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1

Longitudinal plasma proteome profiling identifies protein clusters associated with overall survival in glioblastoma

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Background: Glioblastoma (GBM) is the most malignant brain cancer. Almost inevitably fatal, it has limited treatment options, few clinically useful biomarkers, and short overall survival (OS).

Materials and methods: Combining high-resolution isoelectric focusing fractionation with liquid chromatography and mass spectrometry (HiRIEF LC-MS) and using proximity extension assays (PEA), we longitudinally analysed the plasma proteome dynamics of 53 GBM patients before surgery and at three time points after surgery. To identify patient clusters and potential biomarkers, we used unsupervised and supervised machine-learning (ML) methods. To explore associations with OS, we performed survival analyses, estimating hazard rates (HR) and 95% confidence intervals (CI) with Cox models.

Results: Through consensus clustering, based on treatment-naïve plasma protein levels, we identified two plasma proteome patient clusters (PPC). Patients belonging to PPC2 had a more oncogenic plasma proteome profile than PPC1 patients and a shorter OS. The association between PPC2 status and shorter OS (HR = 3.011, 95% CI: 1.463-6.196) remained consistent after adjusting for age, sex, and treatment. Supervised ML identified protein panels that separated the patient clusters and may serve as prognostic biomarkers. Largest alterations in the plasma proteome of GBM patients occurred on average one month after surgery, whereas the plasma protein levels at later time points had no difference compared to the pre-surgery levels. We observed a decrease in glioma-elevated proteins in the blood after surgery, identifying potential monitoring biomarkers.

Conclusion: We performed longitudinal in-depth analyses of the plasma proteome in GBM and identified potential prognostic and monitoring biomarkers.

2

OCT1 (SLC22A1) transporter kinetics and regulation in primary human hepatocyte 3D spheroids

Evgeniya Mickols, Alina Meyer, Maria Karlgren, Per Artursson

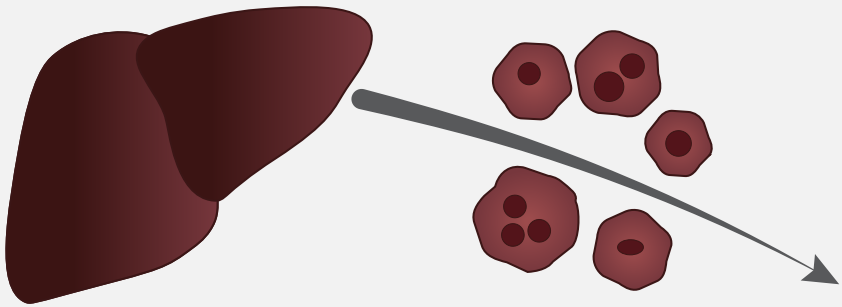
3D spheroids of primary human hepatocytes (3D PHH) cultured in physiologically-relevant conditions keep a differentiated phenotype and proteome fingerprint for weeks in culture. Nonetheless, the transporter function remains to be investigated in PHH spheroids. We demonstrate the applicability of 3D PHH for organic cation transporter 1 (OCT1/SLC22A1) transporter kinetics and long-term drug exposure studies.

3D PHH were cultured in high-throughput 384 well-plate format, and OCT1 transport activity was assessed using the fluorescent model substrate ASP+. Moreover, the ASP+ assay was used together with global proteomics to evaluate the potential modulation of OCT1 expression after prolonged exposure to selected xenobiotics.

In total, 3533 proteins were identified, and 2875 proteins were quantified. 3D PHH proteomes clustered first based on the donor variable and then on the compound exposure factor. We detected compound-specific changes in proteomes. For instance, in the proteomes of 3D PHH exposed rifampicin, a well-known CYP3A4/5 and MDR1 inducer, we observe these proteins to be upregulated; elafibranor, a dual PPARα/δ agonist, caused a statistically significant upregulation of the fatty acid b-oxidation pathway. Lastly, 224 ADME-related proteins were quantified in the dataset, and around half of them were differentially expressed between the PHH donors. Interestingly, no significant change in the hepatic uptake transporters was detected.

Our results show for the first time that 3D PHH spheroids express fully active OCT1 and that transporter kinetics can be studied in individual spheroids using fluorescent probes.

Primary human hepatocytes

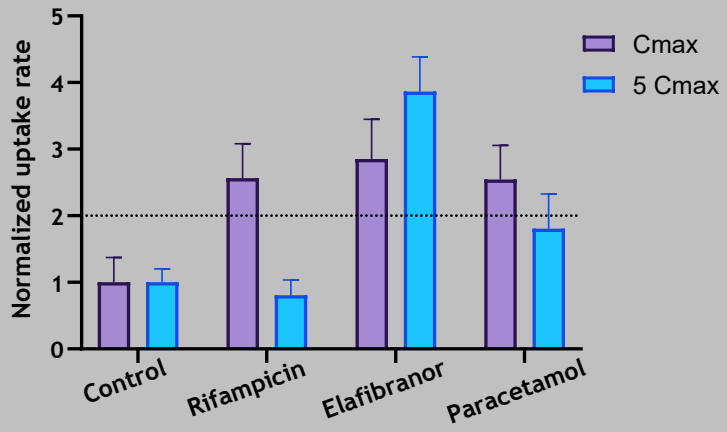


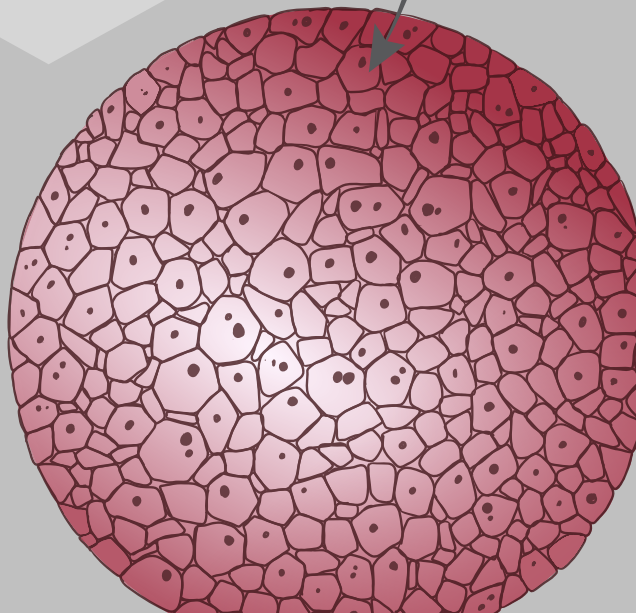
Spheroid formation
384-well plate

in-vivo like conditions

ASP+ kinetics

Long-term
OCT1 modulation





3

Comprehensive proteome and proteoform profiling of childhood acute lymphoblastic leukemia cell lines supports precision medicine insights

Isabelle Leo[1], Nils Kurzawa[2,3], Luay Aswad[1], Matthias Stahl[1], Elena Kunold[1], Tom Erkers[1], Nona Struyf[1], Päivi Östling[1], Olli Kallioniemi[1], Janne Lehtiö[1], André Mateus[2,4], Mikhail Savitski[2], Rozbeh Jafari[1]

[1] Karolinska Institutet, [2] European Molecular Biology Laboratory (EMBL), [3] Institute for Research in Biomedicine, Barcelona, [4] Umeå Universitet

Background: We developed an integrated approach to elucidate drug sensitivity and disease biology in childhood acute lymphoblastic leukemia (ALL), utilizing cell line models for multi-omics, drug response, and proteoform profiling. Our goal was to advance understanding of molecular mechanisms and propose precision medicine strategies.

Materials and Methods: In Study 1, we conducted a multi-omics analysis of 49 ALL cell lines, assessing protein and RNA abundance, cytogenetic fusions, and sensitivity to 528 drugs. Study 2 implemented thermal proteome profiling to distinguish proteoforms in 20 pre-B ALL cell lines, capturing 15,846 proteoforms.

Results: In Study 1, multi-omics phenotyping uncovered post-transcriptional regulation and drug-protein correlations. Notably, we identified bryostatin-1 as a potential therapy for MEF2D-HNRNPUL1 fusion cases and proposed a sensitivity mechanism linked to developmental state. Study 2 revealed proteoform co-aggregation patterns linked to disease biology and utilized proteoforms as drug sensitivity biomarkers. By annotating proteoforms, we revealed cleavage products, ligand associations, and protein interactions, enhancing functional profiling.

Conclusion: Our integrative strategy supports comprehensive molecular profiling of childhood ALL and showcases the applicability of thermal proteoform detection as a global and untargeted method uncovering biological distinctions and drug mechanisms. The identified molecular mechanisms have potential to lead new approaches in precision medicine and serve as a resource for ongoing research.

References: 1) Leo, I.R., Aswad, L., Stahl, M. et al. Nat Commun 13, 1691 (2022). https://doi.org/10.1038/s41467-022-29224-5

2) Kurzawa, N., Leo, I.R., Stahl, M. et al. Nat Chem Biol 19, 962–971 (2023). https://doi.org/10.1038/s41589-023-01284-8

4

Revealing a Novel Interaction between Streptolysin O and Human Plasminogen through Integrated Proteomics

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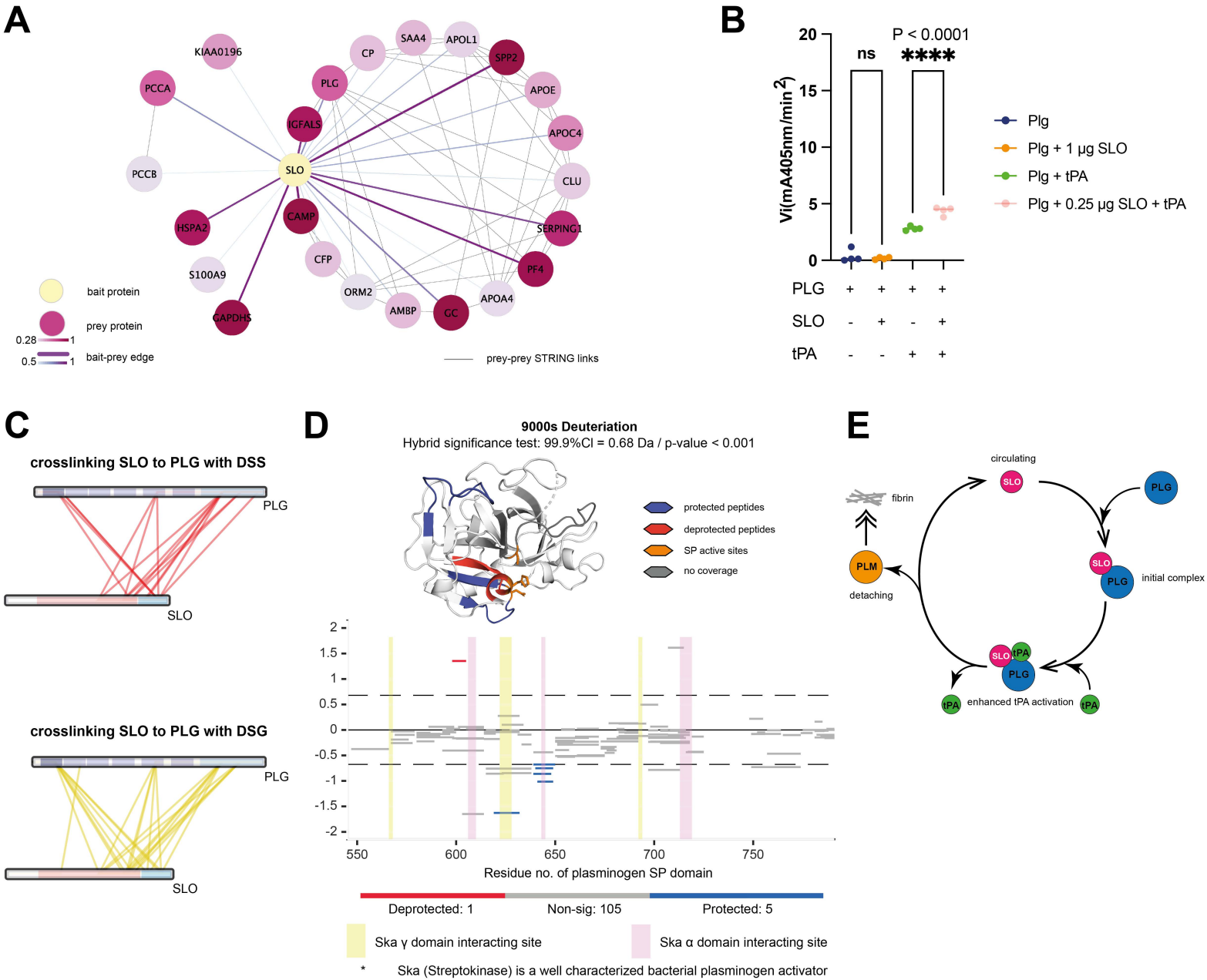
Background: The understanding of host-pathogen interactions is pivotal for bacterial pathogenesis studies. Streptolysin O (SLO), a major virulence factor in Group A Streptococcus (GAS), is known for its pore-forming ability. Here, we employed comprehensive proteomic techniques to investigate SLO's uncharacterized role in interacting with host human plasminogen (PLG).

Materials and methods: Potential SLO-binding plasma proteins were identified using affinity purification-mass spectrometry (AP-MS) coupled with differential abundance analysis. Direct binding of SLO to PLG was validated through an enzyme-linked immunosorbent assay (ELISA). Plasminogen activation assays, crosslinking mass spectrometry (XL-MS) followed by information-driven docking, and hydrogen-deuterium exchange MS (HDX-MS) were then utilized to analyze the biological relevance, proximate interaction sites, and protein dynamics of the SLO-PLG binding.

Results: Our study identified PLG as a direct SLO interactor, leading to enhanced host tissue plasminogen activator (tPA)-catalyzed plasmin generation, potentially aiding in bacterial dissemination and invasion. Molecular mechanisms and dynamics were elucidated through XL-MS and HDX-MS, identifying key residues in the SLO-PLG interface formation and consequent conformational changes.

Conclusions: The discovery of a novel SLO-PLG interaction deepens our understanding of GAS pathogenicity. Applying a multi-modal integrated mass spectrometry strategy, including AP-MS, XL-MS, and HDX-MS, uncovered this previously uncharacterized and understudied protein-protein association. Our research emphasizes the efficacy of advanced proteomic methodologies in probing complex host-pathogen interactions, revealing insights into GAS infection. Our work paves the way for potential therapeutic interventions targeting GAS infections and even insufficient thrombolysis, underlining the importance of continued research into bacterial virulence factors using cutting-edge technologies.



A) Network analysis of the streptolysin O (SLO) and plasma protein interaction derived from affinity purification-mass spectrometry (AP-MS) data-independent acquisition (DIA) quantification matrix.
B) Plasminogen activation assay, demonstrating that SLO-bound plasminogen (PLG) is more susceptible to tissue plasminogen activator (tPA) catalysis, as evidenced by the absorbance of the cleaved chromogenic substrate.
C) Linkage maps depicting all identified inter-protein crosslinks between SLO and PLG.
D) Peptide-level significance test of deuterium uptake under maximum labeling time, revealing the dynamics of PLG's serine protease (SP) domain in response to SLO binding.
E) Proposed model illustrating the molecular machinery role of SLO in the human host plasminogen-to-plasmin activation system.

5

A single sample proteome score to determine mucosal damage in celiac disease

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Background

Celiac disease (CeD) is diagnosed and monitored by histological evaluation of duodenal biopsies, but histological measurements require good biopsy orientation and suffer from poor inter-observer reproducibility. Tissue proteome analysis of formalin-fixed paraffin-embedded (FFPE) biopsy sections capture disease processes in the CeD intestine. We aimed to transform complex proteome data into a numerical score for use as an observer-independent measure of gluten-induced mucosal damage in CeD.

Methods

To enable use in clinical settings we developed a simple protocol for MS-based proteome analysis of single, glass-mounted FFPE biopsy sections. Proteome data were converted to numerical scores using two different computational approaches, taking advantage of previously published CeD proteome datasets. The score approaches were validated using an independent patient cohort of CeD patients comparing biopsies collected at diagnosis to biopsies collected after treatment with gluten-free diet (GFD).

Results

Proteome scoring discriminated between biopsy samples taken from CeD patients before and after treatment on GFD. The two proteome score approaches correlated well with each other, and also with histology-derived villus height to crypt depth (Vh:Cd) ratio. Change in proteome score upon treatment with GFD correlated with change in Vh:Cd ratio. Both unstained and HE-stained sections can be used for proteome scoring, and proteome score can determine disease state of poorly oriented biopsies not suitable for image-based histology.

Conclusion

The proteome score represents a much-needed observer-independent molecular histology measure to supplement image-based histology in CeD. The pipeline is simple and can readily be implemented by non-expert laboratories and scaled for clinical trial settings.

6

Estimation of immune cell infiltration via bulk sample mass spectrometry data deconvolution

Måns Zamore[1], Sergio Mosquim Junior[1], Fredrik Levander[1]

[1] Department of Immunotechnology, Lund University

Background: Immune cell infiltration is an important parameter for predicting cancer prognosis and response to various types of treatment. So far, bulk transcriptomics data have been widely used to deconvolute cancer tissue sample content and infer immune cell infiltration. Proteomics data may be more suitable than transcriptomics data, due to its more direct measurement of protein levels. A few reports of usage of proteomics data for bulk sample deconvolution are available, but there are so far no well-established computational deconvolution methods based on proteomics data.

Materials and Methods: In this study, we have evaluated several computational approaches for estimation of immune cell infiltration based on bulk sample proteomics data. Using defined cell mixtures and parallel proteomics and transcriptomics data from breast cancer tumours, the impact of different pre-processing methods and usage of different software tools on the outcome of cell deconvolution were assessed.

Results: The results of the analyses show the feasibility of using proteomics data for bulk sample deconvolution and immune cell infiltration. Consequences of differential pre-processing and differences between the investigated software tools are further presented. Finally, the consequences of using proteomics versus transcriptomics data are further shown and discussed.

Conclusion: This research highlights the potential of proteomics data for sample deconvolution and estimation of immune cell infiltration. Our findings underscore the importance of pre-processing and need for further development of algorithms for proteomics-based deconvolution.

7

Functional Enrichment Metric Score for proteomic analysis

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[1] Gothenburg University, [2] Northwestern University Feinberg School of Medicine

Functional integrative metric score focuses on utilizing statistics as the basis for evaluating the efficacy of enrichment, while also considering the biological relevance of the results. This metric encompasses various aspects, such as statistical significance, size of enriched gene/protein sets, background set and possibility to check them separately in the context of the disease. Additionally, it takes into account the diversity of functional gene categories and their interrelationships to merge statistical measures with biological context. This metric score is mostly dedicated to differential analysis of genes/proteins from multi-omics platforms. Integrating statistical and functional aspects offers a holistic evaluation of the quality of integrative enrichment analysis.

Our results depict evaluation of experimental results from App Knock-In mouse models of Alzheimer's disease and comparing with our functional integrative metric score along with other similar tools. Reducing result redundancy in multi-omics enrichment analysis, can contribute to a better understanding of biological mechanisms and more precise identification of significant functional categories. Our findings demonstrate that our method provides reliable results that have both statistical rigor and biological relevance. Our metric scoring system enables researchers to identify biological significance in functional, biological, molecular and cellular categories that are also statistically significant. This leads to a deeper understanding of the underlying biological processes and mechanisms in the studied disease.

Our functional integrative metric score may serve as tool for researchers in multi-omics bioinformatics field of research.

Our metric score contributes to a better understanding of complex proteins/genes functions for multi-omics enrichment analysis.

8

Leveraging explainable machine learning to improve the molecular definition of sepsis

Aaron Scott[1], Erik Malmström[1], Lisa Mellhammar[1], Axel Gustafsson[1], Anahita Bakochi[1], Marc Isaksson[1], Tirthankar Mohanty[1], Lars Malmström[1], Johan Malmström[1], Adam Linder[1]

[1] Division of Infection Medicine, Department of Clinical Sciences, Lund University

Background: The diagnosis of sepsis is a complex process that involves the clinical evaluation of organ dysfunction and infection. By utilizing high-throughput proteomics and explainable machine learning, we look to further interrogate the unique molecular definition of sepsis to improve diagnosis and personalize patient care.

Materials and methods: 1378 blood plasma samples were analyzed from patients suspected of sepsis using data-independent acquisition trapped ion-mobility mass spectrometry to generate a compendium of proteome maps. Through statistical analysis, explainable machine learning, and novel feature selection methods we investigated the unique proteome signatures associated with different clinical parameters to provide a molecular atlas for the definition of sepsis.

Results: We identified panels of proteins that were predictive for 6 different types of organ dysfunction and infection and analyzed the biological processes associated with each panel to show how their unique proteome signatures influence sepsis. We then combined each protein panel to train a machine learning model that predicts severe sepsis with high accuracy. Finally, we used this classifier to stratify patients into different risk categories and highlight how increasing risk is associated with decreasing survivability.

Conclusion: Through a combination of high-throughput mass spectrometry and explainable machine learning we identified unique biological signatures associated with the clinical manifestations of sepsis to improve our overall understanding of the condition. Additionally, as patients are broadly diagnosed under the current definition of sepsis, these findings have the potential to be applied in a clinical setting as a starting point for better personalized care.

9

Enzymatic Phosphorylation of Oxidized Tyrosine Residues

Jaakko Teppo[1], Juho Heininen[1], Catharina Erbacher[2], Tapio Kotiaho[1], Risto Kostiainen[1]
[1] University of Helsinki, [2] University of Münster

Background

Post-translational modifications (PTMs) alter the function and fate of proteins and cells in almost every conceivable way. Protein modifications can occur as a result of specific regulating actions of enzymes, such as tyrosine kinases phosphorylating tyrosine residues, or by nonenzymatic reactions, such as oxidation related to oxidative stress. While many studies have addressed the multisite, dynamic, and network-like properties of PTMs, only little is known of the interplay of the same site modifications.

Materials and methods

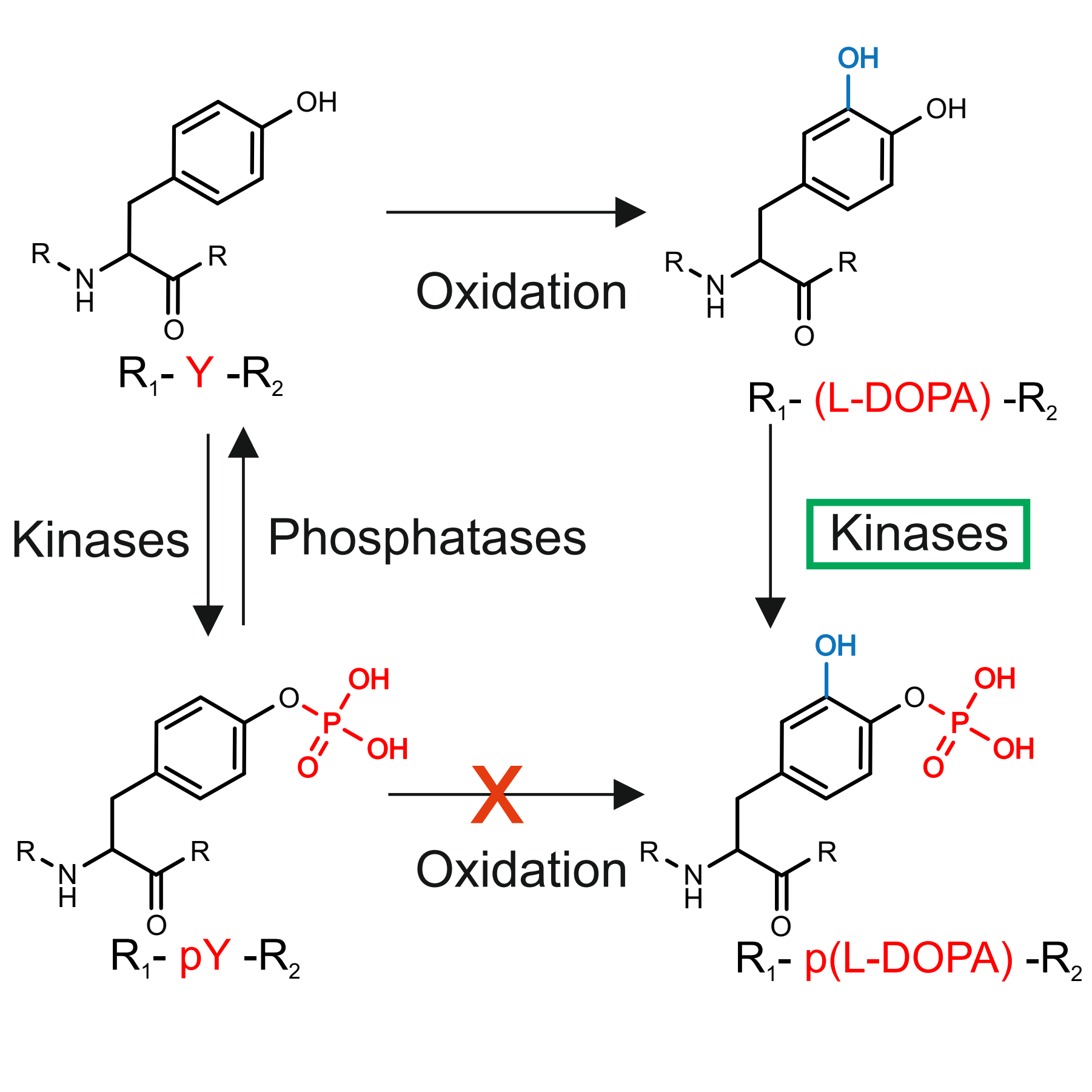
In this work, we studied the enzymatic phosphorylation of oxidized tyrosine (l-DOPA) residues using synthetic peptides, in which tyrosine residues were replaced with l-DOPA. The tyrosine kinase INSR was used to phosphorylate the peptides. The phosphorylated peptides were identified by liquid chromatography-mass spectrometry and the site of phosphorylation by tandem mass spectrometry.

Results

The results show that oxidized tyrosine residues are phosphorylated, displaying a specific immonium ion peak in the MS2 spectra. In peptides with oxidized tyrosine residues, the reaction occurs slightly slower than in peptides in which the tyrosine residues are not oxidized. Furthermore, we detected this novel PTM, where both oxidation and phosphorylation take place at the same tyrosine residue, in our reanalysis of published phosphoproteomics data.

Conclusion

Our data indicate that there can be multiple PTMs (that do not exclude each other) at the same modification site. These results give rise to further questions about the role of tyrosine oxidation, e.g., how widespread it is, how it affects protein functions, or what kind of biological significance it has.


OH
R
N
H
R
O
Oxidation
OH
OH
R
N
H
R
O
R1- Y -R2
R1- (L-DOPA) -R2
Kinases
Phosphatases
Kinases
OH
O
P
OH
O
OH
R
N
H
R
O
X
Oxidation
OH
OH
O
P
OH
O
R
N
H
R
O
R1- pY -R2
R1- p(L-DOPA) -R2

10

Metaproteomics - status, challenges, opportunities, and future directions of the field

Magnus Arntzen[1], Tim van Den Bossche[2], Valerie Schiml[1], Live Heldal Hagen[1], Sabina Leanti La Rosa[1], Phil Pope[1], Vincent Eijsink[1]
[1] Norwegian University of Life Sciences, [2] Ghent University

Omics methods have enabled scientists with a means to interpret the complexity of microbial communities on a scale not attainable before. Individually, the various –omics methods such as metagenomics and metaproteomics can provide great insight, but the true power lies in combining, which can provide detailed understanding of which organisms occupy specific metabolic niches, how they interact, and how they utilize nutrients.

Metaproteomics was first established in 2004 by Wilmes and Bond and involves the global analysis of protein expression within microbial communities using mass spectrometry. In their pioneering work, only a handful bacterial proteins could be identified from a mixed microbial community. Microbial communities can be highly complex and heterogeneous and may contain hundreds-to-thousands of different species. Over the last two decades, and alongside developments in mass spectrometry and sophisticated software algorithms, metaproteomics has evolved into a robust technique often allowing identification of >10.000 microbial proteins in environmental samples. From the 'good and bad' bacteria in the human gut, to the production of biofuels, or controlling nitrogen turnover in soil to tackle climate change - metaproteomics tools have great potential to provide important novel insights with implications for human, animal, and plant health, as well as environmental sustainability.

In this talk I will summarize the status of the field, point at specific challenges and opportunities for metaproteomics using examples from our own research on biogas reactors and nitrogen turnover in soil. I will also introduce the Metaproteomics Initiative and their Critical Assessment of MetaProteome Investigation (CAMPI) studies.

11

Mapping of time-resolved Hsp90-client protein interactions

Kirill Jefimov[1], Elad Noor[2], Evgeny Onishchenko[1]
[1] University of Bergen (UiB), [2] Weizmann Institute of Science

Background: The heat shock protein 90 (Hsp90) chaperone system is an integral part of the proteostasis network, which upholds the functional state of cellular proteins. Yet, neither the inventory of Hsp90 clients nor the biological significance of these interactions are not clear.

Materials and Methods: We employed state-of-the-art proteomic techniques (pulse-SILAC AP-MS, DIA-MS) to uncover the temporal patterns of Hsp90-client interactions in budding yeast.

Results: Our results reveal a clear temporal niche of Hsp90, interrogating the majority of its clients at the early stages of their lifecycle. Striking examples of this age specificity include components of the nuclear pore complex, proteasome, and nuclear-encoded mitochondrial proteome that are significantly younger compared to their average cellular age. We also observe a cohort of constitutive Hsp90 clients that do not show temporal preference and are mainly involved in protein translation and amino acid metabolism.

Conclusions: Our analysis of Hsp90-client interactions, introducing an additional temporal dimension, expands the inventory of Hsp90 clients and reveals functional diversity of the Hsp90 chaperone system linked to the client's age whose biological significance is yet to be fully understood.

12

Tetramodal Chemical Imaging Delineates the Lipid–Amyloid Peptide Interplay at Single Plaques in Transgenic Alzheimer's Disease Models

Junyue Ge[1], Srinivas Koutarapu[1], Durga Jha[1], Maciej Dulewicz[1], Henrik Zetterberg[1,2,3,4,5,6], Kaj Blennow[1,3], Jörg Hanrieder[1,2,3]

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Amyloid-β (Aβ) plaque formation is one of the main hallmarks of Alzheimer's disease (AD). Over the years, mounting evidence that changes in the neuronal lipid biochemistry are associated with protein misfolding and aggregation into mature, neurotoxic plaques. However, the overall molecular events underlying neurodegenerative Aβ pathology in AD are largely unknown. The primary goal of this project is to employ advanced chemical imaging to unravel the exact lipid-Aβ plaque interactions in various AD model systems.Here, we employed a multimodal imaging paradigm combining matrix assisted laser desorption/ionization imaging mass spectrometry (MALDI-IMS) and fluorescent amyloid staining. Tri-modal MALDI-IMS under negative- and positive ion mode lipid analysis and subsequent peptide/protein ion imaging were performed at 10 μm spatial resolution on the same tissue section from tgAPPSwe and tgAPPArcSwe mouse brains. Furthermore, employed LCO-based, hyperspectral, fluorescent amyloid microscopy paradigm provided the insights into degree of Aβ aggregation associated with plaque polymorphism. Multivariate statistical analysis was used to interrogate the IMS datasets and region-specific differences in Aβ peptide pattern were correlated with changes in lipid distributions revealed by MALDI-IMS lipid analysis. MALDI-IMS and fluorescent amyloid staining revealed chemical features associated with heterogeneous distributions of different individual Aβ deposits. For the tgAPPSwe mouse model, it was primarily the wild type peptides that aggregated in Aβ plaques including both diffuse and core formation. For the tgAPPArcSwe mouse model, there were massive, cored plaques containing primarily the C-terminally truncated peptides.

13

Spatial proteome maps to navigate the uncharted subcellular landscapes of non-model organisms

Konstantin Barylyuk[1,2,3], Amandine Guerin[4], Nicola M. Moloney[3], Ludek Koreny[3], Huiling Ke[3], Simon Butterworth[3], Oliver M. Crook[3,5], Imen Lassadi[3], Vipul Gupta[3], Eelco Tromer[3,6], Tobias Mourier[7], Tim J. Stevens[8], Lisa M. Breckels[3], Arnab Pain[7], Paula MacGregor[9], Boris Striepen[4], Kathryn S. Lilley[3], Ross F. Waller[3]

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Research in life sciences has primarily centred on a few model organisms, neglecting the vast diversity of eukaryotes, particularly unicellular microorganisms known as protists. Despite their critical ecological significance, these protists, including apicomplexans like malaria-causing Plasmodium spp., remain understudied. Through diseases in humans and livestock, they threaten healthcare, food security, and economies worldwide.

Although the next-generation sequencing technologies have accelerated genome mapping in non-model species, challenges persist in functionally annotating genes due to the lack of established genetic and cell-biological methods. Moreover, homology-based inference of gene function is restricted to a small subset of conserved genes.

Protists, despite being unicellular, exhibit complex subcellular architectures with unique specialised organelles. In such intricate subcellular landscapes, localising proteins is pivotal in unravelling their function.

Using hyperplexed Localisation of Organelle Proteins by Isotope Tagging (hyperLOPIT), we generated high-resolution maps of protein subcellular localisation for several protists, including the apicomplexan parasites Toxoplasma gondii and Cryptosporidium parvum. These maps provide functional context to hundreds of previously uncharacterised proteins, including those mediating the host-parasite interactions. Notably, in C. parvum, hyperLOPIT revealed a previously unknown secretory compartment.

Integration of spatial proteome maps with comprehensive cell-wide datasets, encompassing gene knockout phenotypes, genetic polymorphisms and mutation rates, co-expression networks, and gene orthology, delivers unprecedented insights into the spatial organization of regulatory programmes, distribution of selective pressures and adaptive responses across organelles, and compartment-specific evolutionary trajectories to parasitism in protists. In summary, spatial proteomics revolutionises the study of non-model organisms, offering a paradigm shift in understanding their biology.

14

Six potential proteins predicting immune checkpoint therapy response in three independent metastatic melanoma cohorts

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Background: What is really problematic in melanoma care that there is unmet need for novel protein discovery with predicting therapeutic efficacy. In our study, we investigate a panel of six proteins targeted at predicting response to ICI therapy in proteomics and transcriptomics data of independent metastatic melanoma cohorts.

Methods: A machine-based learning score system (EaSIeR) was utilized to assess a set of proteins, that was previously reported in one cohort as were associated with immunotherapy response (ITR), based on quantitative protein measurements to distinguish potential responders (Q4) and non-responders (Q1). This evaluation was conducted in two separate melanoma patient cohorts who had not received immune checkpoint inhibitor (ICI) treatment. The identified proteins were validated in separate immunotherapy based transcriptomic datasets.

Results: Six proteins (ITGAX, PSMB5, TNFAIP2, SAMSN1, CD163, MTSS2), identified within immunotherapy treated cohort, were correlated with immunotherapy response. Unambiguously, these proteins exhibited significant dysregulation between predicted responder and non-responder groups in the two untreated melanoma cohorts (Q1Q4TestP < 0.05; PearsonP < 0.05). Furthermore, three among the six proteins were associated with patient survival (Cox, p < 0.05), both at the protein and at the transcript levels.

Conclusions: In summary, in our study we identified and validated potential proteins predicting immunotherapy response in different metastatic melanoma cohorts affirmed by immunotherapy transcriptomic datasets. We suggest that quantifying these proteins could serve as targets in prospective studies and aid therapeutic decision-making for melanoma patients.