointestinal (GI) tract. It gives protection against various pathogens in the lining of the lungs and GI tract, and resists the stomach acids from digesting the stomach itself. Contrary to this, mucus also helps in the selective absorption of nutrients in the small intestine. Mucus consists of mucin a glycoprotein, which imparts the viscous nature of mucus. Along with mucin, various non-mucin proteins such as albumin and lysozymes are also present and they interact with mucin protein for the hierarchical assembly of mucins. Mucus has a unique viscoelastic property, alteration of this property leads to various disease conditions like cystic fibrosis and gastric ulcer, etc. Ionic liquids (ILs) and deep eutectic solvents (DESs) have been applied to alter the viscoelasticity of mucus in order to deliver the drug of interest to the researchers. DES enhances the delivery of certain drugs through the mucus layer preserving the chemical integrity of mucus, which were unable to permeate initially. DESs are very promising due to their effectiveness and biocompatibility. In this work, the effects of DESs on the mucus layer have been thoroughly investigated in terms of changes in the microrheology of the mucus layer by using diffusing wave spectroscopy (DWS). This work focuses on the effects of Choline bicarbonate-maleic acid-based DES on pristine mucin and mucin with non-mucin protein complex in order to know the mechanism of action of DES on the native mucus layer.

P-230

Effect of Multivalent Salts on Protein Diffusion under Crowding Conditions

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The biophysical properties of proteins change in the high-concentration limit of the macromolecules surrounding them. However, many protein studies are performed in dilute solutions. To learn more about physiologically relevant conditions, a comprehensive understanding of the effects is required. We study protein diffusion, focusing on anisotropic protein-protein interactions. Previous studies have shown that there is a coupling between rotational and translational diffusion. The strength of this correlation depends on protein concentration, shape, and charge. Bovine serum albumin shows intermediate coupling, which we alter with trivalent salts, e.g., $LaCl_3$. This leads to changes in phase behavior, including reentrant condensation and LLPS. We measure protein dynamics using a variety of NMR methods. The translational diffusion coefficient is measured by PFG NMR, while information on rotational diffusion is obtained from $T_1\rho$ and T_2 relaxation times. A retardation factor is then calculated, compared with macroviscosity, and analyzed with an appropriate fitting model to quantify the effects of salt and protein concentration.

P-230

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P-231

Scrutinizing the Protein Hydration Shell from MD simulations against SAXS/SANS data from a worldwide round robin benchmark

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The proteins hydration shell plays key roles in protein stability, folding, recognition, enzyme activity and several others. However, it remained unclear whether the hydration shell predicted by explicit-solvent molecular dynamics (MD) simulations matches experimental conditions, while accurate experimental probes of the hydration shell structure remained limited. Small-angle scattering (SAS) in solution using X-rays (SAXS) or neutrons (SANS) in principle provides information on the hydration shell, since both the radius of gyration (R_g) and the zero-angle scattering (I_0) depend on the hydration shell contrast relative to bulk solvent. Using MD simulations and explicit-solvent SAXS/SANS calculations, we computed R_g and I_0 for five different proteins and a set of 18 different combinations of protein force fields and water models, and we validated the simulation results against consensus data from a recent worldwide round robin benchmark (Trewhella, Vachette et al., doi:10.1107/S2059798322009184). Overall, we find remarkable agreement between MD simulations and consensus SAS data, however the agreement is protein and force field-dependent. Beside the influence of the shape, geometry, surface composition and size of the protein under investigation, certain force field/water model combinations are underestimating the hydration layer contrast significantly, while some water models with increased dispersion interactions may, for some proteins, overestimate the hydration layer contrast. Our study shows that explicit-solvent SAS calculation and consensus SAS data provide a novel route for scrutinizing the hydration layer of proteins.

P-232

Free energy landscape of pore formation in complex membranes strongly depends on lipid composition

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Biological membranes exhibit highly heterogeneous lipid composition, comprising hundreds or even thousands of different chemical species. Given that membranes act as barriers between cells, an important question is how this complexity affects the membrane's mechanical properties and its resistance against the formation of an aqueous defects. We calculated free energy profiles of pore nucleation and expansion for a large set of biologically relevant complex membranes using our in-house developed reaction coordinate [1]. The systems containing up to 14 lipid types reveal large variations in their free energy profiles, which likely reflect their biological functions. For instance, the plasma membrane with its rich sterol content are by far more stable as compared to membranes of intracellular compartments. To rationalize the variations in membrane stability by their lipid content, we calculated pore free energies for 21 model membranes with varying sterol content, tail length, tail unsaturation or head group type, as well as varying concentration of curvature inducing lipids, revealing strong dependence of membrane stability on the lipid properties. [1] Joint Reaction Coordinate for Computing the Free-Energy Landscape of Pore Nucleation and Pore Expansion in Lipid Membranes, Hub J. , J. Chem. Theory Comput. 2021, 17,1229-1239

P-233

Unravelling the details of Hsp90's conformational cycle on the single-molecule level

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The heat shock protein 90 (Hsp90) molecular chaperone is an essential proteostasis hub in eukaryotes accomplishing a number of key functions. Amongst its many "client" (substrate) proteins are kinases and steroid hormone receptors, which regulate the cell cycle and are involved in signal transduction making Hsp90 an important therapeutic target. To fulfil its function, Hsp90 interacts with many different co-chaperone proteins, which are involved in regulating its functional cycle. However, many of the details of how Hsp90 interacts with co-chaperones and processes its clients remain elusive. Shedding light on these details is prerequisite for rational therapeutic design. We are currently developing tools which will enable us to obtain a complete quantitative description of the conformational cycle of Hsp90, as driven by co-chaperone proteins, nucleotides and clients using single molecule optical tweezers combined with fluorescence microscopy, mass photometry and standard biochemical techniques. We have recently characterized the interplay between Hsp90 and its cochaperone Aha1 and been able to measure the stoichiometry of the interaction and its impact on the conformation of Hsp90 using the optical tweezers. We have thus learnt more about the role Aha1 plays in regulating the conformational cycle of this important molecular machine. In conclusion, many open questions about Hsp90's functional cycle remain, which we are starting to address with powerful single-molecule techniques.

P-234

Reconstitution of bacterial macromolecular crowding

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The cell interior is a highly crowded space where multiple biochemical reactions take place in a carefully regulated fashion. Disturbances in macromolecular crowding require a response to prevent suboptimal cell functioning. To better understand the role of macromolecular crowding on the cell interior and intracellular organization, we reconstituted macromolecular crowding in artificial cells. We used an emulsion-transfer method to create liposome-based cell mimics that contain Escherichia coli cell lysate. After that, they were controllably concentrated via osmosis-induced shrinkage to ~200 mg/mL, as verified by a macromolecular crowding sensor1. The readout was the same as in E. coli and, therefore, physiologically relevant. We perturbed the organization with small molecule co-solutes and find an influence of the identity of the co-solute on the organization. The observed differences diminish after 200 mg/ml, at which concentration the bacterial lysate does not mimic in-cell behavior. This suggests that cells have additional mechanisms to control the organization of the cell interior after hyperosmotic stress, in addition to the macromolecular crowders and co-solutes present. Hence, macromolecular crowding depends on the presence of co-solutes in concentrated bacterial lysates and possibly, therefore, also in living bacterial cells.

P-235

Characterization of microfluidic cancer-cell sorting using imaging cytometry

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To study how mechanical factors influence the spread of cancer and the surrounding extracellular matrix (ECM), it is crucial that cancer cell populations be sorted without labels in relevant subpopulations with respect to mechanical properties. It is also important to confine subpopulations for long-term observation to track changes, in inherent properties of the subpopulations, and on the influence of the sorting. A deterministic lateral displacement (DLD) device with two inlets and three outlets was used for cell sorting. Performance of the DLD was evaluated using MDA-MB-231 breast cancer cells. Sorting efficiency was evaluated by imaging cytometry after collecting the sorted cells from the three outlets as well as from the inlet population. The result of the sorting is that larger cells and clusters are fully displaced in the direction defined by the array geometry, medium sized cells are partially displaced, while smaller cells travel with the flow. After sorting, cells were subsequently cultured for Ki67 (proliferation) and YAP staining. Through the image analysis, we observe different size distributions of the cells in three different subpopulations. Statistical analysis shows that the mean size of fully displaced cells is 24.0±5.6μm, partially displaced cells to middle outlet is 19.3±4.0μm, non-displaced cells is 16.1± 2.6μm and inlet population is 19.0±3.5μm. We can also observe that subpopulations and inlet populations have different proliferation rates. Future work will focus on deformability of circulating tumor cells under different flow rates and their long-term behavior with respect to proliferation and motility.

P-236

IL-15 Trans-Presentation Is an Autonomous, Antigen-Independent Process Ádám Kenesei1, Julianna Volkó1, Katalin Tóth2, Thomas A Waldmann3, György Vámosi1

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The interleukin-15 receptor (IL-15R) is composed of three subunits (IL-15Rα, IL-2/15Rβ, γc). IL-15 acts mainly via trans-presentation (TP), whereby an IL-15Rα-bound IL-15 expressing professional antigen-presenting cell presents the ligand to a βγc receptor heterodimer on a neighbouring T or NK cell. IL-15 TP is essential for immunological memory and is involved in signal transduction in many autoimmune diseases. Since antigen presentation (AP) is also based on APC - T cell interaction, the question arises whether AP has any effect on IL-15 TP or whether they are independent processes. Our model system consisted of a Raji B cell line expressing IL-15Rα and a Jurkat T cell line expressing IL-2/15Rβ and γc subunits. Raji cells treated with Staphylococcal enterotoxin E superantigen (SEE) form a stable immunological synapse (IS) through interaction between SEE-binding MHC II molecules and T cell receptor (TCR) with Jurkat cells. We used Förster resonance energy transfer (FRET) to measure the intercellular interaction between IL-2/15Rβ and IL-15Rα subunits at the synapse site. An up-regulation of both subunits in the synapse area and increased FRET values were obtained when Raji cells were pre-treated with IL-15, providing direct biophysical evidence for IL-15 TP. We demonstrated that IL-15 TP can induce STAT5 phosphorylation in Jurkat cells. Our results directly demonstrate the existence of IL-15 TP, and although AP increases the association of IL-15 TP complex, it has no significant effect on IL-15 signalling. Thus, we can state that IL-15 TP can be considered as an autonomous, antigen-independent process.

P-237

Cholesterol controls the effects of ionic liquids on a model cellular membrane

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Ionic liquids (ILs) are amphiphilic molecules that have attracted the attention of researchers across the world due to their various superior characteristics, such as low melting point, high ionic conductivity, and good thermal stability. They can be used as potential molecules for the development of novel antimicrobial and anticancer drugs. Present study is focused on the interaction of imidazolium-based IL with a model cellular membrane composed of brain sphingomyelin (SM) in the absence and presence of cholesterol. Thermodynamics and in-plane rheological properties have been investigated on a monolayer of these lipids formed at the air-water interface. Surface area-pressure isotherm results suggested the reduction of area occupied by a lipid molecule on addition of IL. Interestingly, this effect is insignificant in the presence of cholesterol. Further, the IL-induced decrease in the elasticity of the membrane is reversed back in the presence of cholesterol. The X-ray reflectivity (XRR) technique probed the formation of an interdigitated phase in the membrane due to the presence of the IL with a considerable reduction in the bilayer thickness. In contrast, this phase is diminished significantly in the presence of cholesterol. Therefore, the cholesterol molecule has control in deciding the insertion of an IL into a cellular membrane. This study explains why an IL molecule could be devastating to a cholesterol-free bacterial membrane but safe to a human cell.

P-238

Computational characterization of proton coupling in Xyle

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Sugar transporters have the fundamental role to take up energy into cells in the form of glucose and other hexoses; they belong to the major facilitator superfamily (MFS) and follow a rocker-switch alternating access model of transport. Xyle, an escherichia coli homolog, has been used as a model to study functional characteristic of GLUTs. Yet, in contrast to most of the GLUTs, Xyle is a proton-coupled sugar transporter. Previous studies have suggested that substitution of one aspartate residue (D27) mutation with an asparagine might be responsible for the difference in coupling. However, the presence of the same aspartate in GLUT2, despite it not being coupled to proton uptake, suggests that the coupling could involve more than simply this residue. Intriguingly, this aspartate belongs to a network of three protonatable residues, one aspartate, one glutamate and one arginine. We speculate that the proton coupling might be achieved through an interplay of these residues which could all participate in the proton coupling process. Here we use molecular dynamics simulations to investigate the interplay between these residues in the inward- and outward-facing states of Xyle. Different protonation states for the three residues stabilize or de-stabilize binding of the substrate and inhibitor, suggesting a tight coupling between the protonation event and the binding processes. Finally, we show a free-energy landscape of the translocation process suggesting that the stability of different states along the path is itself controlled by the protonation state of these residues.

Poster Presentations

– Session 2 –

P-512

A correlative study using single molecule localization microscopy and atomic force microscopy of collagen fibrils

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Collagen fibrils are the main structural component in tendon, ligaments and bones. Knowing the structure and arrangement of single fibrils remains challenging, especially in physiological conditions. Here, we correlated single molecule localization microscopy methods (SMLM) with atomic force microscopy (AFM) in near-native conditions, revealing insights into the structure of collagen fibrils. Samples were prepared on coverslips and labelled via fluorescent antibodies. Then selected fibrils where imaged with SMLM and AFM, both in wet and dry conditions. We were interested in the differential performance of the two imaging methods and made a comparative analysis of the cross-sectional area of the fibrils as well as a collagen specific structure called D-banding. Our results show that with SMLM we are able to obtain better insights into the cross section of collagen, whereas AFM experiments reveal better details of the D-banding. Thanks to the correlation of the two imaging techniques, we were able to reveal relevant structural features of collagen fibrils in near native conditions. Finally, we compared their performance and suitability to image collagen fibrils.

P-513

Properties of γD-crystallin undergoing Liquid-Liquid Phase Separation studied by EPR and In-situ Raman spectroscopy

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Liquid-Liquid Phase Separation (LLPS) describes the demixing of a homogeneous protein solution into a dense protein-rich and a diluted protein-depleted two-phase system.In contrast to the intrinsic disorder associated to most proteins undergoing LLPS, γD-crystallin is a globular protein with a mass of 20,6 kDa, ubiquitously expressed in the eye lens of vertebrates, which forms molecular condensates in aqueous solutions at relatively high concentration and low temperatures. The presence of co-solutes such as polyethylene glycol and TMAO were shown to stabilize the phase separated state thereby increasing the onset temperature of LLPS.Here, we used γD-crystallin as a model protein undergoing LLPS, to eluicidate the effects of molecular crowding on its dynamics and to address the changes in solvation and protein density upon droplet formation in the presence of co-solutes. In an integrative approach we investigate the effects of co-solutes on the phase diagram by UV-vis spectroscopy, the dynamics and the local protein density of γD-crystallin in the condensed phase by continuous wave and pulsed EPR spectroscopy and we used in-situ Raman spectroscopy to address local protein concentration in the two phases, partitionig of co-solutes and changes in protein hydration.

P-514

Cell behaviour in the field of constant magnetic force

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The influence of magnetic field on living organisms has a long and well documented research history. Scientific literature is full of articles trying to answer how magnetic field influences organisms at macro and micro-level. However, the significant amount of research is focused on investigating the static (constant) magnetic fields, whereas the magnetic force can only act on the object (of nonzero magnetic susceptibility), when a gradient of magnetic field is applied. In such situation, the resulting force is dependent on this gradient. This study is focused on the influence of the homogenous magnetic forces on migrating MEF 3T3 fibroblasts. First part of research is focused on the process of designing a novel type of microscope stage with constant gradient of magnetic field, that is based on the idea of Halbach array of magnets. We present the development of the stage comparing several 3D printing materials and the characterization of the spatial distribution of magnetic field. Then, we applied this stage in cell biology observations, to analyze the migration and morphology of MEF 3T3 cells. We also examined the cytoskeleton structure, focusing on actin filaments, microtubules and intermediate filaments, that plays the substantial role in cellular functioning.

P-515

Impact of polymer thin film structure onto protein adsorption and orientation

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The basis of ongoing research is understanding material properties and detailed knowledge of the interactions between the polymer surface and the biomolecules deposited on it. Research focuses on changes in orientation of protein molecules, whose positioning affects the number of binding sites directed into the bulk solution and has a direct impact on the sensitivity and selectivity of the biomolecular layer under investigation. The main factor affecting the process of adsorption of proteins to the surface is the structure of the polymer layer used, especially the exposure of chemical groups on the surface related to the arrangement of the side groups of the polymer chain. The conducted studies focused on checking the influence of exposure of chemical groups on the surface of polymer thin films on the amount of adsorbed protein and on the orientation of protein molecules. Through the use of secondary ion mass spectrometry (ToF-SIMS) or atomic force microscopy, the polymer layers were fully characterised, with a particular focus on their topography and determination of the chemical structure of the layer. Using ToF-SIMS measurements combined with principal component analysis and knowledge of the protein structure enable to define the orientation of the protein during the adsorption process.

P-516

Coevolution-driven molecular dynamics simulations of the mitochondrial carriers transport mechanism

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The mitochondrial carrier family (MCF; SLC25) members are transmembrane proteins responsible for transporting various metabolites, nucleotides, and cofactors between the mitochondrion and the cytosol. Given their roles, it is not surprising to find that dysregulation of SLC25 members is often the cause of metabolic disorders or that they are involved in the growth and progression of several tumor types. To date, the three-dimensional structure of only two opposite conformational states (cytoplasmic and matrix state) of the ADP/ATP mitochondrial transporter (AAC; SLC25A4) have been experimentally solved. However, little data about the conformational transition or the mechanism of translocation of the substrate is available. In this regard, the combination of coevolution analyses, machine learning algorithms, and advanced MD methods has proven to be able to characterize the conformational transition of membrane transporters efficiently. This approach has been used to investigate the transport mechanism of the SLC25 protein family, using the bovine SLC25A4 as a case study, for which a crystal structure of the cytoplasmic state is already available.

P-517

Reconstituting a minimal ESCRT machinery from archaea

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The Endosomal Sorting Complex Required for Transport (ESCRT) is a highly conserved protein machinery in eukaryotes. The Asgard archaeal clade, recently discovered and thought to be at the origin of modern cells (eukaryogenesis) also contain ESCRT homologs, which may have played a role in this process. These protein complexes in mammals are responsible for many cellular processes such as cytokinetic abscission, cargo trafficking, viral budding and many more. The origin of the developed ESCRT complexes is however much “simpler” in the Asgard archaeal clade, with only 2 ESCRT-III homologs instead of 12 in humans. The metagenomic sequencing and prediction of these earlier evolved homologous proteins are thought to maintain the characteristic structure of canonical ESCRTs. We are therefore suggesting to use these sequences to produce recombinant archaea ESCRTs and explore the potential multitude of functions in a 2-protein system by reconstituting these in vitro. In the following poster, promising preliminary data pointing towards negatively charged membrane affinity, inter- and intra- protein polymerization and membrane deformation can be observed.

P-518

Molecular Insights of the Interplay between SNX9 and Phosphoinositide Lipids: Implications in Endocytosis and Macropinocytosis

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Biological membranes undergo dynamic remodeling to maintain cell homeostasis. Membrane associated proteins from the Sorting Nexin (SNX) family have been implicated in membrane remodeling through their BAR domains, which bind anionic lipids in a non-specific manner. Additionally, SNX proteins possess specific lipid-binding domains that have been proposed to facilitate their temporal membrane targeting. SNX9, known for its role in endocytosis and macropinocytosis, binds to multiple variants of phosphoinositide (PI) lipids via its PX domain. However, it remains unclear whether the PX domain of SNX9 preferentially binds a specific variant of PI lipid, and what the implications of such differential binding are for cellular functions. To address this question, we use a combination of in cellulo and in vitro assays as well as molecular dynamics simulations. We find that during macropinocytosis, membrane-bound SNX9 is spatially and temporally colocalized with PI(3,4)P2, but not with the PI(4,5)P2 and PI3P lipid populations. While in vitro assays show that the binding affinities of SNX9 to model membranes containing PI(3,4)P2 or PI(4,5)P2 are comparable, all-atom simulations indicate a specific binding, mediated by the PX domain canonical binding site to PI(3,4)P2. Our work not only provides insight into the spatial and temporal recruitment of SNX9 during macropinocytic events, but also suggests a possible mechanism by which proto-endosomal identity is retained prior to membrane scission.

P-519

Assessing the role of solvent and supersaturation in formation of pre-nucleation aggregates in Carbamazepine

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A crystalline compound can have more than one crystalline with same chemical composition, called polymorphism. Polymorphs can have different free energy based on molecular structure and orientation. Thus, getting only desired polymorph of a compound is challenging in pharmaceutical industry. In presence of solvent or interfering substance polymorphs get converted. Since all polymorphs have separate physicochemical properties, control polymorphism is difficult. Carbamazepine (CBZ), an anticonvulsant drug, used in the treatment of epilepsy and bipolar disorder. This active pharmaceutical ingredient have been studied with respect to stability and polymorphism. CBZ have four anhydrous polymorphic forms. Form III is the thermodynamically most stable and bioavailable one. Other forms are thermodynamically metastable. Thermodynamics explain the stability of crystal forms, whereas kinetic point of views determine which metastable form will crystallize. Here, we observed the interactions between CBZ with various solvents (Ethanol, Methanol, Ethyl acetate, Ethyl lactate, DMF, acetone) having different supersaturations to understand the aggregation behaviour and topology of the drug molecules. The polymer consistence force field parameter has been used for the simulation, where all atoms were involved in the system using LAMMPS package. The radial distribution function analysis, energy calculations, angle distribution and hydrogen bond distance analysis confirm aggregation of form III of CBZ in ethanol (Supersaturation, S=2.0), ethyl acetate (S=3.5), ethyl lactate (S=3.3) and acetone. It suggests that not only hydrogen bonding interactions, but also π-π interactions influences the aggregation pattern of form III and enhance the chance of getting thermodynamically stable and bioavailable desired polymorph.

P-520

Molecular characterization of pathologic and tissue-specific TRPM2 cation channel variants

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TRPM2 is a temperature-sensitive, non-selective, Ca2+-permeable cation channel, activated by the simultaneous intracellular presense of ADPR, Ca2+ and PIP2. TRPM2 has important roles in body temperature regulation, cytokine production, oxidative stress response, inflammation, apoptosis. Point mutations in TRPM2 may play a role in bipolar disorder (D543E, R755C) or amyotrophic lateral sclerosis and Parkinson's dementia (P1018L). Alternative splicing results tissue-specific variants in healthy neutrophils (ΔC-TRPM2) and in the striatum (SSF-TRPM2) as well. The goal of this study is to investigate the molecular characteristics of these variants in a cell-free environment using inside-out patch clamp configuration. This enables to define the ADPR- and Ca2+-sensitivity, gating parameters, inactivation kinetics and temperature dependence of each TRPM2 variants in a yet unprecedented way. This detailed knowledge is essential for a comprehensive understanding of the role of these mutants in pathomechanisms and tissue-specific variant functions.

P-521

Incorporating temperature measurements in mechanobiology studies using diamond color centers

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The monitoring of experimental conditions is a crucial aspect of cell biology studies. One of the important environmental parameters is temperature, which influences the behavior of cells in organisms. In this study, we developed a novel experimental setup to monitor a local temperature in Traction Force Microscopy (TFM) experiments. We employed the Optically-Detected Magnetic Resonance (ODMR) effect in nitrogen-vacancy (NV) color centers in diamond. This technique relies on the decrease of fluorescence when interacting with a microwave field of a resonant frequency that changes with temperature. Typical experimental ODMR setups use an intensive light excitation source, strong microwaves, and significant signal averaging (that increases the experiment duration), which are harmful for living cells. We developed a hybrid ODMR-TFM substrate that contains the NV- diamond particles and optimized the ODMR acquisition conditions, to find a satisfactory interplay of excitation, microwave sequence and experiment duration. This resulted in an accuracy of temperature measurement <1K in a relatively short time experiment and low illumination conditions. We also present exemplary ODMR-TFM measurements for heating, cooling, and constant temperature, which show significant differences between the actual local temperature measured by diamonds and the global temperature of the cell medium measured by a thermocouple.

P-522

Permeability Properties of Cx26 and Cx30 Hemichannels: Insights into their Distinct Roles in the Cochlea

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Cx26 and Cx30 are the main connexins expressed in the cochlea with overlapping expression patterns. Cx26 is crucial for cochlear development and it is believed that hemichannels formed by Cx26 play a role in ATP release from cells in the cochlea. Moreover, connexin-formed gap junction channels may be important for Ca2+ wave propagation in the tissue. Currently, it is accepted that Cx26 hemichannels are permeable to both cations and anions, while Cx30 hemichannels are permeable to cations. Although the sequences of these connexins are quite similar, they differ significantly in their functions. Mutations in Cx26 genes result in severe deafness, while mutations in Cx30 genes are associated with milder forms of deafness. This indicates that they play different roles in the cochlea. One of the key differences between these connexins may be the amino acid at position 49, which is one of the constituents of the channel pore. At this position, Cx26 has a neutral alanine, while Cx30 has a negatively charged glutamate. In this study, we use dye uptake method to investigate the permeability of hemichannels. Preliminary data suggest that the amino acid at position 49 may have a special importance for hemichannel permeability.

P-523

Unravelling biological mechanisms by looking at single molecules in real-time

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Imagine you could directly see the location and dynamics of individual proteins binding to a piece of single DNA. What if you could assemble your biological complex step by step and see it in action in real-time? What if you could manipulate the structure of your biomolecule and quickly change buffer conditions to test your experimental hypotheses? By using "molecular tractor beams", the C-trap makes that a reality. Essential biological processes performed by proteins interacting with DNA or cytoskeletal protofilaments are key to cell metabolism and life. In order to understand the molecular basis of life, as well as the pathological conditions that develop when processes go wrong, it is critical to get detailed insights into these processes at the molecular level. Not only at the highest resolution, but also in real time. Here, we will take you through an exciting journey of different research topics in which dynamic single molecule studies revealed remarkable insights. In each study, the C-Trap system was used; our easy-to-use platform that combines optical tweezers with fluorescence microscopy. We will explain how the ability to control, visualize and manipulate single molecules in real time, changes the way we answer tough scientific questions in the field of DNA processing, single-protein dynamics, liquid-liquid phase separation (LLPS) and beyond.

P-524

Protein photodegradation in the visible range? Insights into protein photooxidation with respect to protein concentration

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Visible light (400–800 nm) can lead to photooxidation of protein formulations, which might impair protein integrity. However, the relevant mechanism of photooxidation upon visible light exposure is still unclear for therapeutic proteins, since proteinogenic structures do not absorb light in the visible range. Here, we show that exposure of monoclonal antibody formulations to visible light, lead to the formation of reactive oxygen species (ROS), which subsequently induce specific protein degradations. The formation of ROS and singlet oxygen upon visible light exposure is investigated using electron paramagnetic resonance (EPR) spectroscopy. We describe the initial formation of ROS, most likely after direct reaction of molecular oxygen with a triplet state photosensitizer, generated from intersystem crossing of the excited singlet state. Since these radicals affect the oxygen content in the headspace of the vial, we monitored photooxidation of these mAb formulations. With increasing protein concentrations, we found (i) a decreasing headspace oxygen content in the sample, (ii) a higher relative number of radicals in solution and (iii) a higher protein degradation. Thus, the protein concentration dependence indicates the presence of higher concentration of a currently unknown photosensitizer.

P-525

Insights into the coupling between membrane protein and its membrane environment

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We are interested in understanding the biophysics of membrane proteins and the coupling between membrane protein and its membrane environment. The photocycle kinetics of bacteriorhodopsin (bR) includes the events of a wide range of time scales, which make it a great model protein for studying this subject. We used pump-probe spectroscopy to probe the photocycle kinetics of bR embedded in nanodiscs made of different lipid compositions. Our study demonstrates that the coupling between membrane protein and its environment depends primarily on the timescale of a biology process in consideration. The photocycle kinetics and the temporal photocurrent profiles of bR were significantly altered as the content of negatively charged lipids, DMPG or DOPG, was reduced. We further probed membrane protein function on the timescale of μs-to-ps and showed that the M-rise process of monomeric bR was independent of the lipid environment. . This finding provides researchers a guideline to think about the properties of membrane proteins.

P-526

Probing limitations of free-energy calculations based on molecular dynamics simulations

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Molecular dynamic simulations are nowadays one of the key methods to investigate the dynamics of protein-ligand binding at atomic resolution. The calculation of binding free energies of charged species are an encountered problem in molecular dynamic simulations. This is due to the approximation of the long-range electrostatic interaction. Here, we explore the discrepancies and biases of different approaches, and whether and under which circumstances robust and reliable free-energy differences can be obtained using alchemical methods. Testing various setups and well-established approaches show that the obtained free energies are strongly dependent on the initial setup choices. Alternative approaches, for example placing more copies of perturbed species in a box, were tested. These schemes still suffer from several pitfalls, thus presenting a promising solution approach in addressing the challenges related to non-neutral perturbations.

P-527

Investigating Membrane Integration and Folding of NALCN DI Domain: Implications for CLIFAHDD Mutations

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The sodium leak channel NALCN plays a crucial role in the central nervous system, and mutations contribute to neurodevelopmental disorders, including CLIFAHDD syndrome. Recent structural investigations confirmed four homologous repeats (domains I-IV) in NALCN, each with six transmembrane (TM) segments (S1-S6). Importantly, one-third of CLIFAHDD-related mutations occur in domain I (DI) of NALCN. This study focuses on investigating the early stages of the NALCN DI domain biogenesis within biological membranes. Initially, we examined the translocon-assisted insertion of individual TM sequences using a glycosylation-based reporter system. Subsequently, we characterized NALCN DI's membrane integration and topology using truncated C-terminal reporter tag fusions both in prokaryotic and eukaryotic cells. Furthermore, we explored the impact of described CLIFAHDD mutations on S6's insertion. Finally, by taking advantage of dynamic simulations we analyzed TM helix contacts, with specific emphasis on CLIFAHDD mutations within NALCN DI domain. We conclude that the hydrophobicity of each of the six TM segments has implications on the stability and proper topology of DI in NALCN. Mutations within the TM segments may not alter their insertion or topology but can affect intrachain contacts and, consequently, functionality.

P-528

EFFECT OF STAPHYLOXANTHIN ON BIOPHYSICAL PROPERTIES OF MEMBRANE MODELS: A SPECTROSCOPY STUDY

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Staphylococcus aureus (S.aureus) is a pathogenic gram-positive bacterium that normally resides in the skin and nose of the human body. Pathogenic S.aureus strains produce a higher concentration of carotenoids; the main component is staphyloxanthin. This molecule has been extensively related to modulating the physical properties of the S.aureus cell membrane. In this work, the staphyloxanthin molecule was purified using PTLC and identified by HPLC-MS. The modulatory effect of staphyloxanthin was studied on representative lipid models of the S.aureus cell membrane, prepared based on the main lipids of the bacteria. For that purpose, phosphatidylglycerol and cardiolipin (80:20) were evaluated in the presence of different concentrations of staphyloxanthin (5, 10, 15, 20%). In addition, anisotropy and generalized polarization measurements by fluorescence spectroscopy using an ISS-PC1 spectrofluorometer were performed. The phase transition temperature of the synthetic lipid systems was determined by infrared spectroscopy using a BioATR II unit. The results showed that staphyloxanthin induced changes in the thermotropic behavior of the lipid models, particularly in the liquid-crystalline phase. On the other hand, increasing concentrations of staphyloxanthin induced significant fluidization of the membrane: The most relevant change in the evaluated parameters was obtained at 15% of staphyloxanthin. The results suggest that staphyloxanthin plays a modulatory role at phospholipid bilayers, which could indicate that the evaluated biomolecule modulates the physical state of biological membranes.

P-529

A point mutation turns an inactivated potassium channel into a hyperpolarization activated cation channel.

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In potassium channels, the slow inactivation (C-type) process decreases the ionic conduction by affecting the selectivity filter. The W434F mutation in Shaker potassium channel stabilizes it in the inactivated conformation. The recent determination of the structure of the Shaker W434F channel shows a dilated selectivity filter, compared to Shaker WT structure. The P475D mutation in the bundle crossing region stabilizes the activation gate in the open conformation over a wide voltage range (-140 mV to +100). Sh-P475D was expressed in Xenopus oocytes and inactivation was favored by removing external potassium. Under these conditions, the channel displays robust hyperpolarization activated currents. Similar currents are observed when the W434F mutation is added in Sh-P475D background. The currents elicited by hyperpolarizing voltages in the Sh-P475D mutant are non-selective (PK/PNa~1) and permeate large quaternary ammonium derivatives. We analyzed the role of residues known to affect pore inactivation, the contribution of the selectivity filter, and the influence of the voltage sensor movement on the kinetics and steady state characteristics of these inactivation-induced hyperpolarization activated currents. Our results suggest a mechanism of hyperpolarization gating mediated by an expansion of the selectivity.

P-530

Structure function relationship of bacterial mechanosensitive channel MscS gating

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Assigning solved membrane protein structures to functional states can be complicated by potentially non-native conformations resulting from the removal of stabilizing lipids. This is especially applicable to mechanosensitive channels that gate in response to subtle changes in membrane tension, such as MscS. Cryo-EM structures of MscS fall into two categories depending on the method of solubilization: (1) nonconductive (lipid-reconstituted or mixed micelles) characterized by kinked pore-lining helices and splayed lipid-facing helices, or (2) semi-open (pure detergent or short-chain lipids) satisfying ~70% of experimental conductance. However, MscS has 3 functional states: open, resting, and inactivated. Based on patch-clamp electrophysiology, we suggest that the existing non-conductive structures satisfy the criteria for inactivation. We hypothesize that MscS bears internal stress that is finely balanced by lateral pressure from lipids, giving it the discrete tension sensitivities for the opening or inactivation transitions and leading to the observed structural categories. Using MD simulations, we generated models for the missing resting and open states and validated them through mutagenesis and patch-clamp. We aim to capture these conformations using cryo-EM through mutations stabilizing the missing states. So far, we have solved structures for five mutants: a non-inactivating mutant, two cysteine substitutions for crosslinking salt bridges, another cysteine substitution to crosslink a hydrophobic contact, and a constitutively open mutant, all with resolutions of 2.4-3.4 Å. We are also exploring methods for retaining the native lipid environment such as polymer solubilization as well as in-situ structural studies, which would eliminate the lipid removal step common to all previous structures.

P-531

Absolute binding free energy calculations for membrane targets in the TRPC3/6 subfamily of proteins

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Predicting the binding affinity of a protein-ligand complex is among the key problems in structure-based drug design and several computational methods exist to address this issue. Among the existing simulation-based methods, alchemical absolute binding free energy calculations have shown great potential in accurately predicting free energies. However, the accuracy of these calculations with lipid-exposed binding sites remains unknown. We aim to characterize and understand the effects of lipids in predicting binding free energies by using the TRPC3/6 subfamily of channels as a model system. TRPC3/6 are cation channels that play a key role in several neurodegenerative diseases and have several known antagonists and agonists with lipid-exposed binding sites. We have performed extensive ABFE calculations for these protein-drug complexes and have characterized the performance of these calculations. Our study gives further insight into the factors affecting the accuracy of ABFE calculations on membrane-exposed binding sites and will help guide the refinement of these FEP-based protocols for better binding affinity predictions in the future.

P-532

Ascorbate pharmacodynamic model for anticancer therapy designing

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Vitamin C selective toxicity to cancer cells has been reported by number of in vitro studies, which is explained by ferroptosis induced by ascorbate. There are also clinical trials of anti-cancer therapy by intravenous infusion of high doses of ascorbate (IV therapy), which produced different results. This is likely a result of sufficient conceptual model allowing proper design of such therapy, which in addition would be cancer specific. Such theoretical model has been proposed. The model is based on compartmental pharmacokinetics, Michaelis-Menten kinetic of ascorbate active cellular transport and our previous model of ascorbate homeostasis which considers pH gradient and membrane potential effect on the passive transport. The aim of the computational simulation was to determine optimal therapy protocol for IV delivery route and to compare it with other ascorbate delivery pathways: oral free and liposomal nano-formulations. Conclusions derived using the model are confronted with clinical results of ascorbate IV therapy for selected cancer type.

P-533

CW-EPR spectroscopy as a powerful tool for comparative binding studies on human fatty acid binding proteins

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Fatty acid binding proteins (FABPs) are a family of cytosolic transport proteins showing a high diversity in terms of their amino acid sequences and binding preferabilities. Many aspects of their binding mechanism and functions remain unclear. We analyzed the binding characteristics of FABP3, FABP4 and FABP5 under different physical conditions via continuous-wave electron paramagnetic resonance (CW-EPR) spectroscopy. For this purpose, the spin probes 5- and 16-doxyl stearic acid (5/16-DSA) were provided as model ligands, investigating their binding dynamics and chemical environment inside the binding pockets of the proteins. The proportion of bound 5/16-DSA depends dramatically on the protein concentration and the temperature, but with remarkable differences between the three FABPs. EPR simulations enabled the construction of binding curves, revealing two differently bound states with distinct rotational dynamics and environments. The more dynamic state ('intermediately bound') seems to dominate at body temperature. The EPR results were supplemented by binding affinity measurements via microscale thermophoresis (MST) and analyses of the self-aggregation of the FABPs via dynamic light scattering (DLS). Our results provide innovative insights into the binding mechanism of FABPs on a molecular level and attest the power of simple CW-EPR spectroscopic experiments applied on biological transport systems.

Poster Presentations

– Session 3 –

P-793

QM/MM MD simulations of horizontal proton transfer pathways in the antiporter-like subunits of mitochondrial respiratory complex I

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Molecular mechanism of respiratory complex I has remained one of the biggest mysteries in bioenergetics. Being the largest enzyme in the respiratory chain, it couples the electron transfer process to proton pumping across the inner mitochondrial membrane, spatially separated by ~200 Å from one another. This phenomenon raises an enigmatic question: how does the energy released from the redox reaction can be transmitted for such long distances? In our work, we advocate the hypothesis that protons can translocate along the central hydrophilic axis of the enzyme - a highly hydrated region revealed by the structural data. We apply state-of-the-art quantum mechanical/molecular mechanical (QM/MM) free energy simulations to the high-resolution structure of respiratory complex I from Yarrowia lipolytica and show low energy barriers for the proton pathway laterally spanning the entire ND2 subunit. For the first time, we emphasize the critical role of tyrosine residues in coordinating the transmission and demonstrate reasonable energetics for the proton transfer in interfacial regions of the ND2 subunit with the adjacent ND4L and ND4 subunits. Our results elucidate the long-range energy translocation processes in the membrane part of the enzyme, casting doubts on the canonically admitted proton pumping models in respiratory complex I.

P-794

User-unbiased method for molecule detection in SMLM algorithms enables transferability and reproducibility

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We present an optimal method for user-unbiased primary detection of molecules in single molecule localization microscopy (SMLM) algorithm. Currently, the SMLM algorithm detects active fluorophores by finding local maxima over segmented images, typically using thresholding. The abundance of options and non-trivial settings can pose challenges for non-expert users, software developers, and hinder the transferability of results. To solve this problem, we have designed an optimal detection algorithm based on signal detection theory. It offers maximum detection probability for a given probability of false detection, which is the only parameter specified by the user. Consequently, probability of false detection becomes the sole parameter necessary to reproduce conditions across different samples, setups, and software. Our implementation is robust and computationally efficient, surpassing currently popular methods especially for low signal-to-noise ratio (SNR). We compared the performance of the detectors using simulated datasets and SMLM challenge dataset. We propose the establishment of detection derived from signal detection theory as a standard tool in SMLM software, offering enhanced performance and addressing the challenges associated with algorithm settings and result reproducibility. Looking beyond SMLM, our method can be beneficial in other imaging techniques suffering from low SNR like iSCAT or particle tracking.

P-795

Proton-coupled electron transfer dynamics in the mycobacterial respiratory supercomplex III2IV2

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Biological energy conversion is catalyzed by membrane-bound enzyme complexes that establish functional electron transfer chains. Recent studies show that these enzymes form higher order supercomplexes (SC), comprising large assemblies of the individual respiratory enzymes, but despite resolved atomic structures, their functional role remains poorly understood and highly debated. Here, we study the dynamics of the obligate M. smegmatis III2IV2 respiratory SC by combining large-scale molecular dynamics simulations with DFT-based QM/MM models and cryo-electron microscopy (cryoEM) data. We describe putative quinone-binding sites and the energetics of the proton-coupled electron transfer reactions between quinone and heme bL and heme bH in both Qo and Qi binding sites of Complex III. Our structural and computational data provide evidence of possible proton release pathways as well as mechanistic principles unique to this mycobacteria supercomplex with relevance to drug targeting against tuberculosis.

P-796

Surface–particle interactions control the escape time of a particle from a nanopore-gated nanocavity system: a coarse grained simulation

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Nanopores and cavities are promising single molecule tools for investigating the behavior of individual molecules within confined spaces. For single molecule analysis, the total duration the analyte remains within the pore/cavity is highly important, and is determined by a complex interplay among particle–surface interactions, external forces and Brownian diffusion, making predictions of the dwell time challenging. Here, we show how the dwell time of an analyte in a gated-nanocavity depends on the sizes of the nanocavity/nanopore, and on particle–wall interactions. For this purpose, we used a coarse-grained model that allowed us to simulate hundreds of individual trajectories within a nanocavity volume. We found that by increasing the attraction between the particle and the wall, the diffusion process transforms from a usual 3D scenario to a 2D motion along the cavity surface, resulting in a significant reduction of the average dwell time. Additionally, the comparison of our results with existing theories on narrow escape problems allowed us to quantify the reliability of theory derived for ideal conditions for geometries more similar to actual devices.

P-797

Elucidating Water Molecule Rearrangement in Phytochrome Proteins Upon Light Absorption

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Phytochromes are light-detecting proteins found in plants, fungi, and bacteria. They undergo conformational changes upon light absorption, affecting their signaling capacity. Therefore, understanding the chemical mechanism of light absorption in these proteins is essential. In a recent study, we recorded femtosecond time-resolved serial crystallography (SX) data of the phytochrome protein derived from D. radiodurans. The study reveals significant conformational changes in both the chromophore and its surrounding protein environment following photoexcitation. Additionally, the time-resolved structures reveal the reorganization of adjacent water molecules, which play a critical role in the hydrogen bond network between the chromophore and conserved histidine and aspartate residues surrounding the chromophore. However, the atomistic-scale understanding of these water molecule reorganizations remains limited, and we hypothesize that these changes are caused by the protonation changes of the histidine residue. To investigate the underlying chemical mechanism, we employed molecular dynamics simulations on both excited and native state structures, considering three distinct protonation states the histidine residue. By calculating difference water density maps of the chromophore region, we compare our computational results with our experimental findings. Our initial results allow us to identify the potential impact of the conserved histidine on the rearrangement of water molecules around the bilin chromophore.

P-798

Single-molecule imaging to simultaneously monitor multiple processes during protein-RNA complex assembly in real-time

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Protein-RNA complexes (RNPs) are indispensable in all life forms. RNPs are made up of many components assembled by multiple synchronized processes. Ribosome assembly is one example where processes of rRNA transcription, folding, processing, modification and binding of r-proteins occur simultaneously. It is imperative to understand how these processes are functionally coupled to get mechanistic understanding. We use multi-color single-molecule imaging to monitor multiple processes simultaneously in real-time. We developed a co-transcriptional assay to probe rRNA structure, r-protein and assembly factor binding in real-time. Dynamic RNA structure probing informs if specific regions on the RNA are single-stranded or structured, similarly binding of r-protein to the same RNA molecule tells if a particular rRNA structure is natively formed. We also monitored binding of RNA modification enzymes to understand how RNA modification is functionally coupled to other processes. Our final aim is to directly and simultaneously track processes of ribosome assembly to study how multiple processes in RNP assembly cooperate for efficient and regulated assembly.

P-799

Understanding the Structural Behavior of PsbS in Thylakoid Membrane: Insights from Molecular Dynamics Simulations

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PsbS is a protein that plays a central role in the photoprotection of plants. However, studying PsbS in its natural environment poses challenges due to the complexity of thylakoid lipids. Moreover, previous computational studies have predominantly focused on POPC membranes. To address this gap, we developed a coarse-grained model of the thylakoid membrane based on the latest advancements in the Martini 3 forcefield. Molecular dynamics simulations were conducted to compare the structure of PsbS in both POPC and the thylakoid membrane. Our results reveal that the flexible regions of the protein, particularly the upper loops, exhibit decreased flexibility in the thylakoid membrane due to their integration into the membrane, unlike in POPC where they remain exposed to the solvent. Furthermore, our investigations demonstrate that PsbS predominantly interacts with MGDG and DGDG lipids, highlighting them as the primary interaction partners. These findings provide crucial insights into the structural behavior of PsbS in the thylakoid membrane and shed light on the intricate interactions between PsbS and thylakoid lipids. Understanding these interactions is fundamental for comprehending the role of the thylakoid membrane in the photoprotection mechanisms in plants.

P-800

Structure-function study of an obligate respiratory chain supercomplex from M. smegmatis

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Tuberculosis is one of the most common causes of death world-wide. The causative agent of this disease is the gram-positive bacterium Mycobacterium tuberculosis from the phylum Actinobacteria. The increased occurrence of multidrug resistant strains is a main motivator for researching new possible drug targets. Respiratory chain complexes present promising candidates for such research. In the genus Mycobacterium soluble cytochrome c is absent and complex III and IV are organized into an obligate supercomplex, as opposed to their eukaryotic homologs which can function as individual complexes. In this study we used biochemical, structural and computational approaches to obtain a better understanding of the functional and mechanistic working of the M. smegmatis respiratory chain supercomplex, which is homologous to the one from M. tuberculosis. We observed several structural and functional differences including modifications in the Qo site of complex III and the occurrence of new menaquinol binding sites with unknown function. Mutants of these sites were designed to study their structure-function properties.

P-801

Structure-function studies of non-heme diiron alkane hydroxylases from diverse bacteria

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n-Alkanes are the main component of natural gas and crude oil. The conversion of these fairly unreactive compounds into high value-added products, forming the technological basis of chemical industry, is an energy-intensive process. As the use of petroleum products increases, hazardous pollutants are released into the environment. The integral membrane metalloenzyme alkane monooxygenase (AlkB) initiates the first step of the alkane metabolic pathway by terminal C–H bond hydroxylation and thus has a potential for applications, such as the bioremediation of oil-contaminated soil or as ecological modification of the mentioned energy-intensive industrial processes. AlkB has been reported in different arrangements: as a sole protein and fused to its electron transfer partners - a small iron-sulfur protein and a flavin-dependent reductase AlkT. Our study includes AlkB representatives in all arrangements from various organisms and focuses on their structure determination by Cryo-EM and X-ray crystallography, elucidation of the electron transfer path from AlkT to AlkB and a thorough understanding of the alkane activation mechanism.

P-802

Dimer formation process of Aβ40 and Aβ42 using the Hamiltonian replica-permutation method

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Amyloid-β peptides (Aβs) tend to form oligomers and amyloid fibrils, which are associated with the Alzheimer's disease. Aβ has two isoforms, Aβ40 and Aβ42, and the difference between these isoforms is only two additional C-terminal residues. However, Aβ42 rapidly forms aggregates. To investigate the difference in aggregation process between Aβ40 and Aβ42, we performed the Hamiltonian replica-permutation molecular dynamics simulations for these Aβs. Experiments on Aβ aggregation were also conducted to validate of the computational results. We will discuss the role of the two additional C-terminal residues in the aggregation process.

P-803

Structure of Telomeric Chromatin and Interactions with Shelterin Components TRF1 & TRF2

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Mammalian telomeres consist of the tandemly arranged DNA sequence repeats TTAGGG that act as a platform for recruitment of the capping structure at the end of the linear chromosomes, which protect their ends from incorrect DNA repair and degradation. Telomeres consists of the six-protein Shelterin complex as well as nucleosomes that package 5–15 kbp of telomeric DNA into chromatin. Aberrant regulation of telomeres has been linked with cancer and ageing. Almost nothing was known about the structure of telomeric chromatin at the molecular level. Recently (Soman et al, Nature, 699, 1048, 2022), we characterized telomeric chromatin fibres with EM. We determined the Cryo-EM structure of the telomeric tetra-nucleosome fibre, which revealed a novel columnar architecture. The structure of the di-nucleosome unit at 3.9 Å resolution displays about 130 bp DNA wound in a continuous super-helix around the closely stacked histone octamers. We also characterised the interaction of telomere repeat binding factors 1 and 2 (TRF1 & 2) with telomeric chromatin, which reveals that TRF2 induces the columnar form while TRF1 mediated chromatin fibre crosslinking. Both TRF1 and TRF2 induce liquid-liquid phase separation when interacting with the telomeric chromatin fibers (Telo-10, with 10x157 bp of DNA comprised of TTAGGG repeats).

P-804

Thermott – Tool for Protein□ligand Interactions and Protein–Ligand Binding Database (PLBD) of Thermodynamic and Kinetic Intrinsic Parameters

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Thermal shift assay (TSA, ThermoFluor, differential scanning fluorimetry, DSF) is a technique to determine protein melting temperature for optimal conditions and protein-ligand binding affinities. The technique is easy to perform, but difficult to obtain the dissociation constant. There is a lack of user-friendly tools for regression analysis of complex thermodynamic equations. We developed Thermott — a free and open-source tool for analyzing TSA protein–ligand binding data. The tool is accessible at https://thermott.com. We have developed and introduce here a Protein-Ligand Binding Database (PLBD) that contains thermodynamic and kinetic data of reversible interactions between proteins and small molecule compounds. The manually curated binding data are linked to protein–ligand crystal structures, enabling the structure-thermodynamics correlations to be determined. The database contains over 5500 binding datasets of 556 sulfonamide compound interactions with the 12 catalytically active human carbonic anhydrase isozymes defined by TSA, ITC, inhibition of enzymatic activity, and SPR. In the PLBD, the intrinsic thermodynamic parameters of interactions are provided which account for the binding-linked protonation reactions. The database provides calorimetrically measured binding enthalpies for additional mechanistic understanding. The PLBD is available at https://plbd.org can be applied for small molecule drug design.

P-805

Electroformation of giant unilamellar vesicles from damp lipid films formed by vesicle fusion

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Giant unilamellar vesicles (GUVs) are artificial membrane models that are of particular interest to researchers because of their similarity in size to eukaryotic cells. The most commonly used method for producing GUVs is electroformation. However, the traditional electroformation protocol involves a step where the organic solvent is completely evaporated, leaving behind a dry lipid film. This leads to artifactual demixing of cholesterol (Chol) in the form of anhydrous crystals. These crystals do not participate in the formation of the lipid bilayer, resulting in a decrease of Chol concentration in the bilayer compared to the initial lipid solution. We propose a novel electroformation protocol which addresses this issue by combining the rapid solvent exchange, plasma cleaning and spin-coating techniques to produce GUVs from damp lipid films in a fast and reproducible manner. We have tested the efficiency of the protocol using 1/1 phosphatidylcholine/Chol and 1/1/1 phosphatidylcholine/sphingomyelin/Chol lipid mixtures and were able to produce a GUV population with an average diameter of around 40 μm. Furthermore, compared to protocols involving the dry film step, the sizes and quality of vesicles determined from fluorescence microscopy images were similar or better, confirming the benefits of our protocol in that regard as well.

P-806

Preparation of giant unilamellar vesicles with high cholesterol content from damp lipid films formed by rupturing large unilamellar vesicles by method of electroformation

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The artificial models most frequently used to study the properties of biological membranes are giant unilamellar vesicles (GUVs) commonly prepared by electroformation method. The traditional protocol includes a step in which the lipid film is completely dried. This results in the precipitation of cholesterol (Chol) in the form of anhydrous crystals (Chol demixing artifact). These crystals do not participate in the formation of the lipid bilayer, resulting in a decrease of Chol concentration in the bilayer compared to the initial lipid solution. This issue can be avoided by producing multilamellar liposomes using the rapid solvent exchange method, extruding them to form large unilamellar vesicles. A damp lipid film was obtained by spin-coating these vesicles on a hydrophilic surface. Special attention was paid to high Chol concentrations. The experiments were performed using Chol/phosphatidylcholine mixtures and results were assessed by fluorescence microscopy. The modified approach appears to be superior to the traditional protocol in terms of GUV yield and size, especially when high Chol concentrations are present. We concluded that the optimal duration of spin-coating is 30 s. The average diameter of GUVs is about 40 μm at low (< 40 mol%) and about 30 μm at higher Chol content.

P-807

Role of Lipid Nanodomains for Inhibitory FcγRIIb Function

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The Fcγ receptors are single-pass transmembrane proteins, responsible for the immune response, that bind to the Fc part of IgG antibodies. In humans, the only inhibitory Fcγ receptor is the FcγRIIb, equipped with an immunoreceptor tyrosine-based inhibitory motif (ITIM), countering the only activating function of the other FcγRs. Malfunctioning of this inhibiting receptor can lead to a myriad of autoimmune diseases such as systemic lupus erythematosus (SLE), which may lead to joint pain, anaemia and inflammation. Atomistic molecular dynamic simulations on the μs timescale show that - contrary to previously proposed models - FcγRIIb (and its mutant) do not reside in so called lipid rafts (highly ordered membrane nanodomains rich in cholesterol and sphingolipids). Instead, the immediate vicinity of the receptors was depleted of cholesterol and the receptor interacted favourably with poly-unsaturated fatty acyl chains and poly-anionic lipids within the inner membrane leaflet. It was shown that FcγRIIb monomers do not reside in a conformation suitable to bind with IgG and likely need to form a complex with multivalent IgG molecules in order to be brought to binding-competent conformation. It is suggested that the I232T mutation alters the receptor complex formation within the membrane, leading to the receptor's altered function.

P-808

Structure and pH stability of the Minimal Progenitor Toxin Complex B

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Botulinum neurotoxins (BoNTs) are the most dangerous biological toxins known to man. BoNT is naturally co-expressed with its non-toxic non-hemagglutinin (NTNH) partner protein which forms a tight complex with the toxin, called minimal progenitor toxin complex (M-PTC). NTNH serves a protective role in shielding the toxin from various harsh environmental conditions. Because of their specific and effective mechanism of action, BoNTs, M-PTC/A, and M-PTC/B especially, are already used for the treatment of involuntary or excessive muscle contractions. Here, we present a structure of BoNT/B-NTNH/B (i.e., M-PTC/B) at 2.7 Å resolution determined by single-particle cryo-electron microscopy. Moreover, we discuss the pH stability of the complex by conducting structural analysis to identify residues involved in its pH-sensing mechanism as well as by performing a pH stability assay to determine that the complex starts to dissociate already at a pH greater than 6. As the M-PTC/B is already an FDA-approved therapeutic compound, we believe that its structure is crucial for a thorough understanding of its mechanism of action as well as for further development of therapeutics utilizing specific properties of the toxin.

P-809

Interplay of polysaccharides and colloidal clay: A model system to understand carbon sequestration in the ocean

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The vertical flux of organic particles from the surface to deeper layers of the ocean creates a major sink in the global carbon cycle. Primary producers, such as microalgae, sequester atmospheric carbon dioxide in the sunlit layer of the ocean and produce particulate organic matter (POM). Because POM has little negative buoyancy, the rate and speed with which organic particles sink and eventually settle on the ocean floor is highly dependent on the aggregation of POM with heavier ballast such as inorganic minerals. We present a model to characterize the interaction and aggregation of polysaccharides with different types of colloidal clay particles. We tested the aggregation behaviour under various environmentally realistic conditions (ionic strength, pH, dissolved salts). Small-angle X-ray scattering and dynamic light scattering were used to evaluate polysaccharide structures and the colloidal stability of clay-polysaccharide mixtures. We find an influence of the polysaccharides on the aggregation state of colloidal particles. Thus, the model system sheds light on which polysaccharide structural features in combination with colloidal properties influence aggregation and sedimentation of marine POM.

P-810

A set of NMR experiments to determine Proline cis/trans isomer specific properties of intrinsicallydisordered regions

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Proline (P) is unique among the proteinogenic amino acids in that the energy difference between trans and cis XP peptidyl bonds is small and therefore the cis state is significantly populated (3-40%, depending on the peptide sequence). Furthermore, the activation energy for isomerization is relatively high (~20kcal/mol) and for intrinsically disordered regions (IDRs) with x prolines there are theoretically 2^x different isomers present, which are slowly interconverting. Many biophysical methods are averaging the properties of this heterogeneous ensemble, while NMR has the unique power to extract isomer specific information without the need for additional labels, since the chemical shifts of nuclei in and around the XP peptidyl bond are sensitive to the isomerization state. Here we present a selection of NMR experiments to extract isomer specific properties of peptides and IDRs as well as interconversion rates between the isomers. We show that binding partners can have very different affinities (up to 100-fold) for the cis and trans isomer respectively. This might have big consequences for the stochastic nature of protein interaction inside the cell, considering the high abundance of prolines in small linear interaction motifs (SLIMs) and IDRs in general.

P-811

Mismetallation or regulation? Role of manganese ions in tuning the radical generation in ribonucleotide reductase

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Ribonucleotide reductase (RNR) is essential for DNA synthesis and repair across all living organisms. The E. coli RNR requires a diiron cofactor to form a tyrosyl radical (Y•), vital for the catalytic reaction. However, the enzyme can also bind manganese, which leads to Y• inhibition. How does nature ensure proper metal incorporation? To address this, we investigated the impact of manganese mismetallation on Y• concentrations using advanced electron paramagnetic resonance (EPR) spectroscopy combined with UV-Vis and TXRF measurements. Our studies revealed an unprecedented asymmetry in the metallocofactor assembly of the homodimeric protein: diiron-Y• cofactor on one monomer and a dimanganese-cofactor on the other. Time-dependent EPR distance measurements unveiled a complex regulatory mechanism where iron displaces manganese, resulting in radical yield recovery. These results indicate that Y• concentrations, and thus RNR activity, are regulated via control of metallation. Understanding these regulatory mechanisms could pave the way for future studies aiming to manipulate RNR activity for therapeutic purposes.

P-812

Molecular principles of protonation dynamics and hydrogen evolution in membrane-bound hydrogenase

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The membrane-bound hydrogenase (Mbh) from Pyrococcus furiosus is an archaeal member of the complex I superfamily. Mbh catalyzes the reduction of the protons to form H2 gas, which is further functionally coupled to the ion pumping (Na+/H+) across the membrane. The [NiFe] cluster in the hydrophilic domain of Mbh is responsible for the synthesis of H2 gas, with both similarities and key differences to the canonical soluble [NiFe] hydrogenases. In this work, we elucidate the molecular principles of H2 production using quantum density functional theory, and quantum-classical (QM/MM) calculations in combination with large-scale molecular dynamics simulations. We investigate the complete catalytic states of the [NiFe] active-site as well as the effect of different spin-states. We identify how the protonation states of surrounding conserved residues modulate the spin-state energetics and show that the protons required for the catalysis are transferred through the extended hydrogen- bonding network between the Cys374L and Glu21L, bridged by a water molecule. We suggest that the position of this key water molecule within the hydrogen bonding network plays functional and regulatory roles. In addition, we showcase the role of the electric fields around the [NiFe] cluster in dictating the proton-transfer energetics and H2 formation during catalysis.

P-813

Unraveling the multivalent and rapid interactions between Musashi-1 and RNA

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Musashi 1 (MSI1) is an RNA binding protein (RBP) with two different RNA recognition motifs (RRMs) and a disordered tail. It is widely involved in oncogenic and neurodegenerative processes which makes it a relevant pharmacological target. In this project we present for the first-time kinetic RNA-MSI1 binding studies of the single RRMs and a construct with both RRMs. Results were obtained through surface plasmon resonance (SPR) and evaluated with different models, being a 1:1 interaction for the single RRMs, and initially a bivalent interaction for the construct with both RRMs, due to the multivalent characteristics of the protein. The intrinsic complexity of the bivalent model, however, resulted in a cumbersome process to accurately fit experimental data for the rapid interactions observed for MSI1-RNA binding. Therefore, we develop a new and more robust analysis method based on rate constants derived from applying a 1:2 model to estimate the affinity of the bivalent interaction. In summary, we present an array of new information about the MSI1-RNA interaction kinetics alongside with an alternative method to calculate the affinity of a bivalent interaction through the use of kinetic parameters derived from a 1:2 model.

P-814

Waveguide scattering microscopy enables size, refractive index, and time-resolved label-free biomarker determination of single extracellular vesicles

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Extracellular vesicles (EVs) are biological vesicular nanoparticles carrying biomolecular cargo, such as proteins and nucleic acids. EV heterogeneity in size, content, and membrane composition after different purification steps complicates determination between physicochemical properties and biological function. In this work, we demonstrate the capabilities of microfluidics assisted label-free waveguide scattering microscopy combined with simultaneous fluorescence detection by comparing two different blood-derived EV populations, platelet and red blood cell EVs.

P-815

Signal peptide interaction with the yeast Sec61 translocon at the single-molecule level

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Signal peptide (SP) of translocating proteins interact with Sec61 to initiate translocation into the reticular lumen. Two pathways exist: co-translational (coupled to translation) and post-translational (occurring after protein translation). Hydrophobicity of SP mostly determines the choice, with yeast post-translational SPs being less hydrophobics. Does the interaction time with Sec61 differs for post- and co- SPs? The force required to break the interaction of Sec61 and SPs was measured using Optical Tweezers. Substrates used were Prepro-alpha-factor (PpαF, post-translational SP) and a chimera protein with the SP of PpαF replaced by the co-translational SP of Dipeptidyl aminopeptidase B. Experimental cycles involved microsphere approach, interaction, and separation. Contact time was ~2 s, with separation at a speed of 100 nm/s and a trap hardness of 0.1 pN/nm. Parameters obtained were: PpaF [τ0 (s) = 17±1, koff (s-1) = 0.060±0.005, $\Delta x^{\ddagger}$ (nm) = 0.34±0.05], Chimera [τ0 (s) = 7±2, koff (s-1) = 0.114±0.04, $\Delta x^{\ddagger}$ (nm) = 0.19±0.07]. These results indicate a longer interaction time (τoff) between the SP and Sec61 for post- translational pathway substrates.

P-816

New nanopores sensors for the detection of DNA

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Aerolysin-like proteins are a family of β-pore-forming toxins (PFTs) widely present in all kingdoms of life. Recently, this family of proteins is gaining attention because of their biotechnological application as nanopore sensors for biomolecules sensing and sequencing. To identify novel and more sensitive PFTs for biomolecules sensing, we explored the possibilities of using the knowledge of the sequence and structure of proteins to screen for potential new nanopore candidates. In spite of the conserved structural fold, the sequence identity is low and complicates their sequence alignment, hindering into the understanding of their pore structure and properties, limiting further biotechnological applications. We analyzed the pore structure of three family members, Clostridium perfringens epsilon toxin (ETX), Laetiporus sulphureus lectin and Bacillus thuringiensis parasporin-2 and compared it to aerolysin. Their structure and sensing capabilities for ssDNA were assessed by in silico methods and explored experimentally for ETX. We found that different types of ETX pores with different conductance can be formed and that one of them can translocate DNA. Moreover, the depth of the current blockage is higher compared to aerolysin, indicating a higher sensitivity for DNA sensing. Our findings open new venues for improving and diversifying nanopore capabilities for molecular sensing.

P-817

The Minimal Cell under a Computational Microscope using the Martini Force Field

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Molecular dynamics (MD) is a well-established simulation method that has successfully been applied to study a wide range of biomolecular processes. As a result of continuous improvements in both computational infrastructure and modeling methods, we currently observe that the study of mesoscopic, multi-component systems has become attainable.Here we present a selection of tools, i.e. the Martini ecosystem, which enable the construction of these complex systems. Their capabilities are demonstrated in an ongoing effort to simulate a Martini model of a genetically minimal cell: JCVI-syn3A. Although some key components of the cell composition have been simplified, this model serves as a starting point for whole-cell simulations with molecular detail. Studying entire cells under the computation microscope will hopefully provide valuable insights into a wide range of problems, ranging from drug design to understanding the internal organization of cellular environments.

P-818

Following the co-translational folding of a multidomain protein

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Tandem repeat proteins composed of multiple copies of similar domains have a high risk of forming non-native inter-domain contacts during folding that can lead to misfolded states. Therefore, a strong selective pressure may exist to minimize misfolding interaction between adjacent domains during co-translational folding. To explore how this occurs we monitored oscillations on pulling force exerted on the nascent chain as it emerges from the ribosomal exit tunnel during vectorial elongation of a multi-domain protein. The protein PimA is a essential enzyme of 386 residues responsible for the initial mannosylation of phosphatidylinositol in Mycobacterium smegmatis. The PimA structure consists of two Rossmann-fold domains with a deep fissure at the interface forming the catalytic center, a long a-helix connects the C-terminal domain with the N- terminal one. By the use of a set of gradually longer fragments of PimA fused to a translational arrest peptide in the E. coli-based PURE in vitro translation system we generated a series of co-translational force-profiles. The force profile analysis reveals that while the c-terminal domain is able to fold co-translationally the N-terminal domain requires the complementation of the central B-sheet by the very C-terminal portion.

P-819

Bayesian Structure Determination from Fluctuation X-ray Scattering Experiments

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Fluctuation X-ray scattering is an emerging method for biomolecular structure determination, where scattering data of an ensemble of molecules in a dilute solution is collected using ultra-short X-ray pulses below rotational tumbling times. This allows to capture structural information in angular intensity correlations that are absent in traditional solution X-ray scattering methods, while also keeping the more biological conditions in solution compared to single-molecule X-ray scattering. However, the reconstruction of the molecular structure of the sample using the scattering images poses a significant challenge, since the orientations of the individual molecules in the solution are unknown and the low signal-to-noise ratio has to be overcome. We present a rigorous Bayesian framework that finds the molecular structure that has the largest probability given all recorded scattering images. In this approach, the orientation of each molecule explicitly appears in the likelihood function. We formally integrate out the unknown individual orientations, and to reduce the computational cost, this integration is approximated by a finite sum over randomly chosen orientations of each molecule. We show that our method can recover the molecular structure of a fictitious 12-atom molecule up to 4Å resolution, using 2000 synthetic noise-free images of 50 randomly oriented copies each.

P-820

Thermal Stability of Peripheral Myelin Protein 22 and its Glycosylation Deficient Mutant

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Peripheral myelin protein 22 (PMP22) is a tetraspan integral membrane protein that comprises approximately 2-5% of the total protein content of Schwann cell myelin in the peripheral nervous system. Under normal physiological conditions, approximately 20% of expressed wild-type PMP22 properly folds and traffics to the plasma membrane of cells. Charcot-Marie-Tooth (CMT) disease is a neuropathy of the peripheral nervous system that results in progressive loss of muscle tissue as well as defects in motor and sensory perception. In CMT, an additional copy of the wild-type PMP22 allele causes overexpression of the PMP22 protein, resulting in the formation of cytotoxic aggregates. A glycosylation deficient mutant of PMP22, N41Q, traffics approximately three-fold more efficiently compared to wild-type PMP22. Using the Cellular Thermal Shift Assay (CETSA), the thermal stability of both wild-type and N41Q PMP22 is compared. Additionally, lead hit compounds from an expansive DNA-Encoded Library (DEL) are assessed to determine their ability to modulate the thermal stability of PMP22 using CETSA. Results from experiments presented here give key insights into how chemical chaperones can stabilize PMP22 in a cellular environment and enable the protein to correctly traffic to the plasma membrane, thus abating symptoms of CMT.

P-821

Investigating Cell-Particle Dynamics: Insights into particle uptake and transport and role of Myosin in these processes

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Diamond particles, with their biocompatibility and fluorescent color centers, have found utility in cellular studies for drug delivery and sensing. Yet, the intricate mechanisms underlying cell-particle interactions, encompassing uptake, transport, and intracellular localization, are still not fully comprehended. In our study, we examined the interactions between MEF 3T3 cells and 1µm microdiamonds (MDs) containing NV centers as fluorescent markers. Our primary objective was to investigate the impact of cell-MDs interaction on specific cellular processes, such as MD uptake and subsequent intracellular transportation. Our findings showed a novel cell behavior wherein cells actively searched for and exhibited a preference for MD uptake compared to latex beads (LBs). Furthermore, MDs were actively transported within the cells. We explored the potential roles of myosin family motor proteins (Myosin-II and Myo10) in MD uptake and intracellular transport. Our results demonstrated the involvement of Myo10 in particle transport within cells. Interestingly, inhibiting Myosin-II activity did not reduce the search, uptake, and final destination of diamond particles. These findings can help for a better understanding of the mechanisms of cell-MDs interactions which can enhance drug delivery strategies and improve the efficacy of diamond particle-based sensing techniques.

P-822

Time-resolved solution X-ray scattering of AsLOV2 at the European XFEL

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Photosensory proteins form the molecular basis that enable plants to respond to varying environmental conditions. Phototropins are a class of blue-light photoreceptors responsible for a range of essential regulatory mechanisms including chloroplast motility, phototropism, and stomatal opening. Such functions involve transmitting light-driven molecular signals across a protein scaffold that encompass a series of conformational changes. A classic example occurs in the light-oxygen-voltage sensor from Avena sativa (AsLOV2), where light triggers unfolding of a large C-terminal helical domain. Its photoactivation mechanism has been widely studied, but the exact structural transformations accompanying this event are poorly understood. To investigate this, we performed transient solution X-ray scattering at the European X-ray Free Electron Laser (EuXFEL). The experiment achieved exceptionally low noise levels which enabled resolution of structural changes on the µs timescale. We applied two modeling strategies to understand this response: (i) a machine-learning routine based on AlphaFold to generate several conformations of the unfolded protein that could replicate the time-resolved experimental data and (ii) ab initio modeling based on direct Fourier transforms to retrieve the density maps of the unfolded state. This work provides a direct structural characterization of AsLOV2, while also highlighting enhancements in experimental precision enabled by XFELs.

P-823

Structure and Organization of Monoclonal Antibodies at the Air/Water Interface in Presence of Pharmaceutical Polymers

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Understanding structure and organization of monoclonal antibodies (mAbs) at the air/water interface is important for stability and effectiveness of protein drug formulations used in pharmaceutical industry. To prevent mAb adsorption, the surfactants Poloxamer 188 (P188), Polysorbate 20 (PS 20) and Polysorbate 80 (PS 80) are commonly used. This study focuses on the effect of P188 and PS 20. The twofold aim was to firstly prevent mAb adsorption, and secondly, to desorb mAb by subsequent surfactant addition. The experiments were conducted by Langmuir Film Balance measurements, Drop Shape Tensiometry and Infrared Reflection-Absorption-Spectroscopy (IRRAS). P188 exists in different phases depending on its surface concentration. Our experiments show, that the phase state of P188 has a significant impact on mAb adsorption. The presence of P188 in a concentration above 0.3 mg/L consistently inhibited mAb adsorption. Desorption of mAb was not caused by addition of P188, whereas it was by addition of PS 20. However, IRRA-spectra reveal that PS 20 is only able to displace freshly formed interfacial mAb layers (2-hour old). A 17-hour old mAb layer could not be desorbed by PS 20. This suggests a time dependent reorganization of mAb at the air/water interface which increases its resistance to desorption.

P-824

Small Angle X-ray Scattering (SAXS) as a Powerful Nano-Analytic Tool for Pharmaceutics

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SAXS offers a wide range of applications in pharmacy and pharmaceutical technology. Here we will try to summarize the most important ones and give some examples. SAXS as a physical method is offered for studying proteins, drug delivery formulations, excipients as well as active pharmaceutical ingredients (APIs). All these materials can be in various aggregate states such as solid, liquid crystals or liquids in solution. Various proteins, peptides, and their buffers solvents, can be successful analyzed in the SAXS solutions analytics. For example, important information about proteins (monomers or dimers) conformation and structure can be served by Xsenocs laboratory SAXS without any X-ray beam damage as it can be the case by using Synchrotrons. Generally, SAXS provides additional physical knowledge about a studded material [1].As far as liquid crystals are concerned, lipids are most often included in them as broad drug delivery systems, which show their importance because of biocompatibility. Lipid nanocarriers are lipid nanoparticles, vesicles, multilamellar and unilamellar vesicles (MLVs, ULVs), and so on [2]. Self- assembly of lipids can also be successfully monitored only by SAXS.

P-825

GM1 Micelles Affect Amyloid β Aggregation by Co-assembly

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Amyloid beta peptide (Aβ) is the crucial component of extracellular plaques in Alzheimer's disease. GM1 is a ganglioside abundant in nerve cells and tends to form micelles even in the excess membrane environment. We focus on this aspect and characterize the interaction between Aβ and GM1 micelles, and whether this interaction influences aggregation. We investigate the co-assembly of GM1 micelles and Aβ by means of microfluidic diffusional sizing, confocal microscopy, and cryogenic transmission electron microscopy. We also monitored Aβ aggregation kinetics under the effect of GM1 by using pFTAA fluorescence measurements. Our results revealed the co-assembly of GM1 lipids with both Aβ monomers and fibrils. The results of the non-seeded kinetic show that Aβ40 aggregation is delayed with increasing GM1 concentration, while that of Aβ42 is accelerated. In seeded aggregation reactions, both Aβ40 and 42 aggregations were delayed by adding GM1. The observed co-assembly might influence Aβ primary nucleation through GM1 micelles incorporating Aβ monomers and small oligomers. Co-assembly may also lead to inhibition of Aβ elongation and secondary nucleation by GM1 micelles blocking the ends and sides of fibrils, respectively. These results shed light on glycolipid-peptide interactions that may play an important role in Alzheimer's pathology.