19th CENTRAL AND EASTERN EUROPEAN PROTEOMIC CONFERENCE

14th–17th Oct, 2025 Budapest, HUNGARY

organized by:

MS Proteomics and Glycan Biomarker Research Groups,
HUN-REN Research Centre for Natural Sciences
Mass Spectrometry Division of the Hungarian Chemical Society
Working Committee on Separation Sciences of the
Hungarian Academy of Sciences
Proteomics Division of the Hungarian Biochemical Society

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Venue:
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WELCOME ADDRESS

19th Central and Eastern European Proteomic Conference,
Budapest, Hungary

Dear Delegates,

On behalf of the Organising Committee, the CEEPC, MS Proteomics and Glycan Biomarker Research Groups, HUN-REN Research Centre for Natural Sciences, Mass Spectrometry Division of the Hungarian Chemical Society, Working Committee on Separation Sciences of the Hungarian Academy of Sciences, and Proteomics Division of the Hungarian Biochemical Society, we welcome you to the 19th Central and Eastern European Proteomic Conference in Budapest, Hungary.

This conference brings together leading experts in proteomics, mass spectrometry, and related fields. We have invited speakers from multiple countries who will present their latest research. Following CEEPC tradition, we also provide a platform for young researchers to present their work and exchange ideas with the community.

The program covers method development, instrumentation advances, software solutions, and the application of mass spectrometry in understanding protein functionality in medicine. Participants will address current scientific, clinical, and proteomic challenges, with the goal of translating research findings into practical solutions and therapies.

We look forward to a successful conference.

László Drahos, Lilla Turiák, Károly Vékey, the Organizing Committee & Suresh Jivan Gadher

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Conference Program

Tuesday, Oct 14, 2025

	16.00-18.00	***Registration***
	18.00-20.00	***Get Together Party***

Wednesday, Oct 15, 2025

	9.00-9.20	***Opening***
	Chair: Lilla Turiák	
WeOr01	9.20-9.40	**György Markó-Varga** Individual Cancer Mapping (ICM)
WeOr02	9.40-10.00	**Christoph Messner** High-Throughput Proteomics to Study Human Diseases
WeOr03	10.00-10.20	**Gabriella Gellén** High-Throughput SPE Membrane Approaches for Peptide Cleanup and Enrichment
	10.20-11.00	***Coffee break***
	Chair: Marek Sebela	
WeOr04	11.00-11.20	**Isabelle Fournier** Mass Spectrometry Imaging and Spatial Proteomics: A Multi-faceted Tool to Challenge Precision Medicine and Surgery
WeOr05	11.20-11.40	**Simon Sugár** Small-scale Multiomics to Investigate Cell State in Breast Cancer Cells
WeOr06	11.40-12.00	**Gergő Kalló** Multiomics Perspectives of Salivary in Oral Squamous Cell Carcinoma

WeOr07	12.00-12.20	**Luka Milivojević** A look on proteomics with new levels of sensitivity, precision, and flexibility
	12.20-14.00	***Lunch***
	Chair: Zsuzsanna Darula	
WeOr08	14.00-14.20	**Johannes Stadlmann** SugarQbits: A Glyco-Dedicated Open Search Approach for Unsupervised Glycoproteomics
WeOr09	14.20-14.40	**Jonas Nilsson** Glycoproteomic Characterization of Chondroitin Sulfate-Glycopeptides of Endogenously Cleaved Aggrecan
WeOr10	14.40-15.00	**Tamás Pongrácz** The Human Blood N-Glycome: A Cross-Disease Study with Highlights
WeOr11	15.00-15.20	**Dalma Dojcsák** Clinical Associations of the Insulin-Specific IgG N-Glycome in Type 1 Diabetes
	15.20-17.00	***Poster session 1 and coffee break with wine***
	Chair: Johannes Stadlmann	
WeOr12	17.00-17.20	**Roman A. Zubarev** Recent Breakthroughs in Chemical Proteomics
WeOr13	17.20-17.40	**Ágnes Révész** Unlocking N-Glycosylation: Adaptive Collision Energy Choice and Digestion Enzyme Effect for Proteomic Discovery
WeOr14	17.40-18.00	**Mirko Marino** Integrating High-Throughput Proteomics with Clinical Data to Stratify Metabolic Risk in Healthy Adults: Findings from The INSTEAD Study
	18.30-19.15	***Dinner Seminar***

Thursday, Oct 16, 2025

Chair: Piotr Widłak

ThOr01	9.00-9.20	**Connie Jimenez** Clinical Proteomics to Improve Patient Diagnosis, Prognosis and Treatment
ThOr02	9.20-9.40	**Michel Salzet** Illuminating the Shadows: Functional Roles of the Dark Proteome in Cancer Progression
ThOr03	9.40-10.00	**Peter Treit** Plasma Proteomics and Metaproteomics Profiling in End-Stage Cirrhosis
ThOr04	10.00-10.20	**Éva Csősz** Multiomics Examination of Obesity and Type 2 Diabetes Using Mass Spectrometry-Based and Mass Spectrometry-Free Technologies
	10.20-11.00	***Coffee break***

Chair: Katarina Davalieva

ThOr05	11.00-11.20	**Piotr Widłak** The Link Between The Proteome and Functions of Lymphocyte-Derived Exosomes from The Plasma of Melanoma Patients
ThOr06	11.20-11.40	**Lilla Turiák** Challenges in Glycosylation Analysis of Extracellular Vesicles
ThOr07	11.40-12.00	**Martin Kolísek** Impact of a Complex Adjuvant Parkinson‘s Disease Therapy on the Profile of Plasma Proteins.
ThOr08	12.00-12.20	**Goran Mitulović** Deep Insights into Complex Proteomics Samples with Advanced timsTOF

	12.20-14.00	***Lunch***

Chair: Éva Csősz

ThOr09	14.00-14.20	**Stephen Pennington** Discovery and Development of Biomarkers for Psoriatic Disease
ThOr10	14.20-14.40	**Katarina Davalieva** Comprehensive Proteomics Investigation of Altered Molecular Pathways in Recurrent Pregnancy Loss
ThOr11	14.40-15.00	**Tímea Körmöczi** The Role of Mass Spectrometry in Advancing Hyperemesis Gravidarum Research
ThOr12	15.00-15.20	**Zoltán Szabó** Quantitative Analysis of The Cell Surface Proteome
	15.20-17.00	***Poster session 2 and coffee break with wine***
	19.00-22.00	***Conference dinner***

Friday, Oct 17, 2025

Chair: Károly Vékey

FrOr01	9.00-9.20	**Mikhail Gorshkov** Direct Identification of Proteins from Peptide Mass Spectra for Biomarker Discovery: The Case of Alzheimer's Disease
FrOr02	9.20-9.40	**Lukáš Žid** Synthesis of Peptide Against Sars-Cov-2 Virus That Can Plausibly Cross BBB
FrOr03	9.40-10.00	**Mangesh Bhide** Proteomic Aspects of The Crossing of The Pathogens and Drugs Across The Blood Brain Barrier
	10.00-10.30	***Proteomics Quiz and Presentation of the best poster prize***
	10.30-11.10	***Coffee break***

Chair: Ágnes Révész

FrOr04	11.10-11.30	**Laszlo Prokai** Identification of Protein Networks and Biological Pathways Driving The Progression of Atherosclerosis in Human Carotid Arteries
FrOr05	11.30-11.50	**Cristina Banfi** Proteomics-Driven Discovery of Novel Disease Regulators: Uncovering Hidden Players in Adipogenesis, Thrombosis, and Atherosclerosis
FrOr06	11.50-12.10	**Petr Gintar** Suppression of Peptide Loss in Autosampler Vials
FrOr07	12.10-12.30	**Suresh Gadher** Multi-Omics' HIV Immune Monitoring of Patients Undergoing Antiretroviral Therapy (ART) and at Increased Risk of AIDS-defining Cancers

12.30-12.40	***Presentation of the 20th CEEPC***
	Closing

INDIVIDUAL CANCER MAPPING (ICM)

György Markó-Varga

Dept. of Biomedical Engineering, Lund University, Biomedical Center, BMC D13, SE-221 84 Lund, Sweden

Tracking tumor evolution in individual melanoma patients, from diagnosis through metastases, reveals the unique ways in which melanoma's aggressive progression impacts each person. This dynamic evolution, driven by genetic and epigenetic changes, produces intratumoral heterogeneity, complicating treatment as different tumor sub-clones respond variably to therapies.
To address this, the Swedish Cancer Moonshot is advancing personalized medicine, developing biomarker-driven treatments and early-phase trials tailored to each patient's molecular profile. Additionally, cutting-edge proteomic and imaging research supports immunotherapy advancements, enhancing immune checkpoint inhibitors through combination therapies designed to heighten effectiveness and reduce resistance. These strategies offer a more targeted approach to resilient treatment for individual melanoma patients.

HIGH-THROUGHPUT PROTEOMICS TO STUDY HUMAN DISEASES

Christoph B. Messner

Precision Proteomics Center, Swiss Institute of Allergy and Asthma Research (SIAF), University of Zurich, 7265 Davos, Switzerland

Recent advances in proteomics have enabled higher throughput and improved quantitative precision, opening the door to a wide range of new applications—particularly for gaining molecular insights into human disease. Our work leverages these technological developments to study a variety of conditions, including inflammatory skin diseases, allergies, and cancer. By analyzing sample materials accessible at large scale we aim to capture disease heterogeneity and treatment response. Additionally, we investigate the role of post-translational modifications, particularly glycosylation, as key modulators of protein function and potential diagnostic indicators. This talk will highlight how large-scale proteomic approaches support biomarker discovery, the development of novel therapeutic approaches, and a deeper understanding of disease heterogeneity.

HIGH-THROUGHPUT SPE MEMBRANE APPROACHES FOR PEPTIDE CLEANUP AND ENRICHMENT

G. Gellén[1)]

1) Affinisep, 10 rue Richard Dufour, 76770 Le Houlme, France

Bottom-up LC-MS/MS workflows typically require multistep sample preparation, followed by peptide cleanup to remove salts that otherwise cause ion suppression and reduce identification rates. For proteoform analysis, enrichment of post-translationally modified (PTM) peptides is also essential to enable reliable identification, localization, and quantification. Conventional cleanup strategies are often labor-intensive and prone to sample loss or elution variability. Developing robust, automatable, and standardized cleanup and enrichment methods is therefore a critical step toward establishing proteomics as a practical tool in diagnostics and drug discovery.

Our SPE membrane technology, developed from two decades of expertise in complex sample preparation, addresses several of these bottlenecks in MS-based proteomics.

One major challenge is high-throughput single-cell and other low-input analyses, where consistent recovery from limited protein material is required. Using optimized membrane workflows, reproducible results were obtained across a 1 ng–10 µg input range, with up to 97% protein identification and <3% RSD, demonstrating reliable analysis even from trace-level samples.

In glycoproteomics, the structural diversity of glycans complicates enrichment and detection. Preliminary studies with SPE membrane-based methods showed on average a five-fold increase in N-glycopeptide identifications from cell lysates and plasma compared to unenriched controls, reaching or exceeding the performance of established HILIC-based methods.

Phosphoproteomics represents another demanding application, as conventional desalting frequently results in the loss of polar phosphopeptides. Membrane-based C18 formats enabled up to 2.4-fold more phosphopeptide identifications with reproducibility below 10% RSD. Optimized acidification conditions further improved recovery of hydrophilic and singly phosphorylated peptides.

These findings highlight how SPE membrane workflows provide efficient, reproducible, and high-throughput solutions for peptide desalting and PTM enrichment, ultimately supporting more robust and sensitive MS-based proteomic analyses in clinical and cancer research.

MASS SPECTROMETRY IMAGING AND SPATIAL PROTEOMICS: A MULTI-FACETED TOOL TO CHALLENGE PRECISION MEDICINE AND SURGERY

Isabelle Fournier[1-2]

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2) Institut Universitaire de France, 75000 Paris

Large-scale MS-based proteomics has transformed the study of proteins. However, conventional strategies lack a spatial dimension, which is crucial for a better understanding of physiological and pathophysiological mechanisms through access to the cell microenvironment. Conversely, MS imaging (MSI) reveals the spatial distribution of molecules at the level of individual cells but cannot provide large-scale identification. Therefore, combining MSI and large-scale omics to perform MSI-guided spatial proteomics opens up new avenues for investigating biological mechanisms and their clinical applications. Indeed, measuring the cell environment is crucial for many applications, including in oncology. The clonal heterogeneity of cancer cells and their interaction with surrounding cells, including immune cells, drive cancer evolution and the fate of both tumour and patient. By using a combination of non-targeted and targeted MALDI IHC and MALDI MS imaging, as well as various spatial proteomics approaches, we were able to study the clonal heterogeneity of different cancers, including brain tumours, breast cancer, and oesophageal cancer. This study demonstrates not only the possibility of finding prognostic biomarkers, but also of moving forward to better select treatments according to the nature of the identified clones. Additionally, our findings suggest that the microenvironment in which the tumour grows mainly drives patient outcomes. This will be demonstrated using a combination of different spatial proteomic strategies developed to address cancer heterogeneity at the single-cell level. Finally, the gradual shift towards direct in vivo and in situ real-time analysis using novel ambient ionisation mass spectrometry technologies, such as SpiderMass, provides new opportunities for characterising the TME during surgery for surgical guidance and improved patient management.

SMALL-SCALE MULTIOMICS TO INVESTIGATE CELL STATE IN BREAST CANCER CELLS

S. Sugár[1)], J. Price[1)], J. Haworth[2)], P. Fullwood[2)], M. P. Smith[2)], JM. Schwartz[2)] and C. Francavilla[1,2)]

1) Department of Biotechnology and Biomedicine, Technical University of Denmark, Kongens Lyngby, Denmark

2) School of Biological Science, Faculty of Biology Medicine and Health (FBMH), The University of Manchester, M139PT, Manchester, UK

Multiomics is becoming a cornerstone of life sciences as it can capture a more holistic picture than any single omics (e.g. transcriptomics or proteomics) by leveraging complementary information from several layers. Integrating these heterogeneous data types is a critical step in gaining meaningful insights into the biological system studied; however, it poses unique challenges especially with limited sample sizes, where statistical power and data sparsity can hinder robust conclusions. Network-based approaches provide a powerful biologically informed framework for integration that can enhance interpretability and robustness by incorporating prior knowledge and enabling the visualization of complex molecular interactions.

In this presentation I will demonstrate how we apply network-based multiomics data integration to characterize cell state under different conditions in breast cancer cells.

MULTIOMICS PERSPECTIVES OF SALIVARY IN ORAL SQUAMOUS CELL CARCINOMA

Gergő Kalló[1,2,3], Mária Golda[4], Andrea Guba[1,3], Zoltán Tóth[5], János András Mótyán[4], Ildikó Márton[1], Mabuse Moagi[1], Csongor Kiss[6], József Tőzsér[1,4], Éva Csősz[1,2,3]

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Oral squamous cell carcinoma (OSCC) represents one of the most prevalent malignancies of the head and neck region. In addition to well-established genetic and environmental risk factors, OSCC has been increasingly associated with microbial infections and/or dysbiosis. Saliva plays a critical role as the chemical barrier of the oral cavity, and alterations in its composition can indicate pathological changes, including OSCC. The primary aim of our research is to investigate the alterations in the salivary molecular profile in OSCC patients.

Saliva is known to contain over 2,700 distinct proteins, with the most abundant ones serving key roles in the chemical defense of the oral cavity. These include antimicrobial and immunomodulatory proteins such as lactotransferrin, lipocalins, and lysozyme-C, as well as information-carrying proteins such as cytokines.

In this study, we analyzed differentially expressed proteins in the saliva of OSCC patients, constructing and examining the protein-protein interaction networks of both upregulated and downregulated proteins using publicly available proteomic datasets. A total of 127 proteins exhibiting differential expression between OSCC patients and healthy controls were identified, with the central hub proteins including IL-6, IL-1B, IL-8, TNF, APOA1, APOA2, APOB, APOC3, APOE, and HP. Furthermore, enriched Gene Ontology (GO) terms were analyzed to uncover biological insights.

Among the differentially expressed proteins, several potential biomarkers have been reported, including IL-6, S100A9, catalase, and various members of the matrix metalloproteinase (MMP) family. Although the differential expression of MMPs in OSCC has been described, their activity in salivary secretions remains poorly understood. To address this gap, our research team is developing a quenched fluorescent peptide-based assay to assess whether changes in MMP expression profiles correlate with alterations in enzymatic activity. Preliminary results indicate that this method is effective for analyzing protease activity in saliva.

Considering that changes in the composition of the chemical barrier can alter the defense function of the saliva, we investigated the antimicrobial activity of saliva collected from patients with OSCC against pathogenic bacteria associated with OSCC.
In addition to protein composition, we also examined the amino acid and biogenic amine content of saliva, employing an in-house validated, targeted LC-MS method. This comprehensive analysis aims to elucidate the broader molecular alterations in OSCC.
Our findings underscore the significant role of the chemical barrier of saliva in the pathogenesis of OSCC, highlighting potential mechanisms that could lead to improved diagnostic and therapeutic strategies for this malignancy.

Funding: This research was funded by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences and the National Research Development and Innovation Office of Hungary, grant numbers K143021 and GINOP-2.3.3-15-2016-00020.

A LOOK ON PROTEOMICS WITH NEW LEVELS OF SENSITIVITY, PRECISION, AND FLEXIBILITY

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Due to complexity, analysis of biological samples has always required high performance instrumentation that can provide high quality of data which then leads to meaningful and confident results. Parallel to that, diversity of such analyses dictates ever growing need for higher sample throughput required to gather enough information from different sources to identify specific molecules or processes that characterize respective conditions. Since its introduction at ASMS 2023 Orbitrap Astral mass spectrometer has shown us new levels in protein analysis. This year we have introduced Orbitrap Astral Zoom, a mass spectrometer that provides even higher performance. With new unique features we can now do more faster even when facing the most challenging workflows like single cell analysis. Through this presentation we will try to uncover some of those features and how they contribute to even better results.

SUGARQBITS: A GLYCO-DEDICATED OPEN SEARCH APPROACH FOR UNSUPERVISED GLYCOPROTEOMICS

F. Hrdina[1], D. Stenitzer[1], S. Walcher[1], S. Yan[2], J. Vanbeselaere[1], K. Paschinger[1], C. Schäffer[1], IBH Wilson[1], and Stadlmann J.[1]

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The characterization of glycoproteomes remains a significant challenge in proteomics due to the structural diversity of glycans and the reliance of many search engines on predefined glycan mass databases. To address these limitations, we recently developed SugarQbits, a glyco-dedicated "open search" approach that enables the unsupervised identification of glycopeptides without the need for prior knowledge of glycan masses. By leveraging the co-occurrence of prominent fragment ion pairs, such as $[peptide]^+$ and $[peptide + HexNAc]^+$, our platform transforms conventional proteomics search engines into powerful tools for glycoproteomics.

Recently, we applied SugarQbits to analyze glycopeptide-enriched fractions from two insect cell lines[1], *Spodoptera frugiperda* (Sf9) and *Trichoplusia ni* (High Five), as well as the bacterial species *Tannerella forsythia*[2]. In the insect cell lines, we identified over 3,400 unique glycopeptides, revealing distinct glycan profiles, including insect-specific di-fucosylated N-glycans and non-canonical phosphorylcholine-modified glycans. In *T. forsythia*, a reconfigured SugarQbits pipeline detected unique glycopeptides containing the species-specific linkage trisacchride GlcA-(α1,2)-[Rha-(α1,4)]-Gal, identifying 921 glycopeptides from 303 proteins.

Our results highlight the versatility of SugarQbits in uncovering novel glycan structures, including glucuronylated and phosphorylcholine-modified glycans, alongside typical oligomannosidic and fucosylated structures. This approach provides a robust, glycan database-independent solution for glycoproteomics, enabling the discovery of previously uncharacterized glycan modifications across diverse biological systems. Future applications of SugarQbits promise to further expand our understanding of glycoproteomes and their functional roles in non-model species biology.

References

1. S. Yan, J. Vanbeselaere, *et al*.; Mol Cell Proteomic, **24 (6)**, **(**2025)
2. S. Walcher, F.F. Hager-Mair, *et al*.; Glycobiology, **34(12)**, (2024)

GLYCOPROTEOMIC CHARACTERIZATION OF CHONDROITIN SULFATE-GLYCOPEPTIDES OF ENDOGENOUSLY CLEAVED AGGRECAN

J. Nilsson[1,2] F. Noborn[2], M. Blomqvist[2] and G. Larson[2]

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2) Department of Laboratory Medicine, University of Gothenburg, Vita straket 12, Gothenburg, Sweden

To better understand the structure, function and biomedical importance of post-translationally modified ECM proteins, the matrisome, it is crucial to structurally characterize them. In the field of glycoproteomics, N- and O-glycosylated peptides, typically released after tryptic digestion, are analyzed with LC-MS/MS, which enables the site-specific glycan analysis of glycoproteins. In line with this concept, we have developed glycoproteomic methods to characterize glycoproteins carrying glycosaminoglycan (GAG) structures, specifically a family of glycoproteins referred to as chondroitin sulfate proteoglycans (CSPGs) present in human adult urine samples. As children have a relatively high concentration of GAGs in urine, we undertook a CS glycoproteomic analysis of urine samples of individuals aged 0.5 to 19 years. The analysis revealed a multitude of endogenously cleaved CS-glycopeptides derived from aggrecan, e.g. S.GLPS.G and G.VEDIS.G, which were not detectable in adult urine samples. Using the Byonic software, we identified 714 unique glycopeptides from 226 different peptides encompassing up to 150 out of the 184 theoretically possible SG and SA CS-glycosites in aggrecan, previously inaccessible for a structural analysis. Fragmentation analysis in negative mode higher-energy collision dissociation (HCD) was used to characterize the sulfate distribution of the CS chains of the dominating S.GLPS.G glycopeptides. The LC-MS methodologies may become useful in future CS glycoproteomics projects, aiming to exploit the aggrecan glycopeptides as biomarkers for osteoarthritis or rheumatic disorders.

THE HUMAN BLOOD *N*-GLYCOME: A CROSS-DISEASE STUDY WITH HIGHLIGHTS

T. Pongrácz[1,2], *O. A. Mayboroda*[2], *M. Wuhrer*[2]

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Proteins in the circulation undergo substantial *N*-glycosylation in hepatocytes and plasma cells, collectively shaping the human blood *N*-glycome. Glycosylation modulates protein function, stability, and clearance, and is shaped by (epi)genetic, environmental and metabolic factors. Consequently, physiological and pathological states are often mirrored in distinct blood *N*-glycomic signatures, some of which may hold potential as clinically translatable biomarkers in areas of unmet diagnostic need.

Proteins in plasma or serum were denatured, followed by enzymatic liberation of *N*-glycans, linkage-specific derivatization of sialic acids, and purification by cotton-HILIC. Corresponding clinical cohorts including malignacies, infectious, autoimmune and metabolic diseases were measured using a largely consistent, high-throughput, semi-automated MALDI-MS-based workflow capable of processing over 500 clinical samples within a day. The data, while generated individually over a decade, was re-analyzed in an integrated meta-analytic framework. Key glycosylation motifs – such as antennarity, linkage-specific sialylation, fucosylation, and expression of different *N*-glycan types – were quantified and compared across, and for selected examples, within cohorts.

Our cross-disease analysis revealed both shared and disease-specific patterns, with linkage-specific sialylation, antennary fucosylation, or antennarity as consistently emerging hallmarks of pathology. Furthermore, our analysis reveals that distinct glycomic profiles characterize the severity stages of metabolic dysfunction-associated liver disease, from early to advanced fibrosis and hepatocellular carcinoma, highlighting blood protein *N*-glycosylation as a potential biomarker axis for this highly prevalent condition.

While there is a lack of standalone technology for fully defining glycans, using a unified analytical approach with sufficient throughput and largely consistent data analysis workflows enables study comparability. Fulfilling these criteria allowed us to explore blood glycomic features in 13 major diseases. We show the perplexing complexity of the glycomic dimension of the studied conditions, and highlight on biomarker candidates that suggest utility in prevalent diseases such as metabolic dysfunction-associated liver disease and cancer.

CLINICAL ASSOCIATIONS OF THE INSULIN-SPECIFIC IGG N-GLYCOME IN TYPE 1 DIABETES

Dalma Dojcsák[1,3], Marco R. Bladergroen[2], Oleg A. Mayboroda[2], Csaba Váradi[3], Tamás Pongrácz[2], Manfred Wuhrer[2]

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Recent advances in glycomics have highlighted the pivotal role of serum N-glycans derived from glycoproteins as potential biomarkers for various inflammation-related diseases, including cancer, metabolic disorders, infections, and autoimmune conditions. Type 1 diabetes mellitus (T1D) is a chronic autoimmune disease that primarily develops during childhood or adolescence and results from the immune-mediated destruction of pancreatic β-cells. This process leads to an absolute deficiency of insulin, the key hormone regulating glucose homeostasis. The autoimmune response is characterized by the activation of autoreactive T lymphocytes and the production of islet autoantibodies against specific β-cell antigens such as insulin, GAD65, and IA-2. As insulin secretion ceases, exogenous insulin therapy becomes essential for maintaining normoglycemia and preventing acute and chronic complications. Today, after insulin therapy is established in children, annual follow-up examinations include monitoring various laboratory parameters such as HbA1c, urea, creatinine, lipids, triglycerides, and TSH to assess glycemic control and to detect potential complications, such as thyroid dysfunction

The aim of this research was to comprehensively map the total serum protein and antigen-specific IgG N-glycosylation profiles observed in T1D using advanced, high-throughput MALDI-FTICR MS technique. The cohort included in the experiment—comprising 144 T1D patients and 41 healthy controls. Only patient subgroup was related with clinical laboratory parameters, for example HbA1c, creatinine, lipids. In the follow-up analyses, special emphasis is placed on the type of sialic acid linkages, as their alteration has been identified as an early biomarker in other diseases such as NAFLD. The differentiation of sialic acid linkages was achieved through specific ethyl esterification derivatization.

Anti-insulin specific IgG (IAA) was captured from T1D serum samples and quantified using the SiluMAb isotopic labeling method. Glycopeptide analysis of IgG1 isoform is also being investigated, given that the inflammatory condition characteristic of T1D. With quantitation and glycopeptide analysis we confirmed that most of the T1D samples contained IAA at different concentration. This variation also correlated with age and certain glycotraits, such as bisection or di-galactosylation on the IAA. These detailed MS-based analyses may contribute to a deeper understanding of the pathomechanism of T1D and the identification of potential glycan-based biomarkers for disease diagnosis and monitoring.

RECENT BREAKTHROUGHS IN CHEMICAL PROTEOMICS

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Chemical proteomics aims at detailed characterization of the interactions between drug molecules and their protein targets, as well as deciphering the mechanism of drug action. We are currently using four dimensions of analysis, assessing drug-induced changes in protein abundance, solubility (PISA [1]), accessibility to protease and redox state.

Recently, we combined three analysis dimensions by multiplexing them in the PISA-REX approach into a single experiment [2]. We have also automated the solubility experiment in an OPTI-PISA set-up [3]. In another effort, molecular weight (MW) information was brought back to proteomics without losing the benefits of the shotgun (bottom-up) approach [4]. The latter method, GAPPIS, is particularly suitable when protein MW changes under drug influence, e.g., in apoptosis-related proteolysis. Two new partial proteolysis-based approaches are being developed that provide structural details on drug-target interactions, and yet work in an unbiased manner at the proteome-wide scale [5-6].

Despite the two decades of intensive development, the arsenal of chemical proteomics tools has not been exhausted, with new and more powerful tools still emerging at a high rate.

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UNLOCKING N-GLYCOSYLATION: ADAPTIVE COLLISION ENERGY CHOICE AND DIGESTION ENZYME EFFECT FOR PROTEOMIC DISCOVERY

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N-glycosylation is a complex, biologically critical post-translational modification, and its comprehensive analysis remains a central challenge in proteomics. The confident identification and quantitation of site-specific glycosylation from biologically relevant samples rely on robust mass spectrometric (MS) strategies and optimized data analysis workflows.

We have systematically investigated how instrumental parameters—particularly collision energy in tandem MS—influence fragmentation patterns and the performance of database search engines for N-glycopeptides, profiling nearly 200 distinct glycopeptide structures from multiple standards. Our comparison of the Byonic, pGlyco, and GlycoQuest search engines reveals that the energy required for optimal fragmentation depends not only on analyte characteristics, such as peptide sequence and glycan mass, but also on the scoring engine's focus (peptide- vs glycan-centric). Building on these insights, we envisioned a workflow in which collision energy parameters are adaptively tuned based on features accessible in real time (e.g., precursor m/z, hydrophobicity/retention time, glycan mass). Implementing this strategy might boost glycopeptide identifications—by up to 100% for challenging, borderline cases—across diverse samples, including blood plasma, HeLa cells, and therapeutic antibodies.

Further, in a comparative study, we are assessing the impact of digesting glycoproteins with different enzymes (e.g., trypsin, GluC, chymotrypsin) on site-specific glycosylation profiles. Early results show that each protease yields distinct glycan distributions, influencing both detection efficiency and structural coverage of glycosylation sites, thereby setting the stage for comparative MS1-based quantitation of glycosylation patterns.

Our findings offer best-practice recommendations for experimental setup and data analysis to fully exploit the potential of advanced instrumentation, ultimately aiming to improve confidence and reproducibility in glycosylation analyses relevant to health and disease.

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INTEGRATING HIGH-THROUGHPUT PROTEOMICS WITH CLINICAL DATA TO STRATIFY METABOLIC RISK IN HEALTHY ADULTS: FINDINGS FROM THE INSTEAD STUDY

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Precision nutrition aims to tailor dietary strategies to individual biological characteristics in order to improve health outcomes and prevent disease. High-throughput proteomic technologies have emerged as valuable tools for capturing the complexity of metabolic responses influenced by diet. However, the potential of circulating protein profiles to classify apparently healthy individuals according to their underlying metabolic risk remains largely untapped. Such stratification could be essential for identifying those who are more likely to benefit from targeted nutritional interventions. In the INSTEAD trial, we analyzed baseline data from a cohort of healthy adults participating in a randomized crossover dietary study. Anthropometric measurements, clinical biomarkers, and plasma proteomic profiles were collected. A Composite Risk Load (CRL) index was computed using conventional clinical parameters. Plasma proteins were quantified using the SomaScan® platform. We conducted correlation analyses, hierarchical clustering, and protein–protein interaction network evaluations to explore relationships between proteomic profiles and metabolic risk.

A subset of clinical and proteomic features showed strong associations with the CRL index ($|r| > 0.50$). A regression model built on the top 30 proteins explained over 83% of the variance in CRL (adjusted $R^2 = 0.831$), and the combination with clinical data further improved predictive accuracy (adjusted $R^2 = 0.880$). Unsupervised clustering of proteomic data identified three distinct participant groups, each exhibiting different CRL levels and unique proteomic patterns. Functional enrichment analysis highlighted biological processes such as lipid metabolism, inflammation, and immune regulation, particularly enriched in the higher-risk subgroup.

This study demonstrates that high-throughput proteomics can effectively differentiate healthy individuals by their metabolic risk profile. The integration of proteomic signatures with clinical data may enhance risk prediction and support the development of personalized nutritional strategies.

Keywords: proteomics, personalized nutrition, SomaScan, risk stratification, biomarker discovery

CLINICAL PROTEOMICS TO IMPROVE PATIENT DIAGNOSIS, PROGNOSIS AND TREATMENT

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Our lab employs label-free proteomics of clinical samples to explore the biological basis of disease, identify new targetable proteins and pathways for therapeutic intervention, predict disease outcome or treatment response, and probe resistance mechanisms. In my presentation, I will highlight on-going efforts employing tissue (phospho)proteomics and biofluid proteomics with focus on cancer and dementia.

ILLUMINATING THE SHADOWS: FUNCTIONAL ROLES OF THE GHOST PROTEOME IN CANCER PROGRESSION

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The classical perception of the eukaryotic proteome has been radically transformed with the recognition that large genomic regions previously categorized as non-coding DNA encode functional peptides, now termed the "dark proteome" [1-3]. These noncanonical proteins, often under 100 amino acids, arise from alternative open reading frames (altORFs), small ORFs (smORFs), untranslated regions (UTRs), and noncoding RNAs (ncRNAs). Their identification and validation have been made possible through technological advances in proteogenomics, notably ribosome profiling [4], deep high-resolution mass spectrometry (MS), and sophisticated bioinformatics. The dark proteome has emerged as a major player in cancer biology, revealing unappreciated layers of regulatory control over oncogenesis.

The study of the dark proteome employs a multidisciplinary approach combining state-of-the-art proteogenomics pipelines. Ribosome profiling is used to detect active translation events across noncanonical coding regions [4]. Deep MS and advanced crosslinking mass spectrometry (XL-MS) facilitate the identification and mapping of protein–protein interaction networks [5-6]. Bioinformatic analysis incorporating evolutionary conservation metrics distinguishes functional peptides from noise. In parallel, machine learning-driven rescoring strategies improve sensitivity and specificity of dark proteome identification from complex MS datasets 1.

Characterization of alternative proteins has revealed their key roles in processes such as cell proliferation, apoptosis, metabolic reprogramming, immune evasion, and genomic stability [7-10]. Notable examples include AltAKT and AltEDARADD, which modulate cancer cell proliferation; HOXB-AS3, which acts as a tumour suppressor by destabilizing oncogenic mRNAs 3,9 and EMBOW, which regulates cell cycle progression via interactions with chromatin modifiers. These microproteins integrate into classical signalling cascades and often function as modulators or gatekeepers of critical oncogenic pathways. Evolutionary analyses reveal both recently emerged, species-specific proteins and ancient conserved microproteins, underscoring their diverse functional relevance. Moreover, their tumour-specific expression and small size present promising opportunities for use as neoantigen sources in cancer immunotherapy and as scaffolds for drug design1.

The systematic exploration of the dark proteome is redefining the molecular landscape of cancer research. While challenges remain in distinguishing biologically relevant alternative proteins from transient translation products, integrative multi-omic approaches and continual improvements in detection and functional annotation methods are driving progress. These efforts are poised to expand the repertoire of cancer biomarkers and therapeutic targets. Ultimately, unveiling these hidden proteins may deliver transformative insights into oncogenesis and precision oncology, offering novel avenues for diagnosis, prognosis, and treatment.

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PLASMA PROTEOMICS AND METAPROTEOMICS PROFILING IN END-STAGE CIRRHOSIS

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Acute-on-chronic liver failure (ACLF) is a life-threatening syndrome characterized by systemic inflammation, immune dysfunction, and high short-term mortality. While immune dysregulation is established, the contribution of the gut microbiome to ACLF development remains uncertain. Mass spectrometry–based proteomics offers a unique window into this problem, as it resolves both host- and microbe-derived proteins in a single experiment, providing functional and mechanistic readouts across clinical stages. Here we present a multi-matrix proteomics and metaproteomics investigation into cirrhosis, within the MICROB-PREDICT project, aimed at biomarker discovery and improved patient stratification.

In plasma, we applied a longitudinal untargeted LC–MS/MS workflow to ~100 patients and ~300 samples across the course of disease progression. The discovery cohort (~90 patients) enabled identification of candidate markers, while an independent validation cohort was profiled on the Orbitrap Astral, confirming robustness across platforms. In total, >1,200 proteins were quantified, with statistical modeling and machine learning approaches identifying 12 reproducible biomarker candidates predictive of ACLF development. Multivariate proteomic scores outperformed established clinical scores in stratifying patients along the AD–ACLF spectrum, highlighting pathways of immune activation, systemic inflammation, and metabolic reprogramming.

Stool metaproteomics was performed in the same discovery cohort (90 patients), necessitating careful reduction of the vast and heterogeneous protein search space. To address this, we curated sample-specific FASTA databases in layers: (i) de novo peptide sequencing provided initial sequence inclusion, (ii) matched metagenome assemblies with prodigal-based gene and isoform

calls were weighted by metatranscriptome TPM values to prioritize biologically relevant entries, and (iii) reference gut microbial catalogs (UHGG, GMGC) supplied taxonomic breadth. This layered strategy improved peptide–spectrum matching, cross-sample comparability, and the resolution of microbial functions in a longitudinal setting.Multi-omics integration complemented metaproteomics by adding quantitative and functional context from metatranscriptomics, metabolomics, and dysbiosis indices.

Plasma proteomics identified systemic progression markers, while stool metaproteomics uncovered microbial pathways with diagnostic potential and complementary predictive value for ACLF risk. Together, these data demonstrate that tailored, multi-matrix mass spectrometry can reveal both systemic and gut-associated biomarkers, advancing clinical stratification in cirrhosis and ACLF.

MULTIOMICS EXAMINATION OF OBESITY AND TYPE 2 DIABETES USING MASS SPECTROMETRY-BASED AND MASS SPECTROMETRY-FREE TECHNOLOGIES

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Obesity and type 2 diabetes (T2D) can be considered as major health-impairing conditions affecting millions of people worldwide.

Blood samples collected from patients with obesity, and/or T2D were examined by mass spectrometry (LC-MS). DIA and DDA proteomics datasets were acquired and phosphoproteomics analysis was carried out. The concentration of more than 500 analytes from the MxP® Quant 500 XL Biocrates panel were studied in targeted LC-MS approach. In parallel the non mass spectrometry-based proximity extension assay (PEA) was applied and the relative changes of 366 proteins related to cardiovascular diseases were examined. Along with sample collection a thorough clinical examination was carried out, including classical laboratory tests (lipid profile, liver, and kidney metabolism, etc.), gene polymorphism analyses, and imaging. The data were integrated into the UDBD Health BigData repository. Besides the complex statistical analyses, machine learning was applied to find the most important features for disease prediction. Network analysis was carried out using various network models and IPA. The complex examination of proteomics and metabolomics data along with network analysis identified pathways that are characteristic to obesity and T2D. At the same time, we could observe that at molecular level obesity and T2D are more similar than different highlighting the importance of giving greater attention to obesity and obesity-related complications.

THE LINK BETWEEN THE PROTEOME AND FUNCTIONS OF LYMPHOCYTE-DERIVED EXOSOMES FROM THE PLASMA OF MELANOMA PATIENTS

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Small extracellular vesicles (sEV), also termed exosomes, a subset of EVs sized at about 100 nm, are produced and released by all cell types and are present in all body fluids. Molecular content of sEV reflects that of the parent cells, and these vesicles are key components of the cell secretome and mediators in intercellular communication. sEV released by T cells play a key role in immune regulation. We have analyzed proteomic profiles of T cell-derived sEV in vivo to address and elucidate the potential influence of cancer on the functions of this subset of immune cells. Immune capture with anti-CD3 antibodies was used to isolate and study T cell-derived CD3(+)sEV from the plasma of patients with melanoma (MPs) or healthy donors (HDs). CD3(+)sEV of MPs induced distinct functional responses in T cells or melanoma target cells than CD3(+)sEV of HDs. Approximating functions mediated by melanoma cell-derived sEV (MTEX), CD3(+)sEV of MPs promoted pro-tumor activities of T cells and failed to induce apoptosis or depolarize mitochondria in Mel526 cell targets. Proteomics profiling confirmed functional differences between CD3(+)sEV of MPs and HDs. Of 294 sEV-specific proteins identified in CD3(+)sEV, 226 were detected in the parent T cell proteome, confirming that the CD3(+)sEV proteome mimics that of the parent T lymphocytes. Among them were 66 differentially expressed proteins (DEPs) that differentiated vesicles from MPs and HDs. These DEPs were associated with the processes linked to cancer-related functions. DEPs upregulated in CD3(+)sEV of MPs were associated with RHO-GTPase, cytokine, and MAPK signaling pathways. The abundance of ITGB3 and YWHAB in MPs, two sEV proteins linked with BRAF-related pathways, correlated with the BRAF mutation status. Thus, T cells of MPs were reprogrammed by melanoma to produce CD3(+)sEV that functionally resembled MTEX, partly recapitulated features of the tumor proteome, and differed from CD3(+)sEV of HDs. In cancer, the TEX-rewired T cells produce CD3(+)sEV that potentially serve as a liquid biopsy of patients' T cells.

CHALLENGES IN GLYCOSYLATION ANALYSIS OF EXTRACELLLULAR VESICLES

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Extracellular vesicles (EVs) are widely studied due to their significant roles in cell signaling and intercellular communication. Protein glycosylation plays a crucial role in cell signaling and cancer-related processes such as metastasis, angiogenesis, and drug resistance. Therefore investigating glycosylation of EV proteins is highly relevant in cancer research, but poses several challenges, as minimal amounts of EV samples are available.

In our first study, our aim was to develop sensitive methods for the *N*-glycoproteomic and chondroitin sulfate glycosaminoglycan profililing of small EVs isolated from A549 lung adenocarcinoma and BEAS-2B non-tumorigenic cell lines. Small EVs were isolated by mini-size exclusion chromatography on in-house prepared columns. Different sample preparation methods were compared for proteolytic digestion and subsequent *N*-glycopeptide enrichment. Peptides, *N*-glycopeptides and chondroitin sulfate disaccharides were analyzed by nanoUHPLC-MS(MS). Over 100 *N*-glycopeptides per sample were identified, including glycopeptides of vesicular marker proteins. Chondroitin sulfate analysis successfully identified the most abundant disaccharides and significant changes were observed between 4S and 6S sulfation in the two investigated EV types. These results highlight the importance of exploring the glycosylation of EV proteins in understanding disease progression.

Currently, we are focusing on the glyco(proteo)mic comparison of EVs and their respective cell membranes and cells from cell lines representing different lung cancer subtypes, as well as on method developments for analyzing the glycome of blood derived EVs.

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IMPACT OF A COMPLEX ADJUVANT PARKINSON'S DISEASE THERAPY ON THE PROFILE OF PLASMA PROTEINS

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Parkinson's disease (PD) is a progressive neurodegenerative disease affecting millions of people around the world. Major progress concerning the therapy of PD has been achieved by introducing levodopa substitution therapy, COMT and MAO-B inhibitors therapy, and deep brain stimulation, all contributing to a significant prolongation of patients' lives. Regardless, the quality of life of people suffering from PD is still of concern. Recently we have developed a complex adjuvant PD therapeutic program in the medical spa in Piestany. Parkinson Spa Recovery (PSR) program consists of balneological, physiotherapeutic, sensoric, and nutraceutical modules, jointly improving quality of life and the performance of the patient. Despite having clinical evidence for the effectiveness of the adjuvant PD program, in our recent work we decided to elucidate proteomic changes in the plasma of our PD patients admitted for the three-weeks-long PSR program. Blood plasma of the patients has been collected before starting and after completion of our three-week program. Plasma proteome profiles have been established by using illumina SOMAmer technology. Our presentation will focus on revealing the identities of the proteins changed by the accomplishing PSR program and the interpretation of their functional clustering in relation to the treatment and the ailment.

DEEP INSIGHTS INTO COMPLEX PROTEOMICS SAMPLES WITH ADVANCED TIMSTOF

G. Mitulović

1) Bruker Austria; 2) Bruker Switzerland

Analyzing complex proteomics samples has become significantly easier and more straightforward thanks to recent developments in chromatography and mass spectrometry hardware, software, and sample preparation methods.

New mass spectrometers, such as the timsTOF Ultra 2 with AIP ion processing, enable the analysis of single cells or even smaller amounts of samples, allowing for the scouting of, for example, cancer tissue in several dimensions.

In addition to the mass spectrometers, improved and optimized separation methods and sample preparation methods significantly reduced sample loss and enhanced the separation of peptides. Here, innovative stationary phases are being used for sample trapping on precolumns, thereby reducing sample loss, and new separation phases are being employed for manufacturing separation columns to achieve improved separation.

DISCOVERY AND DEVELOPMENT OF BIOMARKERS FOR PSORIATIC DISEASE

Stephen R Pennington on behalf of the HIPPOCRATES Consortium

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Psoriatic arthritis (PsA), a chronic immune-mediated inflammatory disease affects peripheal joints and other components of the musculoskeletal system. Around 30% of individuals with skin disease psoriasis go on to develop PsA. It is estimated that 1-2% of the general population have PsA and so in the EU between 5 to 10 million people have the disease. It is increasingly recognised that PsA is associated with co-morbidities, particularly those which promote the development of accelerated atherosclerosis and contribute to cardiovascular morbidity and mortality[2]. PsA is characteristically very heterogeneous in its clinical presentation and the extent to which individuals progress to irreversible joint damage.

Despite many years of significant progress in our understanding of the molecular pathways underpinning PsA and of the management of individuals with the disease, it remains evident that significant unmet needs still exist. Notably, there are still no diagnostic criteria or laboratory tests for PsA. This results in delayed diagnosis and delayed diagnosis is known to result in poorer outcome . So, working with the Group for Research and Assessment in Psoriasis and Psoriatic Arthritis (GRAPPA; https://www.grappanetwork.org/) and individuals with the diseases we identified 4 key unmet needs in PsA.

HIPPOCRATES an EU consortium of 27 partners funded by IMI/IHI aims to address these needs. In this presentation we will articulate the key unmet needs, outline the HIPPOCRATES consortium and introduce our on-going efforts to identify and develop clinical and molecular (‘omic) biomarkers to address them. These efforts include establishing a consortium wide data sharing agreement that underpins a secure database of clinical and molecular data, an EU-wide prospective study to monitor progression from psoriasis to PsA and ‘omics studies (epigenomic, proteomic, metabolomic and lipidomic) to identify and evaluate blood-based biomarkers.

COMPREHENSIVE PROTEOMICS INVESTIGATION OF ALTERED MOLECULAR PATHWAYS IN RECURRENT PREGNANCY LOSS

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Recurrent pregnancy loss (RPL) defined as a loss of 2 or more pregnancies affects 0.5%–1% of women at their reproductive age and poses a frustrating challenge for both patients and clinicians. As more than half of the RPL cases do not have a clearly identified cause, uncovering the mechanisms behind the idiopathic RPL is urgently needed.

Using a highly efficient in-solution digestion method and label-free data-independent LC-MS/MS acquisition with ion mobility, we performed 2 comparative proteomics analyses using: chorionic villi isolated from the products of conception of 13 RPL cases and 10 age and gestational week-matched elective pregnancies and of the decidua tissues from 19 RPL patients and 10 controls. Differentially abundant proteins (DAPs) were compared and correlated with publicly available transcriptomic datasets and the expression of selected biomarkers was tested by qPCR in chorionic villi and decidua from an extended cohort of patients and controls.

The comparative proteomics analyses reveiled 128 and 85 DAPs ((Benjamini–Hochberg $p \leq 0.05$) and fold change ≥ 1.5) in chorionic villi and decidua, respectively. Bioinformatics analysis of DAPs from chorionic villi identified statistically significant enrichment of several pathways among which "Complement and coagulation cascades", "Platelet activation", "TCA cycle", and "Ferroptosis" were with the highest significance. Gene expression analysis of the selected candidates from these pathways by qPCR in general showed that the transcription levels were significantly increased in the RLP group compared to controls and consistent with the proteomics findings. Pathway analysis of DAPs from decidua identified enrichment of "Signaling by ROBO receptors", "RNA degradation" and "Cytoplasmic ribosomal proteins". The correlation between protein and gene expression in decidua revealed downregulation of ribosomal proteins at protein and mRNA level, which was later validated by qPCR. Both studies shared DAPs involved in ribosome pathway and SLIT-ROBO signaling.

In conclusion, our data suggests that the other potential causes of RPL from the fetal side beside altered blood coagulation could be linked to TCA cycle and ferroptosis, for which at present, very limited association exists. On the other hand, RPL causes from the maternal side could be associated with some emerging pathways in reproduction such as signaling by ROBO receptors and impaired RNA processing and protein synthesis machinery. The obtained list of DAPs in RPL represents a source for future investigations in terms of screening genetic variants predisposing to pregnancy failure and for developing monitoring biomarkers for high-risk pregnancies.

THE ROLE OF MASS SPECTROMETRY IN ADVANCING HYPEREMESIS GRAVIDARUM RESEARCH

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Hyperemesis Gravidarum (HG) is a rare but severe pregnancy complication that, although affecting a minority of pregnancies, has a profound impact on maternal health due to severe nausea, vomiting, dehydration, and weight loss [1, 2]. Despite its significant physical and psychosocial burden, HG remains poorly understood at the molecular level, and reliable diagnostic targets are still lacking. Recent genetic studies implicate growth/differentiation factor-15 (GDF-15) as a key protein in HG pathophysiology, but the functional protein-level alterations and their diagnostic relevance remain to be further explained [2, 3].

Liquid chromatography coupled with mass spectrometry (LC-MS) offers distinct advantages for investigating GDF-15 in HG, as well as other relevant proteins, due to its high sensitivity, specificity, and ability to quantify multiple targets simultaneously in complex biological samples. The aim of this study was to utilize these strengths and develop targeted LC-MS method to quantify symptom-related proteins, GDF-15 and insulin-like growth factor-binding protein 7 (IGFBP7), in serum samples from clinically well-characterized HG cohorts. Our MS method facilitates the detection of genotype-related protein variants such as the histidine to aspartate substitution at position 202 in GDF-15. Furthermore, this approach enables simultaneous quantification of GDF-15 and IGFBP7 in clinical samples, with the capacity to incorporate additional protein targets as they are identified in relation to HG. MS-based untargeted proteomics provides a valuable platform for supporting such efforts.

This study is embedded in a multidisciplinary collaboration involving obstetricians, midwives, geneticist, bioanalytical chemists and researchers exploring the lived experiences of women with HG. By combining targeted and untargeted high-resolution MS with clinical insight, this approach has the potential not only to uncover molecular signatures specific to HG, but to advance our understanding of pregnancy-related vomiting and nausea in general.

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QUANTITATIVE ANALYSIS OF THE CELL SURFACE PROTEOME

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Cell surface proteins, the surfaceome, drive key cellular functions, including signaling, adhesion, transport, and represent a source of biomarkers and therapeutic targets. Unlike abundant cytosolic proteins, plasma-membrane associated proteins are typically low in abundance, highly hydrophobic, and heavily glycosylated, which complicates their detection by conventional shotgun proteomics. To address these challenges, a variety of enrichment strategies have been developed, including cell-surface biotinylation with (strept/neutr)avidin capture. This method, however, enriches all extracellular proteins, including proteins originating from cell growth medium and intracellular proteins from damaged cell. In addition to those contaminants, proteins labelled by reagent penetrating cell membrane, or unlabeled proteins unspecifically bound by enrichment resin can also be detected in the samples. Minimizing the abundance of such proteins, using appropriate sample processing protocols, and/or filtering the proteomics data for those false positive identifications is essential to obtain valuable information on the surfaceome. The chemical labeling and enrichment steps can introduce additional level of quantitative variance in the measured peptide intensities and may produce different chemically modified forms of the quantified peptides. These can have negative effects on the quantification reproducibility and reliability.

In this work we evaluate different enrichment methods, mass spectrometry data collection and data analysis approaches, quality control measures to obtain reliable quantitative data for comparison of different cell states or apical/basolateral cell sides.

DIRECT IDENTIFICATION OF PROTEINS FROM PEPTIDE MASS SPECTRA FOR BIOMARKER DISCOVERY: THE CASE OF ALZHEIMER'S DISEASE

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Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) is the main technology for protein identification and quantification in complex biological samples. Previously, we developed DirectMS1, a novel approach for ultrafast proteome analysis. Unlike conventional MS/MS-based methods reliant on peptide fragmentation, DirectMS1 identifies proteins directly from MS1 spectra, enabling a significant reduction in proteome analysis time (approximately an order of magnitude). Beyond its utility in ultrafast proteome profiling, the DirectMS1 search engine can re-process data-dependent and data-independent acquisition (DDA and DIA, respectively) datasets. By extracting peptide MS1 spectra directly from data and identifying proteins from all detected peptide-like features, this approach enhances protein sequence coverage, leading to improved quantitative accuracy and a more comprehensive pattern of differentially expressed proteins associated with disease states or therapeutic interventions.

To demonstrate these capabilities, we re-analyzed a DIA dataset from brain samples related to multiple system atrophy (MSA), acquired using short LC gradients and a state-of-the-art Orbitrap Astral mass spectrometer. The re-analysis, by DirectMS1, of DIA data uncovered previously missed proteins. To validate our results, we compared protein identifications with those reported in a recent study of the same sample cohorts using a standard 3-hour LC-MS/MS DDA workflow. The DirectMS1 search engine identified proteins not only detected in DDA, but not detected by initial DIA analyses, and further reported five proteins previously implicated in MSA through non-proteomic studies.

Furthermore, we report novel biological insights into Asymptomatic Alzheimer's disease obtained through analysis with DirectMS1. We applied our search engine to the largest collection of post-mortem human brain proteomic data currently available. To enhance generalizability and robustness of the results across multiple datasets, we developed an AI-based workflow to identify key proteins associated with Asymptomatic Alzheimer's disease. Our analysis revealed previously undetected proteins, implicating ferroptosis as a critical mechanism in the pathogenesis of Alzheimer's disease.

In this presentation, we will report on the fundamentals of the DirectMS1 method, demonstrating its capabilities for ultrafast proteome analysis and identification of differentially expressed proteins, as well as present results from proteomic data processing related to Alzheimer's disease.

This work was performed with financial support from the Russian Science Foundation, grant no. 23-45-00012.

SYNTHESIS OF PEPTIDE AGAINST SARS-CoV-2 VIRUS THAT CAN PLAUSIBLY CROSS BBB

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Since the global spread of COVID-19 caused by SARS-CoV-2 virus there are still limited therapeutic options, especially when the virus crosses the blood-brain barrier (BBB) and invades the CNS. The CNS is protected by the BBB, which restricts entry to immune cells, antibodies, and many therapeutic drugs, complicating treatment. Therefore, biodistribution of the therapeutics across the BBB is crucial for efficient treatment of neuroinfection
caused by SARS-CoV-2. In the present work, we're using peptides derived from the CDR3 region of the heavy chain of the llama antibody that can bind the SARS-CoV-2 and neutralize it; however, it is not able to penetrate through the BBB. Thus, using solid-phase peptide synthesis, we synthesized a fusion peptide comprised of CDR3, GGGS linker and TGN, the CNS homing moiety. The solubility of peptide was increased by adding RKRK sequence at the C-terminal. CDR3-GGGS-TGN was purified by reverse-phase chromatography and the quality was assessed by MALDI-TOF mass spectrometry. A peak at 4156 Da confirmed the synthesis of the fusion construct. CDR3-GGGS-TGN was tested for cell toxicity on the Vero E6 cells, which showed no signs of the toxicity even at 10 ug/ml concentration. Peptide was also tested for virus neutralization, wherein it neutralized the virus completely at 187.5 ng/well containing 200 µl of medium with 75 pfu. Finally, the peptide was incubated in the luminal chamber of the in-vitro BBB model for 5 hrs and content from abluminal chamber was collected and tested for virus neutralization. It appeared that the peptide was able to cross the BBB model, as 100% virus neutralization was observed for the content of the abluminal chamber. CDR3-GGGS-TGN holds merit for further development for its translation into the drug for in vivo trials. Research supported by APVV-22-0084 and VEGA 1/0381/23.

PROTEOMIC ASPECTS OF THE CROSSING OF THE PATHOGENS AND DRUGS ACROSS THE BLOOD BRAIN BARRIER

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Organisms that have crossed the species barrier from animals to humans include several pathogens like viruses (such as Hendra, Nipah, SARS, and influenza), as well as bacteria (*Borrelia*, *Streptococcus*, etc.) and others. It is still unclear why new pathogens like SARS-CoV-2, which appears to cause respiratory symptoms, manage to cross the blood-brain barrier (BBB) and enter the brain. And what exact mechanisms do they use to get over the BBB, after all? Since infectious diseases of the central nervous system (CNS) are very hard to treat, they generally continue to be a significant cause of morbidity and mortality. A major obstacle for curing brain diseases is the blood-brain barrier (BBB), which impedes therapeutic agents to reach the brain and target the pathogens. The second part of the talk will provide an overview of our efforts in developing a proof-of-concept drug delivery nanosystem coated with CDR3-peptides or pathogen-specific nanobodies (Nbs) to target brain infections. The nanosystem is equipped with BBB-homing moiety, which can enhance its translocation across BBB via the receptor-mediated transcytosis. The nanosystem is loaded with antiviral or antibacterial agents to allow specific release of drug-payload in CNS. Finally, some exciting results will be presented to demonstrate how nanosystems are non-toxic to cells, how they effectively kill antibiotic-resistant bacteria, how they kill pathogens in less time than traditional antibiotics, how they achieve better biodistribution in the CNS, and how they save animals with CNS infections more effectively than traditional drugs. We anticipate attracting an audience in the fascinating field of developing nanosystems for drug delivery to the CNS: the nanosystem which is designed around fundamental principles of host-pathogen crosstalk. Research supported by EURONANOMED2021-105 (Antineuropatho), ICGEB CRP/SVK24-02, APVV-22-0084, APVV-18-0259 VEGA 1/0348/22 and 1/0381/23

IDENTIFICATION OF PROTEIN NETWORKS AND BIOLOGICAL PATHWAYS DRIVING THE PROGRESSION OF ATHEROSCLEROSIS IN HUMAN CAROTID ARTERIES

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We present proteomics analyses of human carotid artery samples collected from patients with atheromatous and vulnerable atherosclerotic plaques, respectively. Proteins extracted from the samples were analyzed by bottom-up shotgun approach that relied on nanoflow liquid chromatography–tandem mass spectrometry analyses using both data-dependent and data-independent acquisitions. Differentially expressed proteins were examined using Ingenuity Pathway Analysis® with focus on pathological and molecular processes driving atherosclerosis. From the more than 150 significantly regulated canonical pathways, atherosclerosis signaling and neutrophil extracellular trap signaling were verified by protein-targeted data extraction. Results from our study are expected to facilitate a better understanding of the disease progression's molecular drivers and provide inspiration for further multiomics and hypothesis-driven studies.

This work was supported by the Hungarian Government grants OTKA-K-132828 (J.B.) and NKFIH FK 134605 (É.C.), the Hungarian Government grant NKFIH 149734 (J.B.), Thematic Excellence Programme of the Hungarian Ministry for Innovation and Technology (TKP2020-NKA-04 and TKP2021-EGA-18 to J.B., and TKP2021-EGA-20 to J.T.), the UD Space Sciences Thematic Program, the EU and the European Social Funds (GINOP-2.3.2-15-2016-00043 and GINOP-2.3.2-15-2016-00044), the 2022 Distinguished Guest Scientist Programme of the Hungarian Academy of Sciences (É.C. and L.P.) , and The Welch Foundation (BK-0031, L.P.).

PROTEOMICS-DRIVEN DISCOVERY OF NOVEL DISEASE REGULATORS: UNCOVERING HIDDEN PLAYERS IN ADIPOGENESIS, THROMBOSIS, AND ATHEROSCLEROSIS

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The discovery of novel molecular regulators is essential to advancing our understanding of complex diseases such as obesity, thrombosis, and atherosclerosis. Proteomics has emerged as a powerful technology for unbiased identification of proteins involved in pathological processes, enabling the elucidation of disease mechanisms beyond traditional candidate-based approaches. Recent research utilizing label-free quantitative proteomics has led to the identification of previously uncharacterized proteins with key regulatory roles in adipogenesis, vascular inflammation, and thrombosis [1-3]. In adipose biology, the silencing of one such protein, prenyclcysteine oxidase 1, was found to disrupt the differentiation of preadipocytes by downregulating essential adipogenic transcription factors, ultimately impairing fat accumulation in vivo. In the vascular system, deficiency of this protein conferred protection against arterial thrombosis, with reduced platelet activation and aggregation responses, despite normal hematologic parameters. Furthermore, prenyclcysteine oxidase 1 was shown to be enriched in atherosclerotic lesions and associated with pro-inflammatory and pro-oxidative pathways, contributing to lesion vulnerability and lipid peroxidation. These effects were validated through proteomic and functional analyses in both cellular models and gene-deficient mice. The integration of proteomics with genetic manipulation thus offers a robust platform for identifying unexpected disease mediators and potential therapeutic targets. This case highlights the transformative role of proteomics in uncovering multifunctional proteins implicated in metabolic and cardiovascular diseases, and demonstrates how such approaches can reshape our understanding of disease pathophysiology. Ultimately, proteomics-driven discovery paves the way for novel strategies in diagnosis, risk assessment, and therapeutic intervention across a wide spectrum of chronic conditions.

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SUPPRESSION OF PEPTIDE LOSS IN AUTOSAMPLER VIALS

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The bottom-up proteomics is a widely used approach for protein characterization. Various sample preparation protocols have now been developed, but regardless of their differences, all of these protocols consist of key steps, including protein extraction, purification and digestion, during which proteins undergo either chemical or proteolytic cleavage resulting in peptides. These peptides are then stored in acidic pH in vials prior to final analysis by LC-MS/MS. In all these steps, sample loss is caused by protein or peptide adsorption on contact surfaces. Therefore, there is a continuous effort to minimize this adverse effect.

In our work, we focused on the final stage of sample preparation protocol, when the peptides are transferred into autosampler vials and waiting for the measurement in the autosampler. For this purpose, we used a commercially available HeLa digest. Samples, 10 nanograms of peptides per injection, were measured by LC-MS/MS using an Orbitrap Exploris 480 in data-independent acquisition (DIA) mode. We tested injection of different sample volumes, the type of acid used for sample acidification, and the addition of two types of additives. Based on evaluation of the number of identified proteins and peptides, their signal intensity as well as other parameters (such as MW or Gravy) the optimal combination was determined.

Our results show, that proposed combination, mainly selection of the additive minimize the adsorptive losses in autosampler vials regardless the injection volume. This approach can increase reproducibility in situations where different injection volumes need to be used.

Acknowledgment

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'MULTI-OMICS' HIV IMMUNE MONITORING OF PATIENTS UNDERGOING ANTIRETROVIRAL THERAPY (ART) AND AT INCREASED RISK OF AIDS-DEFINING CANCERS.

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The ability to simultaneously measure multiple secreted proteins and the corresponding gene expression levels of HIV patients receiving continuous antiretroviral therapy (cART) is prerequisite to ensuring patient benefits and long-term effectiveness of ART treatment. Interrogation of such patient samples for molecular signature of the disease together with clinical parameters such as mitochondrial mass, incomplete immune reconstitution, and CD4+ T-cell counts in people living with HIV (PLWH), may help better understand disease progression and efficacy of treatment.

Here, we demonstrate messenger RNA (mRNA) expression using QuantiGene™ assay on a multiplexing platform. Human peripheral blood mononuclear cells (hPBMCs) stimulated with phytohemagglutinin (PHA) were harvested at 24 hours and assayed for specific mRNA targets on a QunatiGene Human 8plex panel. Quantification of CCL2, CLDN5, CSF2, CXCL8, IL17F, IL25, IL6 and OCLN genes were performed on a multiplexing system.

Additionally, whole blood samples from PLWH on continuous ART (A, n=15), with CD4AC >500 cells/µl and CD4/CD8 ratio > 0.9, or untreated (B, n=10) were collected during routine immune monitoring. Mitochondrial mass (MM), mitochondrial membrane potential (MMP) and mitochondrial superoxide levels (MSL) in CD4 and CD8 T cells were determined using flow cytometry

Conclusion
Increased MSL in T lymphocytes of cART- PLWH suggested elevated oxidative stress and possible mitochondrial damage. Additionally, higher MM found in cART- suggested mitochondrial health disruption as well as on-going biogenesis. Complementing whole blood assays with hPBMCs based Quantigene mRNA assay, provided a more holistic view of the antiretroviral therapy for HIV by helping to screen for molecular signature of the disease at gene level. Such a combined approach can elevate patient blood sample screening to a 'multi-omics' level, providing a high-level interrogation of clinical parameters concurrently with gene expression networks of the disease.

Wednesday posters Oct 15, 2025

WePo01	**Susraba Chatterjee**	Clinical Proteomics Profiling of Circulating Immune Complexes for Biomarker Identification in Indian Patients with Tuberculous Pleural Effusion and HIV Co-Infection
WePo02	**Sonia Eligini**	Oxidized Albumin Proteoforms as Biomarkers and Therapeutic Targets
WePo03	**Stepanka Kuckova**	Harmonizing Proteomics Workflows for Cardiovascular Biomarker Discovery: A Cross-Laboratory Study of Dried Blood Spots and Plasma
WePo04	**Liudmila Smirnova**	Proteomic Markers in Affective Disorders: Proteins of Inflammatory and Immune Response
WePo05	**Patrik Kovács**	Analysing The Surfaceome During Chondrogenesis: Searching for Novel Biomarkers
WePo06	**Katalin Kuffa**	Side-Specific Cell Surface Labeling of Transmembrane Proteins in Polarized Cells
WePo07	**Minh Ngoc Nguyen**	The Surfaceome of Human Epidermal Melanocytes and Melanoma Cells
WePo08	**Boglárka Cziráki**	Proteomic Analysis of Lung Adenocarcinoma Subtypes with Different Genetic Alterations
WePo09	**Krisztián Márk Karvaly**	Comparison of Prostate Adenocarcinoma and Benign Prostatic Hyperplasia by Proteomic and Phosphoproteomic Analysis
WePo10	**Petr Lapcik**	LC-MS/MS-based Terminomics Unravels Carboxypeptidase B1 Role in Luminal A Breast Tumor Progression
WePo11	**Hui Lu**	Profiling of CDCP1 Glycosylation in Ovarian Cancer and Its Potential Role in Cancer
WePo12	**Mahshid Moballegh Nasery**	Quantification of Glycosylated Complement Component 9 Protein Epitopes In Patients With Lung Cancer
WePo13	**Muhammad Rashad**	Proteomics Profiling of Snail Slime Treated Human Dermal Fibroblast Using High-End Mass Spectrometry

WePo14	**Ombretta Repetto**	Prognostic Role of Plasma Proteomics Signature in Oligorecurrent Prostate Cancer Patients Undergoing SBRT
WePo15	**Nokhoijav Erdenetsetseg**	Unsupervised Cluster Analysis Reveals Subgroups in Obesity and Type 2 Diabetes with Proteomics Profiles
WePo16	**Sivabalan Vairamuthu**	Development of A Targeted Drug Conjugate Against Borrelia
WePo17	**Jakub Víglaský**	Interaction between Sars-Cov-2 S and N Proteins Mediate Recruitment of Soluble Complement Regulatory Proteins on The Virion
WePo18	**Klára Vlčková**	Protein Extraction from Ancient Material
WePo19	**Gabriella Gellén**	Fast and robust phosphoproteomics sample prep with AttractSPE® Disks Tips C18 for high phosphopeptides recovery and identification
WePo20	**Bence Samu Szőcs**	Boosting The Performance: Platform-Specific Collision Energy Optimization in Bottom-Up Proteomics

Thursday posters Oct 16, 2025

ThPo01	**Rachma Dessidianti**	Miniaturized Size Exclusion Columns as an Enrichment Strategy to Improve HPLC-MS-Based Proteoglycan Analysis
ThPo02	**Akshay Dhingra**	Streamlined and Reproducible Proteomic Sample Preparation from FFPE Tissues Using AFA® Technology
ThPo03	**Virág Nikolett Horváth**	Analytical Method Development for The Proteomic and Glycomic Characterization of Extracellular Vesicles
ThPo04	**Viktória Kiss**	Effect of Culture Conditions on The Proteomics Profile of A549 Cells
ThPo05	**Mabuse Moagi**	Formalin Fixed Paraffin Embedded (FFPE) Proteomics from A Method Development Point of View – Which Is The Best Method for Protein Identification from FFPE Tissue Samples?
ThPo06	**Alexandra Molnár**	Magnetic Particles in Proteomics: Comparison of Magnetic Bead-Based Sample Preparation Methods
ThPo07	**Pierre-Olivier Schmit**	Enhanced Immunopeptide Identification Using MIDIA-PASEF: A Novel timsTOF Scan Mode
ThPo08	**Ruben Szabo**	Taylor-Aris Dispersion Assisted Mass Spectrometry (TADA-MS): Direct Injection Analysis of Proteins with High Matrix Content
ThPo09	**Istvan Szepesi-Nagy**	Fragflow: Automated Worfklow for Large-Scale Quantitative Prteomics in High Performance Computing Environmnets
ThPo10	**Renata Biba**	Directed Fragmentation of Peptides Maximizes b- and y- Ion Yield and Sequence Coverage in Data Independent Analysis Mass Spectrometry
ThPo11	**Daniela Evdokimova**	Changes in Protein and Lipid Composition of Human Lysosomes During Starvation Induced Autophagy
ThPo12	**Mansi Jain**	Phosphoproteomic Analysis of Thermophilic Prokaryotes: Expanding The Phosphorylation Landscape in Extreme Environments
ThPo13	**István Kató**	Replication Independent Function of TLS Polymerases

ThPo14	**Vivien Miczán**	The Proteomic Map of Mitosis
ThPo15	**Michaela Rašková**	Comparative Mass Spectrometric Analysis of Proteolytic Activity in Wine and Wine Vinegars
ThPo16	**Domonkos Pál**	HPLC-MS Analysis of Chondroitin/Dermatan Sulfate and Heparan Sulfate Glycosaminoglycan Disaccharides from Lung Tumor Tissues
ThPo18	**Marcell Cserhalmi**	Neuronal DNA Repair in Huntington's Disease

CLINICAL PROTEOMICS PROFILING OF CIRCULATING IMMUNE COMPLEXES FOR BIOMAKER IDENTIFICATION IN INDIAN PATIENTS WITH TUBERCULOUS PLEURAL EFFUSION AND HIV CO-INFECTION

Susraba Chatterjee[1)], Sudeshna Mallik [2)], Akrite Mishra[1)], Prantiki Halder[2)], Boudhayan Bhattacharya[3)], Bibhuti Saha[3)], Sumi Mukhopadhyay[1)]*

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Introduction

The prevalence of tuberculous pleural effusion (TPE), the second most common manifestation of extrapulmonary TB (EPTB), has doubled since the onset of the HIV pandemic [1]. HIV-TPE co-infection significantly alter blood and clinical parameters associated with increased mortality [2]. Plasma circulating immune complexes (CICs) are important contributors to the immunopathology of TB. Therefore, the aim of this study was to investigate the proteome profile of CICs to unravel biomarkers associated with pathomechanisms of the disease.

Method

Blood samples from patients with HIV-TPE, TPE with healthy controls were collected. CICs were isolated by ammonium sulphate cut from pooled plasma. Highly abundant plasma proteins were removed using depletion column resins. CICs were treated with acid dissociation to dissociate immune complexes following Protein A Sepharose 4B column purification. Antibodies free CICs were now subjected to m-phenyl boronic acid column to obtain glycated antigenic fractions. In-sol digestion of antigenic fractions was performed and analyzed through nano LC-1200 coupled with Q-Exactive plus Orbitrap MS [3] (PRIDE accession-PXD066085).

Results and Discussions

Comparative proteome profiling between TPE-HIV and TPE showed high upregulation of collagen-binding extracellular matrix proteins. These increased expressions may contribute to bilateral pleural fluid accumulation, a prominent clinical feature of the TPE-HIV cohort. Additionally, the upregulation of HIF-1 signaling–associated proteins suggests involvement of the Warburg effect contributing to disease progression in the coinfected group. Upregulation of platelet degranulation–associated proteins reflects the systemic inflammatory response characteristic of the disease.

Innovative Aspects

- Proteomic profiling of CICs to unravel disease specific antigens.
- Foremost attempt to investigate blood-based biomarkers in HIV-TPE coinfection.

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OXIDIZED ALBUMIN PROTEOFORMS AS BIOMARKERS AND THERAPEUTIC TARGETS

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Oxidative stress contributes to the pathogenesis and progression of various chronic diseases, including cardiovascular disorders, heart failure, and aortic valve stenosis. Human serum albumin (HSA), the most abundant circulating protein, plays a central role in extracellular antioxidant defense, primarily via the redox-active cysteine-34 (Cys34) residue. The redox state of albumin gives rise to distinct proteoforms, among which mercaptoalbumin (HSA-SH) retains antioxidant and anti-inflammatory functions. Recent advances in proteomics have enabled the characterization of these albumin isoforms in pathological contexts, revealing a shift toward oxidized forms (e.g., HSA-Cys) in patients with redox imbalance [1-2]. This oxidative modification correlates with disease severity and impaired physiological function. Utilizing mass spectrometry-based proteomic analyses, recent studies have demonstrated that thiol-containing compounds such as N-acetylcysteine (NAC), its amide derivative (NACA/AD4), and thioredoxin mimetic peptides (TXM) can selectively regenerate mercaptoalbumin by breaking thiol-disulfide bonds. The restoration of HSA-SH is associated with enhanced antioxidant capacity and partial recovery of antiplatelet activity, suggesting therapeutic implications. These compounds exhibit superior bioavailability and efficacy compared to NAC alone, offering new avenues for intervention in oxidative stress-driven diseases. This research underscores the utility of proteomics not only in identifying oxidative modifications of key plasma proteins but also in evaluating redox-targeted therapeutics. By focusing on albumin as both a biomarker and a functional antioxidant modulator, proteomic approaches provide critical insights into systemic oxidative stress and its mitigation. This paradigm exemplifies how the integration of redox biology and proteomics may lead to precision strategies for disease diagnosis, monitoring, and treatment.

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HARMONIZING PROTEOMICS WORKFLOWS FOR CARDIOVASCULAR BIOMARKER DISCOVERY: A CROSS-LABORATORY STUDY OF DRIED BLOOD SPOTS AND PLASMA

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Inter-laboratory variability remains a critical barrier to the standardization and large-scale applicability of mass spectrometry (MS)-based proteomics in clinical research. Within the framework of the AtheroNET COST Action, we conducted a systematic comparison of proteomic outputs generated by two independent laboratories (in the Czech Republic and Portugal) analysing aliquots of identical clinical blood plasma samples, with the primary objective of evaluating reproducibility in protein identification and quantification across distinct experimental platforms and protocols.

Despite inherent methodological differences – including instrumentation, digestion strategies, and data acquisition modes – both laboratories consistently identified a substantial overlapping subset of the plasma proteome. This shared proteomic core encompassed numerous clinically relevant cardiovascular biomarkers, such as apolipoprotein A1 (APOA1), transthyretin, and complement component C3. Quantitative inter-site correlation of protein intensity values demonstrated a high degree of concordance (Pearson $r > 0.85$), indicative of robust reproducibility under real-world, heterogeneous laboratory conditions.

Nevertheless, subtle inter-laboratory discrepancies were evident in the total number of proteins identified and in the representation of specific functional categories, particularly those associated with immune response and cytoskeletal organization. These differences likely reflect variations in sample processing workflows and acquisition depth, which in turn impact downstream biological interpretations.

This comparative analysis underscores the feasibility of obtaining reproducible proteomic profiles across laboratories, even when distinct methodologies are employed. At the same time, our findings highlight the need for harmonized protocols to minimize variability and enhance data comparability in multicentre proteomics-driven biomarker studies.

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PROTEOMIC MARKERS IN AFFECTIVE DISORDERS: PROTEINS OF INFLAMMATORY AND IMMUNE RESPONSE

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Currently, there is an increase in various affective disorders, one of the most severe of which is bipolar disorder (BD), the diagnosis of which is a difficult task. The presented analysis revealed BD-specific proteins responsible for the inflammatory and immune response, which have recently received much attention in the pathogenesis of affective disorders.

The study included 22 patients with BD (mean age 40.33±14.1 years, disease duration 10.4±6.2 years). The control group consisted of 17 healthy individuals matched for age and gender with the subjects. For mass spectrometry, blood serum was purified from 14 major proteins using affinity chromatography and separated by 1D electrophoresis according to Laemmli. After trypsinolysis and peptide extraction from the gel, proteins were identified by HPLC/MS on a TimsTof Bruker instrument based on the Advanced Mass Spectrometry Core Facility of Skolkovo Institute of Science and Technology. Protein identification was performed using the Mascot Ver. 2.8.1 search engine and the UniProtKB/Swiss-Prot database. The relative content of proteins was determined using the Advanced Label-free assay based on the emPAI index. Statistical analysis was performed using a two-tailed unpaired Student's t-test (FDR 0.05 and S0 = 2). In the BD group, the lowest p-value ($p = 0.0003$) was for the protein Transforming growth factor-beta-induced protein ig-h3, which plays a role in cell adhesion and is a structural component of the extracellular matrix. The next 5 proteins in order of significance of differences were: Disabled homolog 2-interacting protein, coiled-coil domain-content protein 80, B-cell CLL/lymphoma 9 protein, coatomer subunit gamma-1, ras GTPase-activating-like protein IQGAP1 ($p = 0.001$), which were also components of the extracellular matrix and regulators of a wide range of signaling pathways, primarily regulating the immune response, cellular differentiation and apoptosis. Proteins: ectonucleoside triphosphate diphosphohydrolaseб adhesion G protein-coupled receptor B1 and 14-3-3 protein zeta/delta ($p=0.002–0.005$) provide phosphorylation and dephosphorylation, hydrolyze ATP, regulate apoptosis and ubiquitination, and regulate the formation of dendritic spines in neurons. In healthy people, 168 proteins have been identified, most of which are responsible for maintaining homeostasis of the immune system and have antioxidant and anti-inflammatory properties. These are followed by proteins regulating the processes of transcription, translation, and protein synthesis. Several proteins are responsible for the activation of neurogenesis during damage repair.

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ANALYSING THE SURFACEOME DURING CHONDROGENESIS: SEARCHING FOR NOVEL BIOMARKERS

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Purpose: Chondroprogenitor cells differentiate into chondroblasts and then chondrocytes during the complex process of chondrogenesis. Given that the extracellular matrix (ECM) undergoes profound changes between early and late stages, it is plausible to hypothesise that chondroprogenitors differentiating into chondrocytes are characterised by a dynamically changing surfaceome – i.e., the assembly of plasma membrane ion channels, transporters and other cell surface molecules that regulate their function and phenotype, and maintain communication with the ECM. Therefore, the purpose of this study was to determine the qualitative and quantitative composition of the surfaceome at the earliest stages of chondrocyte differentiation, and to identify potential biomarkers characteristic to each major step of the process.

Methods: Chondrifying primary micromass cultures were established from distal parts of limb buds of chicken embryos (Hamburger–Hamilton stages 22–24). On specific days of chondrogenesis (days 1, 3, 6, 10 and 15), membrane proteins were labelled, enriched and isolated using an aminooxy-biotinylation (AOB) technique and analysed by mass spectrometry using high throughput shotgun proteomics.

Results: Using the AOB labelling approach, a total of 241 unique proteins could be identified reliably ($P<0.05$). According to UniProt database entries and Gene Ontology (GO) annotations, 154 proteins were surface proteins (64%). The remaining 87 proteins were non-surface proteins. 95 (62%) were common between all culturing days. We identified podocalyxin (PODXL) and ciliary neurotrophic factor receptor (CNTFR) as novel components of the chondrogenic cell surfaceome. Their expression on the surface of chondrocytes were validated using western blotting, immunocytochemistry.

Conclusions: Our results provide, for the first time, a repository for proteomic data on differentially expressed low-abundance membrane proteins on the surface of differentiating chondrocytes during the course of normal chondrogenesis. Identifying specific biomarkers characterising the main stages of cartilage formation may offer novel ways by which osteo-chondroprogenitor stem cells could be preferentially directed towards the chondrogenic lineage, thus generating better cartilage for tissue engineering applications.

SIDE-SPECIFIC CELL SURFACE LABELING OF TRANSMEMBRANE PROTEINS IN POLARIZED CELLS

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Cell surface proteins play a crucial role in many biological processes, including immune response, signal transduction, cell-cell interactions and cell migration. The expression of these proteins is affected by diseases such as infection, cancer and genetic disorders, and is intricately linked to epithelial cell polarity, in particular the differences in protein composition between the apical and basolateral membranes. Despite various enrichment methods aimed at exploiting their biochemical and biophysical properties, the identification of the cell surface proteome remains a challenge, especially in polarized epithelial cells.

We present a novel method for enriching proteins located on the apical and basolateral surface of epithelial cells. This new approach was developed based on our highly optimized method for labeling the cell surface of MDCKII (Madin-Darby canine kidney) cells cultured in transwells. We achieved the side-selective labeling with a cell membrane-impermeable biotinylating agent (sulfo-NHS-SS-biotin) applied separately to the apical and basolateral sides of the cell monolayer. The resulting biotinylated peptides or proteins were then enriched using neutravidin-containing resin and subsequently analyzed by liquid chromatography-tandem mass spectrometry (nanoHPLC-MS/MS).

By direct detection of biotinylated lysines from the MDCKII cell line, we identified 1334 peptides biotinylated on the apical cell surface and 1122 peptides biotinylated on the basolateral cell surface. These peptides were derived from 485 and 344 proteins, respectively.

Building on the success of the developed side-specific surface labeling method, we have extended it to other medically relevant epithelial cell lines, such as HCT116 and HT29 (colorectal carcinoma) cells. We detected an average of 1500-1800 labeled peptides (450-500 proteins) from HCT116 cells and 2200-2500 peptides (650-730 proteins) from HT29 cells from the apical or basolateral surface. Our results may help to elucidate the role of cell surface proteins on the apical and basolateral surface and to understand the molecular mechanisms of cell polarization, which may help in the treatment of various diseases.

THE SURFACEOME OF HUMAN EPIDERMAL MELANOCYTES AND MELANOMA CELLS

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Cell surface proteins collectively constitute the surfaceome, a special subset of the plasma membrane proteome. Currently, data concerning the complement of cell surface proteins in healthy and pathological human pigment cells is unavailable. Therefore, this project aims to characterize the surfaceome of human epidermal melanocytes and melanoma cells by different isolation techniques and mass spectrometry analyses.

Three human pigment cell cultures were used in our experiments: primary epidermal melanocytes (from skin samples), WM35 cell line (from in situ melanoma), and A2058 cell line (from metastatic melanoma). In the first approach, sialic acid residues of cell surface proteins were selectively labelled with aminooxy-biotin, after which Streptavidin-conjugated beads were used to isolate the labelled proteins. After trypsin digestion, peptides were analyzed by high-throughput shotgun mass spectrometry. The enrichment ratios were 70.1%, 63.1% and 56.9% for melanocytes, WM35 and A2058 cells, respectively. The combined list of surfaceome proteins consisted of 470 different proteins, among which 112 were detected only in melanocytes, whereas 186 proteins were specific to the melanoma cells and 95 proteins were identified in all three cell types. Notably, validation of initial findings highlighted basigin (CD147) as a promising surfaceome-related biomarker. In our novel method, hydrazide-based enrichment was performed to specifically target oxidized glycan chains of cell surface proteins following whole-cell lysis and protein digestion with Trypsin/Lys-C mix. Selected peptides were enzymatically deglycosylated using PNGase-F and detected by LC-MS/MS. However, data evaluation is still in progress.

Cell surface proteins represent key targets in biomedical research due to their potential as biomarkers and therapeutic alternatives. The differences found in the surfaceome of healthy vs malignant pigment cells can lead to the discovery of highly selective biomarkers of disease onset and progression.

PROTEOMIC ANALYSIS OF LUNG ADENOCARCINOMA SUBTYPES WITH DIFFERENT GENETIC ALTERATIONS

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Lung cancer is the second most commonly diagnosed cancer and the leading cause of cancer-related mortality worldwide, underscoring the need to better understand its underlying biological mechanisms. Lung adenocarcinoma (LUAD), the most widespread subtype of non-small cell lung cancer, is characterized by high heterogeneity and a wide variety of genetic alterations. These features hinder early and accurate diagnosis and complicate treatment selection. To better understand biological differences, we aimed to perform a comprehensive proteomic analysis of tissue samples from multiple LUAD subtypes defined by different genetic alterations.

We analyzed formalin-fixed, paraffin-embedded (FFPE) tissue samples from 69 lung cancer patients. The samples were classified into four groups: tumors with alteration in one of three key oncogenes – epidermal growth factor receptor (EGFR), Kirsten rat sarcoma viral oncogene homolog (KRAS) or anaplastic lymphoma kinase (ALK) – or lacking alterations in these genes (triple wild-type, WT). Following deparaffinization, antigen retrieval, on-surface digestion and C_{18} purification, peptides were analyzed by nanoUHPLC–MS/MS. Protein identification and quantification were performed by Byonic and MaxQuant, respectively, and statistical analysis was conducted using custom R code.

Out of the examined 1711 proteins, 492 were found significantly altered. Hierarchical clustering based on these proteins revealed that lung tumors with different genetic alterations display unique proteomic profiles, although their separation is not complete. Pairwise comparisons revealed that triple wild-type samples differ more from the genetically altered groups than these groups do from each other. The largest divergence was observed between EGFR-mutant and WT tumors, with 168 significantly altered proteins, whereas ALK- and KRAS-mutant samples showed the greatest similarity. Many of the differentially expressed proteins between LUAD subtypes play a role in RNA binding and are involved in cellular component organization or

biogenesis. Notably, several mini-chromosome maintenance (MCM) proteins showed altered expression across multiple comparisons. Given their essential role in DNA replication and their prior identification as potential prognostic biomarkers in LUAD, our findings suggest that investigating MCM protein expression across genetic alteration based subtypes may yield important biological and clinical insights.

COMPARISON OF PROSTATE ADENOCARCINOMA AND BENIGN PROSTATIC HYPERPLASIA BY PROTEOMIC AND PHOSPHOPROTEOMIC ANALYSIS

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Alterations in protein content and/or post-translational modifications (PTMs) may arise during disease onset and progression. Phosphorylation is among the most commonly investigated PTMs. Among males, prostate cancer (PCA) is one of the most prevalent cancer; in addition, the risk of developing PCA increases with age. Owing to limitations in contemporary diagnostic techniques, new approaches are needed. Potential biomarkers may be revealed by proteomic analysis of prostate cancer tissue samples.

In our research, on-surface proteolytic digestion was performed on 77 formalin-fixed paraffin-embedded tissue samples derived from patients with PCA (n = 62) or benign prostate hyperplasia (BPH, n = 15). Peptides extracted from the tissue surface were purified using solid phase extraction (SPE). Phosphopeptides were subsequently enriched using TiO_2 enrichment and the flow-through and wash fraction was collected for proteomics analysis. SPE was applied to purify fractions prior to HPLC-MS/MS measurements. Proteomic fractions were analyzed using data-independent acquisition, whereas data-dependent acquisition was employed for phosphoproteomic fractions.

We investigated protein, phosphorylation site alteration between BPH and PCA samples in general, and PCA samples with different risk stratifications, such as Gleason, D'Amico stratification. The proteomic analysis revealed 903 significantly differently expressed proteins between BPH and PCA samples. The upregulated proteins have a major role in cytoplasmic translation whereas the downregulated proteins have a key role in cytoskeleton organization processes. We found that serine/arginine-rich splicing factor 2 and histone H4 proteins were upregulated in PCA samples, as reported previously [1]. In addition, 378-1008 proteins were significantly altered between BPH and PCA samples with different risk stratification; for

example, 845 proteins between high-risk CAPRA and BPH samples. In the phosphoproteomic analysis 42 phosphorylation sites showed significant differences between BPH and PCA samples. Furthermore, 10-30 phosphorylation sites were significantly different between BPH and PCA samples with different risk category, e.g. 23 phosphorylation sites between BPH and the low-risk Gleason group.
Cancer specific survival analysis was performed on the identified proteins. The univariate Cox-proportional hazards model revealed 112 proteins significantly associated with survival. By fitting Multivariate Cox-proportional hazards models, 89 out of 112 proteins were found to be potentially significant independent predictors of survival after adjusting for the CAPRA score, while 83 out of 112 proteins when the model was adjusted for the D'Amico score.

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LC-MS/MS-BASED TERMINOMICS UNRAVELS CARBOXYPEPTIDASE B1 ROLE IN LUMINAL A BREAST TUMOR PROGRESSION

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Carboxypeptidase B1 (CPB1) is a metalloprotease which cleaves arginine and lysine residues from protein C-termini. Elevated CPB1 expression was previously associated with lymph node metastasis in low-grade luminal A breast tumors [1]. Here we aim to better understand the molecular role of CPB1 in breast cancer development. To identify CPB1 protein substrates, we analyzed lysates of lymph node positive luminal A breast tumors with CPB1 (vs. CPB1 negative control) via trypsin digestion and timsTOF Pro 2 LC-MS/MS system. The data were processed in Spectronaut software with semispecific search settings and in Fragterminomics package in R. Among 76,368 peptides identified in terminomics experiment (FDR=0.01), 17,748 peptides possessed a C-terminal non tryptic cleavage, including a set of top 23 proteins with C-terminal peptides with max. 2 arginine or lysine residues removed from the C-terminus specifically in CPB1-cleaved samples. These CPB1 substrates, including PDXDC1, GANAB and NID2 proteins, were associated mainly with extracellular localization, cytoskeleton and cell motility. To functionally confirm CPB1 role in metastatic potential of cancer cells, a 3D invasion assay was performed, showing increased volumes of spheroids formed by MCF7 cells overexpressing CPB1 compared to CPB1 negative control. To further analyze CPB1 association with clinical-pathological parameters, CPB1 immunohistochemistry was performed in the set of 441 breast tumors. CPB1 staining was significantly associated with lymph node status and relapse in patients of all subtypes, and specifically also with relapse of luminal A tumors ($n=251$; $p\leq0.05$). CPB1 histoscore was connected with a shorter relapse-free (RFS) and distant metastasis free survival in the set of 441 breast cancer patients. Multivariable Cox analysis confirmed CPB1 as the most significant factor associated with RFS in luminal A breast cancer patients. In conclusion, CPB1 seems to play a significant role in the progression of luminal A breast tumors which could be mediated by cleavage of specific protein substrates. Supported by the National Institute for Cancer Research (Program EXCELES, ID LX22NPO5102) – Next generation EU.

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PROFILING OF CDCP1 GLYCOSYLATION IN OVARIAN CANCER AND ITS POTENTIAL ROLE IN CANCER

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My study characterizes the N-glycosylation features of CDCP1, a transmembrane glycoprotein that is commonly upregulated in various epithelial cancers and its upregulation often predicts poor clinical outcome. Using representative ovarian cancer cell lines and patient-derived xenograft (PDX) tumour models, in contrast to normal ovarian surface epithelial cell lines, the overall glycosylation profiles and site-specific glycosylation patterns were examined, aiming to uncover glycan differences and define their association with disease state.

I analyzed the total N-glycome of CDCP1 purified from four ovarian cancer cell lines, in parallel with two immortalized ovarian surface epithelial cell lines. The results showed that complex-type glycans were the predominant class across most samples. Notably, the complexity of CDCP1 glycosylation and the linkage types of sialic acids varied significantly between cancer and non-cancer cell lines. In particular, the TYK-nu and HEY ovarian cancer cell lines showed higher levels of tetra-antennary glycans and α2,6-linked sialylation.

And site-specific glycosylation analysis of CDCP1 revealed two distinct modification patterns among its 14 consensus N-glycosylation sites. Six sites (N122, N310, N512, N577, N639, and N642) were consistently occupied by oligomannose-type glycans across all samples. The remaining eight sites were mainly modified by complex-type glycans in all cell lines and in PDX model LP28. Interestingly, the N205 site displayed marked glycan heterogeneity between cancer and normal tissues. In high-grade serous ovarian cancer (HGSOC) models—TYK-nu, HEY (need further validation), and PDX LP28—this site was enriched with highly sialylated tetra-antennary structures.

This project aims to define the glycosylation features of CDCP1 in ovarian cancer in comparison with normal tissue and, more importantly, to evaluate whether the unique glycan structure of CDCP1 can be targeted to develop peptides for cancer detection and treatment.

QUANTIFICATION OF GLYCOSYLATED COMPLEMENT COMPONENT 9 PROTEIN EPITOPES IN PATIENTS WITH LUNG CANCER

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Current top-down or native proteomic research focuses mostly on quantification of proteins, while there are less studies dedicated to monitoring proteome variability, proteoforms. Epitopes of proteins are defined as sites involved in noncovalent interactions of mAbs and their cognate antigen. Epitomics is the large scale characterization of epitopes that shed light on structural heterogeneity of proteoforms.

In this study, we used previous results (1) of Epitomics; Protein Epitome Profiling (PEP) technology that is built upon diverse monoclonal antibody (mAb) libraries generated by Biosystems Immunolab Zrt. against natural human plasma immunogens that represent the immunogenic epitome. Profiling the epitome with PEP allowed identifying specific antibodies, such as BSI0449, BSI0581, and BSI0639, that recognize epitopes on complement component 9 (C9). Although epitope-specific immunoprecipitates of plasma C9 seem to be identical regarding peptide sequence, epitope-specific posttranslational modifications were observed between samples originating from controls and patients with lung cancer. An example is the presence of N-glycosylation at position 415 of C9.

To obtain more insight on the distribution of the modification, we designed a targeted mass spectrometry approach for monitoring the non-glycosylated and glycosylated forms of the AVNITSENLIDDVVSLIR peptide. We ordered the stabile isotope labeled synthetic peptides and performed SRM-based targeted mass spectrometry measurements. We expect that the results of this study will enable the identification of different C9 proteoforms in lung cancer and will provide information on the extent of glycosylation in disease.

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PROTEOMICS PROFILING OF SNAIL SLIME TREATED HUMAN DERMAL FIBROBLAST USING HIGH-END MASS SPECTROMETRY

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Snail slime (SS), a complex viscoelastic fluid secreted by Helix aspersa, has garnered significant scientific interest due to its unique composition and potential applications. Primarily composed of water, it contains diverse biologically active compounds including glycoproteins, proteoglycans, hyaluronic acid, allantoin, glycolic acid, and antimicrobial peptides. Thus, offering a potent blend of moisturizing, exfoliating, skin regeneration and wound healing properties and powerful anti-inflammatory and antioxidant effects. The current study aimed to identify the skin regenerative and wound healing proteins in SS-treated human dermal fibroblast (HDFs) secretome using LC-MS based proteomics. HDFs were treated with different dilutions of SS (1:40, 1:60, 1:80) in serum-free DMEM for 48 h, supernatant was collected, and proteins were concentrated 10X using MWCO 3 KDa filter. Samples were digested by in-solution digestion and analyzed by nano LC-MS using data-independent acquisition. Results show that SS is capable of stimulating the production and secretion of proteins that are involved in skin regeneration and wound healing. The samples treated with SS 1:80 exhibited a significant increase in protein secretion, with 270 proteins detected, compared to the control. In conclusion, SS is a mixture of multiple molecules that exert their effects at the proteomic level, promote cell migration, collagen-I production and ultimately enhance skin regeneration and wound healing.

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PROGNOSTIC ROLE OF PLASMA PROTEOMICS SIGNATURE IN OLIGORECURRENT PROSTATE CANCER PATIENTS UNDERGOING SBRT

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Purpose/Objective. Improving the prognostic stratification of patients (pts) affected by oligorecurrent hormone-sensitive prostate cancer (orHSPC) and undergoing Stereotactic Body Radiation Therapy (SBRT) is an unmet clinical need. This study aims to evaluate whether a plasma proteomics signature can be identified in these pts and its possible correlation with clinical outcomes. **Material/Methods.** orHSPC pts with 1-3 nodal and/or bone metastases undergoing SBRT were enrolled (n=35) together with a cohort of healthy donors (HD, n=15). After a median follow up of 25.6 months, two subgroups were identified based on the occurrence of biochemical progression (BP): "no-BP" (n=8, no evidence of BP) and "early-BP" (n=7, BP Free Survival<6 months). Moreover, depending on the occurrence of distant progression ("DP"), "no-DP" (n=14) and "polymetastatic-DP" (n=6) subgroups were indentified. Plasma-EDTA was collected before SBRT. Plasma peptides were separated by liquid chromatography tandem mass spectrometry based proteomics. Relative protein amount across our samples was determined through label-free quantification. **Results.** Plasma protein profiles of orHSPC compared to HD differed in 40 differentially abundant proteins. Plasma protein profiles of early-BP pts differed from those of no-BP pts in 20 differentially abundant proteins, among which 6 were more abundant in no-BP compared to early-BP (CA1, CDH5, CFHR4, PRDX2, SAA1 and SOD3). Moreover, plasma protein profiles of polymetastatic-DP pts differed from those of no-DP pts due to the presence of 24 differentially abundant proteins, among which 2 were more abundant (CFHR5 and PZP) and 4 were less abundant (CDH5, HSPD1, PRDX2 and SOD3) in polymetastatic-DP versus no-DP. **Conclusion.** Our work suggests that a plasma proteomics signature in orHSPC pts undergoing SBRT could be identified and used as prognostic indicator for BP- and DP-free survival. The occurrence of impaired vascular integrity (low CDH5 levels), a greater oxidative state (low PRDX2 levels) and an altered redox balance (low SOD3 levels) may correlate with the worsening of the analyzed outcomes. High PZP levels in patients with distant metastasis might indicate an immunosuppressive scenario potentially associated with polymetastatic progression.

UNSUPERVISED CLUSTER ANALYSIS REVEALS SUBGROUPS IN OBESITY AND TYPE 2 DIABETES WITH PROTEOMICS PROFILES

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Olink™ proteomic platforms offer a robust and high-throughput approach for quantitative proteomics, making them highly suitable for the identification of novel biomarkers in biologically complex matrices. Recent advancements in large-scale proteomic technologies, combined with machine learning (ML) algorithms, have significantly enhanced our ability to discover and validate candidate biomarkers for multifactorial disorders such as obesity and Type 2 Diabetes (T2D). This study employed both unsupervised clustering and supervised ML models to investigate intragroup variability within clinical groups using Olink-based serum proteomic data. Proteomic profiling was conducted using the Olink™ Explore Cardiometabolic panel on serum samples from three groups: healthy controls (n = 30), individuals with obesity (n = 48), and individuals diagnosed with T2D (n = 51). The resulting dataset contained 366 protein features expressed as Normalized Protein eXpression (NPX) values. For each group, unsupervised k-means clustering was performed to identify latent subgroups (clusters), with the optimal number of clusters determined via the silhouette method. To pinpoint proteins contributing to intragroup heterogeneity, we implemented a Random Forest (RF) classifier, a supervised ML technique, combined with bootstrap resampling. Protein feature importance was identifed using the Mean Decrease in Gini (MDGini) score, and the top 30 features were selected for each group based on their MDGini rankings and finally stable features were identified as discriminatory features based on their frequency of selection (≥700) across bootstrapped iterations. Along with proteomics data, the clinical parameters such as anthropometric values (age, sex, BMI), blood glucose, cholesterol, trigliceride etc. levels were used for analysis. Unsupervised clustering identified two distinct clusters within each group. In the control group, six proteins (NPDC1, PAM, CD46, PEAR1, PTN, ALCAM) were found to differentiate subgroups. For the obesity group, eleven proteins (ENG, IGSF8, CD46, ALCAM, EPHB4, NOTCH1, IL6ST, CA4, NTRK2, NECTIN2, PEAR1) were identified as important features for subgroup discrimination. In the T2D group, twelve proteins (NOTCH1, IL6ST, CD55, PEAR1, ENG, AXL, DLK1, CD59, IGFBP7, IGFBP6, PCDH17, VCAM1) were associated with intragroup heterogeneity. At the same time, none of the clinical parameters showed statistically significant difference between the clusters. This integrative approach combining unsupervised clustering and supervised ML analysis effectively uncovered distinct subgroups at molecular level within each group. Furthermore, it enabled the identification of protein biomarkers that contribute to intragroup heterogeneity, offering potential insights into the molecular complexity of obesity and T2D.

This research project was supported by the Postdoctoral Fellowship program at the University of Debrecen, Hungary.

DEVELOPMENT AND EVALUATION OF A CDR3 A5 - CEFTRIAXONE NANOCONJUGATE AGAINST *BORRELIA*

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Lyme disease, caused by *Borrelia* species, is one of the most common tick-borne infections. Conventional antibiotic therapies often suffer from limitations such as poor tissue penetration, and lack of pathogen specificity. To address these issues, targeted drug delivery systems using nanocarrier-conjugated antibiotics are emerging. In this study, we developed and evaluated a peptide-drug nanoconjugate system to selectively target *Borrelia*. We employed a nanobody-derived peptide clone CDR3-A5, which demonstrates excellent binding affinity to *Borrelia*, composed of 18 amino acids and contains a C-terminal cysteine residue, which allows for site-specific conjugation. The peptide was first purified using reverse-phase liquid chromatography (RPLC) and its molecular weight was confirmed by MALDI-TOF mass spectrometry (2.2 kDa). In parallel, Ceftriaxone, a widely used antibiotic against *Borrelia*, was functionalized with a heterobifunctional Maleimide-PEG-NHS linker. The resulting Maleimide-PEG-ceftriaxone intermediate was then conjugated to the CDR3 A5 peptide via thiol–maleimide chemistry, targeting the free thiol group of the cysteine residue. The conjugated nanodrug, containing CDR3 A5 (2.2 kDa), maleimide-PEG (3.2 kDa), and ceftriaxone (0.55 kDa), was purified by gel filtration chromatography, and its final molecular weight (6.0 kDa) was determined by MALDI-TOF. A control conjugate without peptide, composed of Maleimide-PEG-ceftriaxone, was similarly prepared and its molecular weight (3.7 kDa) was determined by MALDI-TOF, showing clear differences and confirming successful conjugation. CDR3 A5-Ceftriaxone were tested in vitro against *Borrelia* cultures to evaluate antibacterial efficacy. Our results indicated that the CDR3 A5-Ceftriaxone conjugate exhibited anti-*Borrelia* activity with a MIC of 0.201 µg/mL, while ceftriaxone showed a MIC of 0.161 µg/mL, allowing direct comparison of the conjugate's efficacy. These findings support the potential of this nanoconjugate system as a promising candidate for the targeted treatment of *Borrelia* infections and may serve as a foundation for future therapeutic development in Lyme disease. Research supported by EURONANOMED2021-105 (Antineuropatho), APVV-22-0084 and 1/0381/23.

INTERACTION BETWEEN SARS-CoV-2 S AND N PROTEINS MEDIATE RECRUITMENT OF SOLUBLE COMPLEMENT REGULATORY PROTEINS ON THE VIRION

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The SARS-CoV-2 nucleocapsid (N) protein, found abundantly in COVID19 patient plasma, is increasingly recognized for its immunomodulatory potential. While it has been implicated in proinflammatory signaling, its role in immune evasion via the complement system remains underexplored. In this study, we demonstrate a plausible mechanism by which the N protein may aid viral immune escape: by acting as a bridge between soluble complement regulatory proteins (CRPs) and virion. Using dot blot, ELISA, and biolayer interferometry, we confirmed a high-affinity interaction between recombinant N and S proteins (k_D ~ 86.22 nM), which was further validated on intact SARS-CoV-2 BA.5 Omicron virions. To define the interaction interface, we employed limited on-membrane trypsin digestion coupled with mass spectrometry. This domain mapping approach revealed that the N protein could bind on N-terminal domain (NTD) of the S protein. Additionally, we found that the N protein selectively binds multiple soluble CRPs in human serum, including C1-inhibitor (C1-INH), C4-binding protein (C4BP), factor H (fH), and vitronectin (VTN). Furthermore, we demonstrate that the N protein enables dose-dependent recruitment of these CRPs to the surface of SARS-CoV-2 virions. This tripartite N–CRP–virion complex suggests a novel complement evasion strategy wherein the virus is cloaked in host regulatory proteins to evade complement-mediated neutralization. Our findings position the N protein not only as a structural component and immunomodulator, but also as a viral factor facilitating complement escape. This study provides the first evidence of such a mechanism in SARS-CoV-2 and opens new ways for targeted therapeutic interventions aimed at disrupting this evasion mechanism. This work was supported from APVV-22-0084, VEGA1/0381/23, and ESGD/02/2024

PROTEIN EXTRACTION FROM ANCIENT MATERIAL

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Palaeoproteomics is a dynamically evolving scientific discipline that broadens the analytical toolkit and advances knowledge within anthropology and bioarchaeology. At the same time, palaeoproteomics faces multiple challenges emerging namely from the problematics of protein preservation in ancient materials. These are related to the different efficacy of the isolation protocols compared to processing modern proteomic samples, or the complicated interpretation of the results of bioinformatic analysis.

The presented work focuses on the optimisation of the lysis procedure on a tooth sample dated to the late medieval to early modern period. Tested parameters include the composition of the lysing solution and the duration of its application. Additionally, two sample preparation protocols were compared to assess their efficacy: filter-aided sample preparation protocol (FASP) and single-pot solid-phase enhanced sample preparation protocol (SP3).

The optimised method was applied to multiple dental calculus samples dated to the late medieval to early modern period and to Bronze Age, revealing information on potential dietary sources and pathogens.

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FAST AND ROBUST PHOSPHOPROTEOMICS SAMPLE PREP WITH ATTRACTSPE® DISKS C18 TIPS FOR HIGH PHOSPHOPEPTIDE RECOVERY AND IDENTIFICATION

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Background:
Phosphorylation is one of the most prevalent and important post-translational modifications proteins can undergo. Over 50% of the human proteome is phosphorylated and understanding the dynamic phosphorylation across the proteome can understand the progression of many diseases including cancers. As the stoichiometry of phosphorylation sites is generally very low, enrichment steps, followed by SPE clean-up before LC-MS/MS analysis are required to enhance identification and quantification of each site. However, recovery of phosphopeptides can be greatly affected by the choice of clean-up method, resulting in severe losses.
Methods:
Different SPE C18 options were compared for the purification of phosphopeptides, after automated enrichment using magnetic beads (Ti/Zr-IMAC). Effects of sample acidification prior to SPE clean-up on phosphopeptides detection were also assessed by acidifying the enrichment elution with different percentages (2, 3, 4 and 5%) of phosphoric acid or trifluoroacetic acid (TFA).
Results:
Among all SPE options tested, AttractSPE®Disks Tips C18 provided the highest recovery of phosphopeptides (up to 2.4 times more identifications), with high reproducibility (RSD < 10%). AttractSPE®Disks Tips C18 captured more efficiently hydrophilic peptides, and shorter phosphopeptides were retained compared to other brands.
Quenching the enrichment elution with 3% phosphoric acid provided the highest recovery, with 8% more identifications compared to 5% TFA. Lower acid concentration interestingly provided more singly phosphorylated peptides, while higher acid concentration recovered more hydrophilic peptides. This trend was observed for both acids tested but was more pronounced for TFA.
Conclusions:
AttractSPE®Disks Tips C18 are shown to be the best choice for phosphopeptide purification, offering simplicity of use by centrifugation, high sample recovery, and robustness. These SPE Tips are easily scalable with their availability in different sizes and binding capacities to perfectly adapt to different sample amounts, and can be provided as 96 and 384 SPE well plates for high throughput processing.

BOOSTING THE PERFORMANCE: PLATFORM-SPECIFIC COLLISION ENERGY OPTIMIZATION IN BOTTOM-UP PROTEOMICS

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Collision energy is a key parameter in the tandem mass spectrometric analysis of peptides, significantly impacting the quality of the mass spectra and, consequently, their information content. By selecting the optimal collision energy, more peptides can be identified with higher confidence. The optimal collision energy for a given peptide is the energy at which the peptide can be identified with the highest confidence.[1]

In our study, we compared collision energy-dependent measurement series from various LC-MS/MS instruments produced by different manufacturers to determine the optimal collision energy values for peptides. We conducted a collision energy-dependent measurement series using a standard HeLa tryptic digest on a UFLC-coupled Sciex TripleTOF 5600+ mass spectrometer. For comparison, we used five additional collision energy-dependent measurement series with HeLa tryptic digests available in the MassIVE repository. These were performed on Bruker Maxis II ETD QTOF, Thermo Q Exactive Focus Orbitrap, Thermo Orbitrap Fusion Tribrid, and Waters Select Series Cyclic IMS (trap and transfer cell) mass spectrometers. Peptides were identified using the Byonic and Mascot database search engines. For each platform, an m/z-dependent optimal collision energy setting was determined via a linear fit to the data points of individual peptides.

No significant search engine dependence was observed for the Sciex, Bruker, and Thermo Orbitrap Fusion instruments when using Byonic and Mascot. However, a slight dependence was found for the Thermo Q Exactive and for both collision cells of the Waters instrument.

We found that the optimal collision energy for higher-charged peptides on the Sciex instrument was lower, consistent with recommendations from other manufacturers. The same trend was observed for the other instruments. Therefore, we recommend a charge-dependent optimized collision energy setting for the Sciex instrument as well. For the Waters instrument, no clear charge dependence could be determined due to the high variance of the data points.

Instrument dependence was investigated using doubly charged (2+) peptides. The determined optimal collision energy settings for the transfer cell of the Waters instrument, as well as for the Sciex and Bruker instruments, were similar. In contrast, the optimal collision energy settings for the Waters trap, Thermo Orbitrap Fusion, and Thermo Q Exactive instruments differed significantly. These findings indicate that the optimal collision energy setting can vary between different instruments from the same manufacturer and between different collision cells within the same instrument, highlighting the importance of instrument-specific tuning when transferring protocols.

Reference

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THE DEVELOPMENT OF IN-HOUSE MINIATURIZED SIZE-EXCLUSION COLUMNS AS AN ENRICHMENT METHOD TO ENHANCE HPLC-MS-BASED PROTEOGLYCAN ANALYSIS

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Proteoglycans (PGs), a subset of glycoproteins, consist of a core protein containing covalently linked glycosaminoglycan (GAG) chains in most cases connected by a tetrasaccharide region. Investigation of site-specific PG glycosylation is necessary to understand glycoprotein activities in physiological contexts. The structural complexity, low abundance, and variety of GAG side chains necessitate the development of enrichment techniques to selectively identify these glycopeptide subsets, which is crucial for glycoproteomic studies.

Therefore, our aim was to develop an effective enrichment method for GAG-linker glycopeptides present in PG decorin utilizing miniaturized, in-house-packed size-exclusion chromatography (SEC) spin columns and comparing the method with techniques previously used for enriching linker glycopeptides.

Decorin was enzymatically digested using PNGase F and Lys-C-Trypsin to cleave *N*-glycans and proteins, respectively. GAG-linker glycopeptides were enriched using Sephadex G-25, G-50, G-75, and G-100 SEC spin columns, and the chondroitin sulfate (CS) chains were subsequently cleaved using Chondroitinase ABC. The desalting of GAG-linker glycopeptides was accomplished with C18 spin tips. Measurements were conducted using Waters nanoAcquity UHPLC coupled with Thermo Fisher Exploris 240 Orbitrap MS.

The presence of 5 oxonium ions, $[HexNAc\text{-}2H_2O]^+$m/z=168.066, $[HexNAc\text{-}H_2O]^+$m/z=186.076, $[HexNAc]^+$m/z=204.087, $[\Delta HexAHexNAc]^+$m/z=362.108, and $[HexAHexNAc]^+$m/z=380.119, were identified in the extracted ion chromatograms, indicating that the CS-linker glycopeptides could be enriched using SEC. The database search identified the glycopeptide linker sequence of decorin as well as 9 specific structures of the CS-linker glycopeptides. The use of SEC spin columns, particularly G-75 resulted in the most positive outcomes regarding enrichment.

The enrichment of CS-linker glycopeptides could be enhanced using newly developed, in-house-packed size-exclusion spin columns, compared to existing methods. These findings indicate their possible application to clinical samples.

Funding: The project was supported by the Lendület (Momentum) Program of the Hungarian Academy of Sciences.

STREAMLINED AND REPRODUCIBLE PROTEOMIC SAMPLE PREPARATION FROM FFPE TISSUES USING AFA® TECHNOLOGY

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Formalin-Fixed Paraffin-Embedded (FFPE) samples remain a cornerstone for preserving clinical and research tissues due to their stability and widespread availability. However, extracting high-quality proteomic data from FFPE material continues to present technical challenges. We present a robust, reproducible, and accelerated workflow leveraging Adaptive Focused Acoustics® (AFA®) technology for efficient deparaffinization, protein extraction, purification, and enzymatic digestion.

Starting with 10 µm FFPE tissue scrolls, deparaffinization was achieved using a combination of rehydration buffer and specialized solution with brief heating. Following centrifugation, tissue lysis and homogenization were performed using the Covaris R230 Focused Ultrasonicator. De-crosslinking at 90 °C for 90 minutes was followed by reduction and alkylation. The proteins were purified via a PAC-based magnetic bead protocol and subsequently digested on-bead with Trypsin under AFA-enhanced conditions for just one hour. This workflow was validated across a diverse set of FFPE tissues, including liver, colon, brain, lung, uterus, prostate, and breast. The method yielded high peptide and protein recovery, reproducibility, and robust digestion profiles without increasing missed cleavages. Our results demonstrate the effectiveness of AFA-based workflows in simplifying and accelerating sample prep from FFPE tissues while maintaining high data quality for proteomic analyses.

ANALYTICAL METHOD DEVELOPMENT FOR THE PROTEOMIC AND GLYCOMIC CHARACTERIZATION OF EXTRACELLULAR VESICLES

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Extracellular vesicles (EVs) are small (30–1000 nm) lipid bilayer-enclosed particles carrying proteins, lipids, and nucleic acids released by cells into the extracellular space, playing a fundamental role in intercellular communication. Among them, the small extracellular vesicles (sEVs) with a diameter of 30-150 nm form a distinct subgroup. sEVs are not only key players in signaling processes between healthy cells but may also contribute to the development and progression of several pathological conditions, such as cancer, which makes their investigation highly important. The examination of post-translational modifications of vesicle-derived proteins poses a major challenge due to the limited sample amount, therefore the optimization of sample preparation is crucial.

The aim of this work was to optimize the workflow applied earlier in our research group for the proteomic and glycomic characterization of sEVs isolated from cancerous and healthy cell lines. This included the preparation of sEV samples for proteomic digestion and the enrichment of *N*-glycopeptides. As a first step, we tested the efficiency of various sEV isolation and subsequent digestion methods (e.g. S-Trap, SP3, ethanol precipitation followed by in-solution digestion) compared to the previously used approach using solvent exchange followed by in-solution digestion. Then, we tested different *N*-glycopeptide enrichment methods (e.g. HILIC, PGC) on cell lysate samples and compared them to the currently used enrichment method utilizing acetone precipitation.

The ethanol precipitation method proved superior on a qualitative proteomic level compared to the previous in solution digestion method after solvent exchange. Samples isolated with ammonium bicarbonate solution or prepared with the paramagnetic bead based SP3 method yielded significantly fewer protein identifications. On the quantitative proteomic level, the ethanol precipitation method performed similarly to the control method. The results of the *N*-glycopeptide enrichment methods evaluated based on chromatogram characteristics, purity, and the number of *N*-glycopeptides, indicated that none of the tested alternative enrichment methods outperformed the currently used acetone precipitation method, which therefore remains the most effective approach.

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EFFECT OF CULTURE CONDITIONS ON THE PROTEOMICS PROFILE OF A549 CELLS

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The A549 lung adenocarcinoma cell line is widely used in biological research, yet the influence of cell culture conditions on its proteomic profile has not yet been thoroughly explored. In this study, we investigated how different culture parameters affect protein expression patterns.

Three experimental series were conducted: (1) investigating how proteome changes with passage number using completed medium all the time, or if subjected to repeated nutrient deprivation, (2) comparing different basal media (RPMI, DMEM, and F12), and (3) assessing the impact of fetal bovine serum (FBS) concentration (10%, 5%, 2%, 0%) on the proteome. Cells were lysed with freeze-thaw cycles. and protein extracts were prepared using standard tryptic digestion followed by C_{18} purification and nanoUHPLC/MS-MS analysis.

In the starvation series, principal component analysis (PCA) clearly indicated that nutrient deprivation alters the proteomic profile. Media composition also influenced protein expression, with samples separating distinctly on PCA. Although F12 medium is frequently recommended for A549 cells in the literature, it yielded the poorest results in our hands, with four times less proteins quantified compared to RPMI. The passage number itself had no effect on the cell proteome, but repeated starvation caused systematic changes (repeated shifts in PCA). Across all three experimental series, a total of 623 proteins were quantified. In the experiments with different FBS content, cells cultured without FBS formed a completely distinct cluster, while 10%, 5%, and 2% FBS conditions partially overlapped in PCA. In this series, 101 proteins were differentially expressed, with the most affected biological processes including RNA binding, epithelial cell differentiation, and monocarboxylic acid metabolism.

Our findings demonstrate that variations in serum supplementation, cell culture media and passage number exert a profound effect on the proteomic landscape of A549 cells. These results highlight the importance of carefully selecting culture conditions in experimental design and data interpretation.

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FORMALIN FIXED PARAFFIN EMBEDDED (FFPE) PROTEOMICS FROM A METHOD DEVELOPMENT POINT OF VIEW – WHICH IS THE BEST METHOD FOR PROTEIN IDENTIFICATION FROM FFPE TISSUE SAMPLES?

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Formalin-fixed, paraffin-embedded (FFPE) tissue specimens represent an extremely valuable sample source for biomedical studies as these can be stored at room temperature that is easier and simpler as opposed to dealing with the storage of fresh/frozen tissues. Mass spectrometry (MS) proteomics profiling of FFPE archival tissues holds the promise to finding potential disease biomarkers, with the added luxury of available patient clinical history. Although this preservation technique inherently modifies proteins, several protocols have been developed to take advantage of formalin fixation reversibility. Thus, effectiveness of this approaches relies on proper extraction, denaturation, and digestion. In our study we evaluated the performance of an on-surface digestion protocol along with 5 different extraction/denaturation protocols coupled to in-solution digestion on FFPE tissue samples. The proteomics analysis was performed with an Orbitrap Tribrid (Thermo Scietific) instrument operating on data-independent acquisition (DIA) coupled to EasynLC1200 nanoUPLC. Our results show that the application of different buffers and temperatures resulted in varying protein recovery and profiles. The best method was an off-surface approach, allowing identification to a depth of 1461 proteins from minute amounts of material.

The optimization of sample preparation methods can in turn help pathologists and researchers to find the best method considering time, labor intensity, and protein profile to achieve reliable protein identification and quantification.

MAGNETIC PARTICLES IN PROTEOMICS: COMPARISON OF MAGNETIC BEAD-BASED SAMPLE PREPARATION METHODS

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Proteomic sample preparation traditionally relies on tryptic in-solution digestion followed by C_{18} solid-phase extraction purification (C_{18} workflow), a complex and time-consuming process that can lead to significant sample loss. These limitations motivated the development of alternative strategies to simplify the process and improve recovery. Among the most promising alternatives are the magnetic bead-based single-pot approaches such as SP3[1] and SP2[2]. However, no comprehensive study has systematically compared these methods across diverse biological sample types.

In this study, we examined SP2 and SP3, alongside two modified protocols (SPy and SPx), on samples from various biological sources including cell lysate, plasma, cell membrane, and tissue. Each method was benchmarked against the conventional C_{18} workflow. We investigated the number of identified peptides and proteins, reproducibility, selectivity with respect to isoelectric point, peptide length, and hydrophilicity, and relative protein recovery compared to C_{18}. Our results demonstrated that the magnetic bead-based methods consistently produced cleaner chromatograms than C_{18}. The performance of each method varied depending on the sample type, however, in most cases the SPy protocol outperformed the widely used SP3. The reproducibility of the modified protocols was comparable to SP2, SP3 and C_{18}. Selectivity analyses revealed substantial differences in peptide isoelectric point, length, and hydrophilicity, while recovery distributions indicated biases compared to the C_{18} reference method.

Overall, the SPy workflow proved to be the most versatile and effective for proteomic analysis across diverse sample types, offering a simplified and cost-effective alternative to conventional protocols. For further improvements, we are developing a novel magnetic bead-based protocol, termed optSP. Our future work will focus on evaluating the optSP protocol against the SP2, SP3, SPy and SPx methods, with the aim of achieving even greater performance.

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ENHANCED IMMUNOPEPTIDE IDENTIFICATION USING MIDIA-PASEF: A NOVEL TIMSTOF SCAN MODE

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Mass Spectrometry (MS)-based Immunopeptidomics (Ipep) provides biologically relevant information that cannot be obtained through other techniques. While Data-Independent Acquisition (DIA) methods tend to be more reproducible and sensitive than Data Dependent Acquisition (DDA) methods, the resulting data can be challenging to analyze. Recently, a new acquisition method, midia-PASEF (Distler, 2023), has shown promise for Ipep by acquiring data in the DIA space while processing it in a DDA manner.

Immunopeptides were enriched from varying amounts of B-lymphocytes (from 1x10^6 to 5x10^6 cells) and analyzed using PASEF and midia-PASEF with a timsTOF Ultra 2 system (Bruker). Both acquisition schemes employed ion mobility selection to isolate peptides of interest (+1 for m/z > 600, +2, +3) while minimizing background noise. The resulting data were analyzed with the open-source Sage search engine, combined with MS2Rescore (used as part of the midiaID processing pipeline for midia-PASEF data).

Regarding Class I immunopeptides, we observed a 20% increase of identified peptides identified using midia-PASEF. At each loading level, the amino acid motifs for the 9-mers were similar between both methods, including the preference for E or L at position 2 and V, L, I or A at position 9. This suggests that the additional peptides identified using midia-PASEF are relevant. By combining de novo sequencing with midia-PASEF acquisition, we were able to identify 10% more immunopeptides.

IDENTIFICATION OF DEAMIDATION SITES IN INTACT INSULIN VIA INTERNAL FRAGMENTS PRODUCED BY COLLISION-INDUCED DISSOCIATION

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The prevalence of diabetes is steadily increasing, prompting the widespread use of various commercially available insulin analogs products in clinical practice. Assessing the chemical and structural stability of these pharmaceuticals should be carefully addressed, as their long-term efficacy and safety are intrinsically linked to chemical degradation pathways, such as deamidation [1]. Currently, it is unknown exactly which positions and to what extent deamidation occurs in the various insulin analogues. Therefore, identifying the most frequently occurring deamidated forms and thoroughly investigating their potential physiological effects is of particular importance [2].

Our research group successfully separated the deamidated forms of various insulin analogs, as well as distinguishing them from the native active pharmaceutical ingredient, using capillary zone electrophoresis [3]. We employed collision-induced dissociation (CID) to fragment the deamidated forms of human insulin during their separation. Although CID is a widely used technique, its efficiency is limited in this context: fragmentation events occurring in the region enclosed by the disulfide bridges do not result in a change in m/z, as the disulfide bonds hold the structure together. Terminal fragments outside of this region allow the straightforward identification of some deamidation sites. Appropriate low-abundant internal fragments had to be selected to identify the rest of the deamidation sites. Overall, we were able to separate human insulin from four singly deamidated forms and identified the site of deamidation for each component.

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FRAGFLOW: AUTOMATED WORFKLOW FOR LARGE-SCALE QUANTITATIVE PRTEOMICS IN HIGH PERFORMANCE COMPUTING ENVIRONMNETS

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Although numerous databases exist for various disease contexts, a dedicated and comprehensive resource focused on the proteogenomics of nucleotide repeat expansion disorders (NREDs) remains unavailable. To address this gap, our research aims to perform a systematic analysis of publicly accessible proteogenomic datasets and develop an integrated, standardized database. Given the substantial variability inherent in proteomics data—with differences in instrumentation, experimental protocols, and technical biases—meta-analytical approaches must rigorously account for such inconsistencies. Two predominant strategies are currently employed: (1) re-analysis of raw mass spectrometry (MS) data followed by normalization and statistical integration, and (2) aggregation of differential protein expression results from independent studies, incorporating only consistently reported expression changes. Our approach combines both methodologies by (i) reprocessing raw MS data to derive differential expression profiles and (ii) applying predefined selection criteria to ensure robust and reproducible inclusion in the database.

A central component of this work is the deployment of FragPipe (FP), a widely used proteomics analysis suite, within a high-performance computing (HPC) environment to enable efficient processing of large-scale datasets. Due to the computational demands of proteomics workflows, FP has been adapted for parallel execution on UNIX-based HPC clusters with optimized resource management. While FP supports multiple acquisition modes, its command-line interface presents challenges for full-scale automation. To address this, we developed FragFlow, a Nextflow-based pipeline that automates MS data processing and integrates downstream statistical analysis via the FragPipe-Analyst platform.

The resulting workflow enables reproducible, scalable, and low-intervention proteomics analysis, supporting comprehensive meta-analyses across diverse datasets. By leveraging HPC infrastructure and workflow automation, our project seeks to systematically characterize proteomic alterations in NREDs, thereby contributing to the elucidation of disease mechanisms.

DIRECTED FRAGMENTATION OF PEPTIDES MAXIMIZES B- AND Y- ION YIELD AND SEQUENCE COVERAGE IN DATA INDEPENDENT ANALYSIS MASS SPECTROMETRY

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Accurate protein identification by tandem mass spectrometry is highly dependent on the quality and abundance of fragment ions, which are often generated by the collision-induced dissociation (CID). Chemical derivatization has been widely adopted in proteomics to enhance peptide ionization, generate selective ion series and improve sequence readout for *de novo* peptide sequencing and matching accuracy in database search. We evaluated *N*-terminal peptide derivatization using 4-formylbenzene-1,3-disulfonic acid (4-FBDA), a reagent designed to improve spectral richness in both positive and negative ion modes. Fragmentation efficiency was assessed on a curated dataset of 5454 MS/MS spectra following data-independent analysis (DIA), comparing non-derivatized and derivatized tryptic peptides form 13 proteins (13-240 kDa) in positive and negative ion modes. Statistical analysis of b-, b°-, b*-, y-, y°- and y*- ions obtained after the database match of the most prominent ions showed a near doubling of fragment ion counts upon derivatization. Specifically, non-derivatized peptides on average generated 651 b-ions and 730 y-ions, while derivatized peptides generated 1261 b-ions and 1257 y-ions, underscoring the enhanced and directed fragmentation enabled by the 4-FBDA derivatization approach. Moreover, merged sequence coverage (positive and negative) improved from 77.3-97.5% (86.6% on average) in non-derivatized samples to 88.7-100% (96.4% on average) in derivatized ones. These improvements in fragment ion production and spectral coverage significantly benefit both database-dependent identification and *de novo* peptide sequencing, and are particularly relevant for structural characterization of complex proteins, utterly important in antibody structure analysis.

HETEROGENEITY OF HUMAN LYSOSOMES

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Lysosomes are responsible for the breakdown and recycling of cargo delivered from the cytoplasm by autophagy or from the plasma membrane and outside of the cells via endocytosis or phagocytosis. Cargo is usually transported by vesicles: autophagosomes and endosomes or phagosomes, respectively. The rate of breakdown depends on vesicle biogenesis, motility, lysosomal fusion, and finally the degradation capacity of lysosomes. Each of these steps during autophagy can become rate-limiting and their defects are known to cause diverse diseases including neurodegeneration.

An often-overlooked fact is the extraordinary diversity of lysosomes in animal and human cells. The large variations in their size and shape are in contrast with the relative uniformity of other cellular organelles. The extreme heterogeneity of lysosomes in animals at least in part arises from receiving, digesting, and recycling cargo and resident protein via multiple pathways (the various forms of autophagy, endocytosis, phagocytosis, but also biosynthetic transport routes, lysosome reformation and fusion factor recycling). These must inevitably lead to cycles of activity on the level of individual lysosomes. Differences in the composition of different pools of lysosomes (grouped based of location, size, pH and activity) are unknown.

In this study we investigate how the starvation-induced autophagy affects biochemical and structural composition of these vesicles in human cells. Using proteomics and lipidomics analysis, we found the compounds of the lysosomes in normal and in starved conditions. Our finding show how fluctuating the biochemical composition of the lysosomes is. We aim to connect these changes with the biophysical properties of the membrane to understand the behavior of these organelles in starvation-induced autophagy.

PHOSPHOPROTEOMIC ANALYSIS OF THERMOPHILIC PROKARYOTES: EXPANDING THE PHOSPHORYLATION LANDSCAPE IN EXTREME ENVIRONMENTS

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Protein phosphorylation is a critical post-translational modification that regulates numerous cellular processes, including signal transduction, stress responses, and metabolic control in prokaryotes. Despite its importance, the detailed characterization of phosphorylation in thermophilic species—especially those inhabiting extreme environments such as high temperatures—hasremainedlargelyunexplored.

In this study, we conducted a comprehensive phosphoproteomic investigation of six prokaryotic species selected to represent a wide phylogenetic and physiological diversity. These included four thermophilic species—*Sulfolobus acidocaldarius, Caldimonas thermodepolymerans, Haloferax volcanii*, and *Parageobacillus thermoglucosidasius*—spanning both archaeal and bacterial domains, one mesophilic species, *Escherichia coli*, and one cyanobacterium, *Thermosynechococcus elongatus*. The selection of these organisms enabled us to gain broader insights into how phosphorylation patterns vary between thermophilic and mesophilic species, and how these modifications might relate to thermal tolerance and environmental adaptation. Phosphopeptides were enriched using Ti-IMAC HP beads (ReSyn Biosciences), and the resulting peptide mixtures were analysed using liquid chromatography coupled with electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) on an Orbitrap Fusion Tribrid mass spectrometer. Phosphorylation on serine, threonine, and tyrosine residues was investigated, providing a comprehensive view of phosphorylation events across the selected prokaryotes.

By expanding the phosphoproteome coverage of thermophilic prokaryotes, this work provides a valuable resource for future studies on stress responses, signalling mechanisms, and regulatory networks in extreme environments. It also sets the foundation for comparative analyses aimed at understanding evolutionary differences in post-translational regulation among prokaryotes adapted to varying thermal niches.

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REPLICATION INDEPENDENT FUNCTION OF TLS POLYMERASES

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TLS polymerases play a key role in cellular DNA damage tolerance by allowing synthesis across lesions, enabling cells to avoid repair attempts that could otherwise lead to DNA breaks or apoptosis. However, since most somatic mutations originate from TLS activity, precise regulation of these polymerases is essential for genome stability. Although cells use complex mechanisms to control TLS, many molecular aspects remain unclear especially in non-dividing cell types. We are planning to investigate their potential regulators in G0/G1 phases of the cell cycle to better understand how TLS activity is modulated outside of DNA replication. By combining proteomic profiling with live-cell imaging, we will identify potential regulatory interactors of TLS polymerases. Our results will reveal that TLS in non-dividing cells is not merely a passive damage-bypass mechanism but a tightly regulated process that may contribute significantly to genomic integrity maintenance. These insights might open new possibilities for targeting TLS pathways in diseases where DNA repair regulation is compromised, including cancer and neurodegeneration.

THE PROTEOMIC MAP OF MITOSIS

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Precise spatiotemporal organization of proteins is essential for the regulation of key biological processes, including mitosis[1,2]. Dysregulation of mitotic events and associated factors has been implicated in a range of pathological conditions, such as various cancers [3,4], neurodegenerative disorders like Alzheimer's disease [5], and certain rare genetic diseases [6]. During mitosis, orchestrated spatial rearrangements and regulatory mechanisms ensure the faithful segregation of duplicated sister chromatids, thereby generating genetically identical daughter cells[7–9]. While previous studies have leveraged high-throughput approaches to monitor specific mitotic proteins [10–15], comprehensive systems-level analyses that capture the temporal dynamics of both proteomic and morphological features across mitosis have remained limited.

In this study, we present a high-resolution spatiotemporal characterization of mitosis by decomposing the process into 40 phenotypically defined stages using regression-based deep learning techniques. This framework, integrated within our Deep Visual Proteomics (DVP) workflow [16], enabled the quantification of dynamic proteomic landscapes throughout mitotic

progression. We identified and quantified 4,350 proteins with high confidence, 147 of which exhibited statistically significant temporal changes in abundance. Clustering analyses revealed coordinated regulatory patterns, while network-based approaches highlighted the tightly controlled behavior of core cell cycle regulators and their association with oncogenic mutations. Validation via immunofluorescence confirmed abundance changes and implicated previously uncharacterized proteins—such as C19orf53—in mitotic regulation.

To support data exploration and accessibility, we developed MITO-OMIX, an interactive, web-based platform that integrates morphological and proteomic data across mitosis. Collectively, our dataset provides a comprehensive resource for dissecting the molecular architecture of normal and pathological mitotic events, offering novel insights into the temporal orchestration of cell division.

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COMPARATIVE MASS SPECTROMETRIC ANALYSIS OF PROTEOLYTIC ACTIVITY IN WINE AND WINE VINEGARS

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Plant organisms are a rich source of proteolytic enzymes. Aspartic endopeptidases (e.g., cardosin), serine endopeptidases (e.g., cucumisin), and cysteine endopeptidases (e.g., papain) are well known in the life sciences. Several of these enzymes are widely used as reagents in experimental biochemistry, particularly for protein digestion, as well as in food processing. Bromelain, ficin, papain, and others are effective meat tenderizers, while cardosin A is an excellent milk coagulant and serves as a plant-based rennet substitute in cheese production.

Fresh grapevine juice, fermented juice, bottled wine, and wine vinegar have all been shown to contain a cysteine protease [1,2]. This enzyme (referred to as CYSP) exhibits high sequence similarity to RD21A peptidase from Arabidopsis, oryzain alpha from rice, and many other plant cysteine endopeptidases. In this study, we focused on the proteolytic activity of wine and wine vinegars with potential applications in meat tenderization and milk coagulation. Both commercial and experimental wines were examined. The vinegar samples included commercial white wine vinegars, balsamic vinegar, and a red wine vinegar prepared in the laboratory through fermentation of grapevine juice followed by spontaneous oxidation (acetification).

To study the activity and cleavage specificity of wine and wine vinegar proteases, we used azocasein, casein, gelatin, purified protein standards, and minced beef proteins as substrates. Enzyme activity was assayed by azocasein digestion and by gelatin zymography using native polyacrylamide gel electrophoresis. This approach confirmed the presence of CYSP and suggested the involvement of aspartic proteases.

Digestion of beef proteins and casein with wine and vinegar samples, followed by peptide purification and MALDI-TOF/TOF tandem mass spectrometry, enabled the identification of cleavage sites. The most frequently observed C-terminal amino acids in the resulting peptides were L, F, R, Y, K and E. This cleavage pattern reflects the combined specificity of CYSP and pepsin-like aspartic proteases. CYSP activity was more pronounced in wine, whereas aspartic protease activity predominated in the vinegar samples.

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HPLC-MS ANALYSIS OF CHONDROITIN/DERMATAN SULFATE AND HEPARAN SULFATE GLYCOSAMINOGLYCAN DISACCHARIDES FROM LUNG TUMOR TISSUES

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Lung cancer is the second most frequently diagnosed cancer worldwide and the leading cause of cancer mortality. Since carbohydrates, including glycosaminoglycans (GAGs), can serve as diagnostic biomarkers, we conducted a glycomic analysis of chondroitin/dermatan sulfate (CS/DS) and heparan sulfate (HS) disaccharides in small cell and non-small cell lung cancer tissues, comparing tumor and adjacent normal regions. Using enzymatic digestion and HILIC-WAX HPLC-MS technique, we quantified total GAG abundance and sulfation characteristics, with statistical analyses performed in R and Python. Our results showed that CS abundance doubled in tumor regions, while HS levels remained unchanged, though both GAGs exhibited increased sulfation in tumors. Notably, adenocarcinomas displayed a higher 6-O-/4-O-sulfation ratio for CS, and O-sulfated HS components were elevated. Comparisons across lung cancer phenotypes revealed less striking differences than those between tumor and adjacent tissues. In adenocarcinomas with EGFR, KRAS, or ALK mutations, disaccharide patterns were largely similar to wild-type, except for reduced 4-O-sulfated D0a4 in EGFR-mutant samples. Principal component analysis confirmed minimal separation between genetic subgroups, suggesting that GAG alterations primarily distinguish tumor from adjacent normal tissues rather than cancer subtypes or genotypes.

NEURONAL DNA REPAIR IN HUNTINGTON'S DISEASE

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Neurological disorders, which represent a significant social and economic burden, are among the leading causes of premature death. Neurons are the longest-living cells in our body, constantly being challenged by different forms of stress, including DNA damage. Huntington's disease (HD) is characterized by motor and cognitive decline due to neurodegeneration, primarily affecting medium spiny projection neurons (MSNs) in the striatum and glutamatergic pyramidal cells in the cerebral cortex. The underlying cause of HD is the somatic CAG trinucleotide (Gln) repeat expansion in the huntingtin gene, resulting in the production of toxic mutant huntingtin protein. These glutamine repeats are expressed in terminally differentiated neurons and leading to protein misfolding, synaptic failure, disrupted proteostasis and neuronal cell death. While numerous studies have linked improper DNA repair processes to trinucleotide expansions, the exact underlying molecular mechanisms remain unknown.

The aim of our research is to identify the pathological DNA repair processes that contribute to the expansion of CAG repeats specifically in neurons affected by Huntington's disease. Since current HD treatments only alleviate symptoms, new strategies must target the molecular mechanisms underlying disease progression. CAG repeat expansion represents a promising therapeutic target. By elucidating the role of DNA repair in repeat expansion, our insights may uncover pathways driving HD pathology and explain neuronal subtype vulnerability.

Specifically, we aim to investigate defects in neuronal DNA repair processes through proteomics and genomics analysis, using human iPSC-derived neurons from HD patients versus healthy controls. We have established novel protocols for neuronal differentiation from human iPSCs. HD-relevant cortical glutamatergic neurons can be generated within 20 days through NGN2 induction combined with small molecules, while medium spiny neurons (MSNs) can be produced in a comparable timeframe via the timely expression of ASCL1, DLX2, and CTIP2 using an integrating inducible vector developed in our laboratory. Neuronal subtyping was performed by using various markers in Western blot and immunocytochemistry. Compared to conventional protocols requiring over 50 days, our method offers a significant acceleration and scalability of MSN differentiation, both necessary for cellular proteomics and genomics.