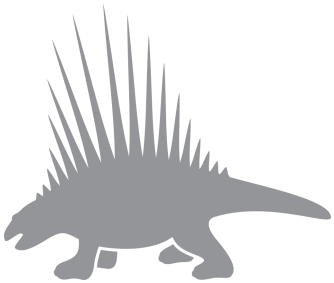

THIRTEENTH ANNUAL CONFERENCE OF THE CZECH SOCIETY FOR MASS SPECTROMETRY

together with

ELEVENTH INFORMAL PROTEOMIC MEETING

(joint meeting)

České Budějovice, November 19 – November 21, 2025

BOOK OF ABSTRACTS

Book of Abstracts from the Thirteenth Annual Conference of the Czech Society for Mass Spectrometry and Eleventh Informal Proteomic Meeting (joint meeting)

Czech Society for Mass Spectrometry

Proteomic section of Czech Society for Biochemistry and Molecular Biology

České Budějovice 2025

Book of Abstracts from the Thirteenth Annual Conference of the Czech Society for Mass Spectrometry and Eleventh Informal Proteomic Meeting (joint meeting)

Authors

Collective authorship

Published

November 2025, first edition

Publisher

Česká společnost pro hmotnostní spektrometrii

Přírodovědecká fakulta, Univerzita Palackého

17. listopadu 1192/12

771 46 Olomouc

Czech Republic

www.czechms.org

ISBN 978-80-907478-5-2

Jihočeský kraj
Tento projekt je podpořen z rozpočtu Jihočeského kraje

České Budějovice
Tento projekt je spolufinancován statutárním městem České Budějovice

Important portion of the financial support needed to organize the conference was kindly provided by the government of the South Bohemian Region and by the city of České Budějovice

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Thirteenth Annual Conference of the Czech Society for Mass Spectrometry
and
Eleventh Informal Proteomic Meeting
(joint meeting)

Date

19th November – 21st November 2025

Venue

Conference hall of the South Bohemian University

Studentská 1410/23

České Budějovice

Czech Republic

Organizer

Czech Society for Mass Spectrometry, Olomouc

Czech Society for Biochemistry and Molecular Biology, z. s., Proteomic section, Prague

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Wednesday 19th November 2025

10:00 – 18:00	Registration
12:45 – 13:45	Lunch – Canteen, Biology Centre CAS
13:45 – 14:00	Conference opening
14:00 – 15:00	**Plenary lecture I: Prof. Alexander Makarov** (Chairperson: Zdeněk Kukačka)
PL-01	*Past, present and future of Orbitrap Mass Spectrometry*
15:00 – 16:00	**Session I** (Chairperson: Michael Volný)
15:00 – 15:20	Elliott James Price
WeO-01	*Towards MS-guided Precision Environmental Health: the Czech node of the European Research Infrastructure for Human Exposome Research*
15:20 – 15:40	Urban Jiří
WeO-02	*Optimization of microflow LC-MS method by design of experiments and its application to proteomics analysis*
15:40 – 16:00	Starovoit Mykyta
WeO-03	*Simultaneous mapping of peptides and released N-glycans by HILIC-FLD-MS for quality control of protein biopharmaceuticals*
16:00 – 16:30	Coffee break
16:30 – 16:50	Sponsor Lecture – Phenomenex Lucia Geis-Asteggiante: Advancing Nano/Micro LC Proteomics with Core-Shell Technology

16:50 – 18:10	**Session II** (Chairperson: Jana Havlíková)
16:50 – 17:10	Melikov Aleksandr
WeO-04	*G-quadruplex – ligand interaction biophysics explored by native mass spectrometry and trapped ion mobility spectrometry*
17:10 – 17:30	Smrčka Tomáš
WeO-05	*Insights into CyRepA2 primase-helicase-DNA interactions using structural mass spectrometry*
17:30 – 17:50	Hájek Jan
WeO-06	*Breaking the Unbreakable: Structural Elucidation of the Hypermodified RiPP Nostatin A by IRMPD Mass Spectrometry*
17:50 – 18:10	Portašiková Jasmína
WeO-07	*Unraveling Oligomerization-Mediated Inhibition in GGPPS by Structural Mass Spectrometry*
18:10 – 18:30	Sponsor Lecture – GeneTiCA A Multi-Approach Exploration of the Proteome: Integrating Three Platforms
18:30 – 19:00	**Flash talks I** (Chairperson: Zdeněk Spáčil)
WeS-01	Hebesberger Philipp: *Quantification of Oxoacids of the Human Metabolome using Liquid Chromatography-Mass Spectrometry*
WeS-02	Škeříková Veronika: *Analyses of human scent samples*
WeS-03	Grepl Jakub: *rDUVLAESCI-MS/MSI: a novel approach for direct analysis of biological surfaces*
WeS-04	Jágr Michal: *Investigation of phenolic compounds in grape pomace through UHPLC-ESI-MS/MS*
WeS-05	Brigante Federico: *Mass Spectral Library Network (MERLIN) To Streamline The Generation Of Public Spectral Libraries*
WeS-06	Mazumdar Aninda: *A Comprehensive timsTOF MS/MS Spectral Library for Improving Metabolite Annotation in Non-Targeted Metabolomics*
19:00 – 22:00	Welcome Reception & Dinner (Canteen, live music from 19:30)

Thursday 20th November, 2025

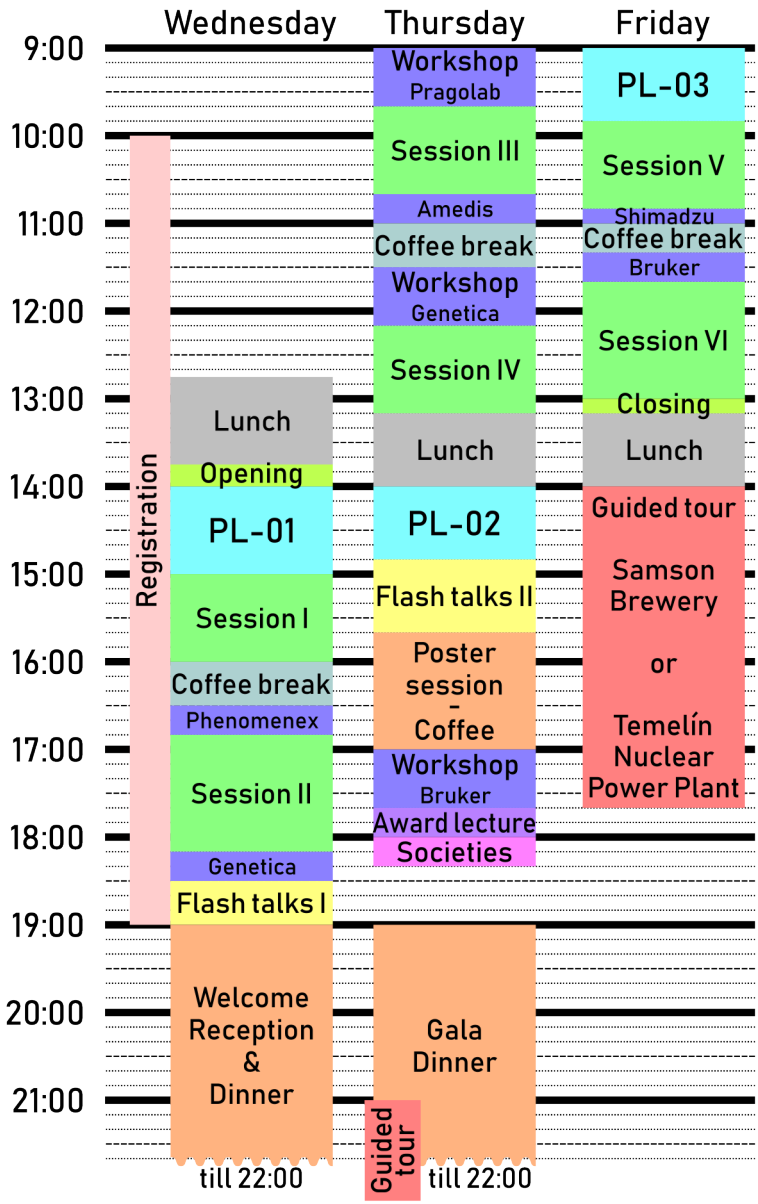
09:00 – 09:40	Workshop – Pragolab
09:40 – 10:40	**Session III** (Chairperson: Tomáš Oždian)
09:40 – 10:00	Talacko Pavel
ThO-08	*Low-input proteomics using the Orbitrap Astral*
10:00 – 10:20	Bouchal Pavel
ThO-09	*Integrated multiomics classification of triple negative breast cancer*
10:20 – 10:40	Konik Peter
ThO-10	*Proteomics of human breast milk*
10:40 – 11:00	Sponsor Lecture – Amedis Tomáš Korba: SCIEX ZenoTOF 8600 – Sensitivity, Speed, Flexibility, Robustness
11:00 – 11:30	Coffee break
11:30 – 12:10	Workshop – GeneTiCA
12:10 – 13:10	**Session IV** (Chairperson: Marek Vrbacký)
12:10 – 12:30	Černý Martin
ThO-11	*Development-dependent molecular strategies of stress adaptation in plants*
12:30 – 12:50	Danchenko Maksym
ThO-12	*Deciphering adaptive shifts in the redox proteome of wheat seedlings under drought*
12:50 – 13:10	Lochmanová Gabriela
ThO-13	*No Mark, No Problem: Alternative Epigenetic Silencing in Algae*
13:10 – 14:00	Lunch

14:00 – 14:50	**Plenary lecture II: Prof. Bernhard Küster** (Chairperson: Juraj Lenčo)
PL-02	*Proteomics advancing pharmacology*
14:50 – 15:40	**Flash talks II** (Chairperson: Petr Novák)
ThS-07	Lenčo Juraj: *Propionic acid boosts sensitivity in high-flow RPLC-MS proteomics compared to formic and acetic acid*
ThS-08	Oždian Tomáš: *Towards Biomarker Discovery: Multiomics analysis of Endometriosis*
ThS-09	Šimoník Jan: *Integrated proteogenomic analysis identifies synuclein gamma as a key target of the mesenchymal stem-like subtype of triple negative breast cancer*
ThS-10	Vít Ondřej: *Human IMPs: A tool for comparing outputs of different tools for transmembrane segment predictions in the human proteome*
ThS-11	Lapčík Petr: *LC-MS/MS-based terminomics unravels role of carboxypeptidase B1 in luminal A breast cancer*
ThS-12	Sysel Jakub: *Mass spectometry in hands of experimental biology: Impaired nucleotide salvage affects de novo synthesis in Drosophila melanogaster*
ThS-13	Hinterholzová Helena: *Bioaccumulation of pharmaceuticals in herbivorous fish: The role of aquatic plants as a dietary source*
ThS-14	Koudelka Štěpán: *LC-HRMS for large-scale chemical exposure studies*
15:40 – 17:00	Poster session + Coffee Break
17:00 – 17:40	Workshop – Bruker Gary Kruppa: Recent developments in proteomics instrument at Bruker: timsTOF AIP and timsOmni for ultra high sensitivity proteomics, PTM analysis and structural proteomics Daniel Vláčil: timsMetabo™ Breakthrough 4D-Metabolomics and 4D-Lipidomics Sensitivity, Specificity and Annotation Confidence – at Speed, Depth, and Scale
17:40 – 18:00	**Award Lecture – Josef Chmelík Prize Laureate** (Chairperson: Pavel Bouchal)
18:00 – 18:20	General Assembly of the Czech Society for Mass Spectrometry (in Czech, lecture room) Informal meeting of Proteomic section of CSBMB (in Czech or English, poster room)
19:00 – 22:00	Gala Dinner – Restaurace Solnice
21:00 – 22:00	Optional guided Walk-through historic center of České Budějovice – start at restaurant Solnice

Friday 21st November, 2025

09:00 – 09:50	**Plenary lecture III: Prof. Jana Roithová** (Chairperson: Anton Škríba)
PL-03	*Mass Spectrometry access to quantitative solution kinetics*
09:50 – 10:50	**Session V** (Chairperson: Kristýna Gloc-Pimková)
09:50 – 10:10	Grabicová Kateřina
FrO-15	*Per- and polyfluorinated compounds (PFAS) in wild common bream*
10:10 – 10:30	Kučera Lukáš
FrO-16	*Spatial mapping of 15N-labeled ammonia with MALDI-FTICR imaging in regenerating liver tissue: Re-directing of toxic byproduct into anabolic pathways*
10:30 – 10:50	Čechová Aneta
FrO-17	*LC-ICP-MS analysis of anti-obesity peptides and their metabolites labeled with lanthanide mass-tag*
10:50 – 11:00	Sponsor Lecture – Shimadzu David Maxa: Advancing Structural Elucidation with Oxygen Attachment Dissociation (OAD)
11:00 – 11:20	Coffee break
11:20 – 11:40	Sponsor Lecture – Bruker Gary Kruppa: Emerging applications of bottom up proteomics in clinical research

11:40 – 13:00	**Session VI** (Chairperson: Volodymyr Pauk)
11:40 – 12:00	Stefani Tommaso
FrO-18	*Advancing Gut Microbiome Metabolomics: A Derivatization-Based Approach*
12:00 – 12:20	Chalupský Karel
FrO-19	*Mass Spectrometry in Drug Development: From Screening to Stability and Pharmacokinetics*
12:20 – 12:40	Kyjaková Pavlína
FrO-20	*Mass spectrometry of human body odor: Signatures of psychophysiological states*
12:40 – 13:00	Coufalíková Kateřina
FrO-21	*Population-scale human chemical exposomics by automated liquid-liquid extraction coupled to gas chromatography – Orbitrap mass spectrometry*
13:00 – 13:10	Closing Remarks and Awards
13:10 – 14:00	Lunch
14:00 – 17:40	Guided Tour – Samson Brewery / Temelín Nuclear Power Plant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Registration
Lunch
Opening
PL-01
Session I
Coffee break
Phenomenex
Session II
Genetica
Flash talks I
Welcome Reception & Dinner
till 22:00
Workshop
Pragolab
Session III
Amedis
Coffee break
Workshop
Genetica
Session IV
Lunch
PL-02
Flash talks II
Poster session - Coffee
Workshop
Bruker
Award lecture
Societies
Guided tour
Gala Dinner
till 22:00
PL-03
Session V
Shimadzu
Coffee break
Bruker
Session VI
Closing
Lunch
Guided tour
Samson Brewery
or
Temelín Nuclear Power Plant

PL-01: Past, present and future of Orbitrap Mass Spectrometry

Alexander Makarov [1 *]

1. Thermo Fisher Scientific GmbH

Since its commercial debut two decades ago, the utility of the Orbitrap™ analyzer has been continuously extended by additional capabilities such as quantitative analysis, new fragmentation methods, diverse vacuum and ambient ion sources, imaging, ion mobility and unprecedented extension of mass range. These enhancements evolved over multiple major families of Orbitrap-based instruments that became a real workhorse of many applications.

As the latest example of such evolution, the asymmetric track lossless (Astral™) analyzer is combining together Orbitrap and time-of-flight features to dramatically improve sensitivity and throughput of proteomic analysis. Performance and operation of this newest analyzer are described in detail, especially for single-cell proteomic experiments.

An overview of main trends in evolution of mass spectrometry instrumentation demonstrates ample reserves in further expansion of analytical capabilities, mainly via combining advanced analyzers with improved ion scheduling and utilization.

The talk concludes with the belief that the latest rapid progress in all aspects of instrumentation and analysis will enable mass spectrometry to remain competitive against alternative technologies and to rise to the challenge of unprecedented throughput of very deep analyses of very complex samples.

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PL-02: Proteomics advancing pharmacology

Bernhard Küster [1 *]

1. Technical University of Munich

This talk will focus on recent chemical proteomics work from my laboratory aiming to advance mechanism-centric pharmacology by merging unbiased target engagement with global proteome response measurements, all in a full dose-dependent manner. Building on dose-dependency as a unifying concept, we established strategies that decode compound action beyond single targets, pathways and transcriptional programs. The decrypt, decryptM and decryptE frameworks align concentration series with proteome-wide responses to infer direct binding, pathway engagement, and adaptive rewiring, enabling mechanism-of-action inference and biomarker nomination in a single experiment. Applying these paradigms to a pharmacologically rich space, we mapped the target landscape of ~1,000 kinase inhibitors, revealing pervasive polypharmacology, unexpected lipid and metabolic kinase engagement, and chemotypes with privileged selectivity patterns. These resources can rationalize phenotypic effects and guide repurposing and lead optimization campaigns. Complementary studies extended the approach to epigenetic modulators, exemplified by quantitative proteomic dissection of lysine deacetylase inhibitors that connected target engagement to dose-dependent proteome remodeling and functional outcomes. Together, these advances operationalize chemical proteomics as an engine for systems pharmacology: they provide scalable, label-free measurements of drug–protein interactions, capture on- and off-targets alongside downstream consequences, and yield actionable maps that bridge medicinal chemistry to cellular physiology. Open data and software from these studies furnish community benchmarks for selectivity modeling, target deconvolution, and network-aware drug design. Looking ahead, integration with high-throughput sample preparation, ultra-fast LC.MS/MS and AI-driven analysis promise routine profiling large compound libraries and anticipatory modeling of drug responses across biological contexts including cancer patients.

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PL-03: Mass Spectrometry access to quantitative solution kinetics

Jana Roithová [1 *]

1. Radbound University

Mass spectrometry is an established tool in reaction monitoring and studying reaction intermediates.[1] The nonlinear ionization response and, thus, the inability to obtain quantitative information about concentrations and kinetics partly cast a shadow on the advantage of the great sensitivity and large dynamic range of mass spectrometry in mechanistic research. To access the quantitative kinetic information about reactions in solution, we have been developing the delayed reactant labeling method.[2] In the lecture, I will introduce the method, explain possible applications, and show how we used the method to study equilibria in solution,[3] solve reaction mechanisms, and predict the reaction selectivity.[4]

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WeO-01: Towards MS-guided Precision Environmental Health: the Czech node of the European Research Infrastructure for Human Exposome Research

Elliott James Price [1,2] *, Jana Klánová [1,2]

1. RECETOX, Facutly of Science, Masaryk University, Brno, Czechia
2. EIRENE-CZ, Czech node of the European Infrastructure for Human Exposome Research

Understanding the molecular determinants of health and disease is essential for advancing precision prevention and treatment. Yet, the current availability of high-throughput assays for quantifying broad panels of clinically relevant proteins, metabolites, and environmental chemicals remains limited. The European Infrastructure for Human Exposome Research (EIRENE) is addressing this challenge by building a harmonized network of laboratories, population cohorts, environmental studies, and databases to elucidate how environmental exposures contribute to chronic disease. This presentation introduces the mass spectrometry (MS) resources developed within the Czech node of EIRENE (EIRENE-CZ). These resources are designed to enable: (i) population-scale profiling of human exposure to environmental chemicals, (ii) quantitative analysis of clinically relevant protein and metabolite biomarkers, and (iii) high-throughput processing of MS data.

A suite of targeted MS assays has been established for the absolute quantification of biomarkers defined at the intersection of the Hallmarks of Health and Hallmarks of Environmental Insults frameworks. We shall report on applications to clinical research studies, evaluation compared to alternative clinical measurement approaches, and welcome input to prioritise key biomarkers and guide future assay development.

In parallel, LC- and GC-HRMS workflows for population-scale chemical exposure profiling have been implemented, and ongoing applications in cohort studies will be highlighted. We will reflect on current technological limitations and prospects for an international precision environmental health monitoring network.

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5. *https://doi.org/10.1016/j.isci.2022.103976*
6. *https://doi.org/10.1016/j.envint.2024.108585*

ACKNOWLEDGEMENT:

The research has been conducted within the European Infrastructure for Human Exposome Research (EIRENE RI), in cooperation with the Network of Exposomics in the US (NEXUS) and within the collaborative framework of the International Human Exposome Network (IHEN; HEU programme grant agreement 101137317). The authors acknowledge the EU's H2020 grant agreements 857560 (CETOCOEN Excellence); the HEU grant agreements 101079789 (EIRENE PPP); and the RECETOX Research Infrastructure (LM2023069) financed by the MEYS (CZ.02.1.01/0.0/0.0/17_043/0009632). The views expressed are those of the author(s) and do not necessarily reflect those of the EU or REA.

WeO-02: Optimization of microflow LC-MS method by design of experiments and its application to proteomics analysis

Jiří Urban [1 *], Jan Valášek [1], Antonín Bednařík [1], Martina Nechvátalová [1], Jan Preisler [1]

1. Masaryk University, Faculty of Science

Due to the reduced radial dilution of analytes, liquid chromatography-mass spectrometry (LC-MS) utilizing narrow columns with an internal diameter of 50 – 75 µm is almost exclusively used in ultra-trace proteomics analysis of limited sample amounts. Micro-flow LC-MS employing columns with an inner diameter of 1 mm is a viable alternative in cases where the sample amount is not limited. It offers robust qualitative and quantitative performance characteristics, simplicity of implementation, and an extensive range of high-quality micro-flow columns. Additionally, micro-column LC analysis is readily applicable to routine clinical applications.

In this work, we have employed the design of experiments protocol to optimize the micro-flow LC-MS method and to investigate the primary factors controlling its sensitivity in bottom-up proteomics analysis.

We divided the optimization process into several consecutive steps planned by Box-Behnken designs. Signal intensity and the number of identified peptides were all used as optimization criteria. In liquid chromatography, we tested the effect of mobile phase flow rate, column temperature, and gradient time. In electrospray coupling, we optimized the position of the capillary, its temperature, voltage, and the flow of sheath, auxiliary, and sweep gases. Finally, we focused on optimizing the ion injection time, automatic gain control target, and Orbitrap analyzer resolution utilizing full MS and MS/MS scans. During the optimization process, the signal intensity and number of identified peptides increased by a factor of four and two and a half times, respectively. After optimizing with a semi-complex proteomic sample, we analyzed the real-life sample and compared the obtained results with those in the literature.

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ACKNOWLEDGEMENT:

Financial support by the Czech Science Foundation project 23-07581S is gratefully acknowledged.

WeO-03: Simultaneous mapping of peptides and released N-glycans by HILIC-FLD-MS for quality control of protein biopharmaceuticals

Mykyta R. Starovoit [1], Juraj Lenčo [1 *]

1. Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University

Protein biopharmaceuticals, especially monoclonal antibodies, have revolutionized therapeutic regimes. However, their structural complexity presents analytical challenges. Modifications such as glycosylation and deamidation affect efficacy and immunogenicity, demanding precise monitoring of critical quality attributes (CQAs). Current multi-attribute methods, based on reversed-phase (RP) LC-MS of tryptic peptides, struggle with hydrophilic peptides and show limited resolution of deamidated, isoAsp-containing, and glycosylated peptides. Glycosylation assessment often requires separate workflows with enzymatic release, fluorescent labeling, and HILIC-FLD analysis. We propose a UHPLC method that combines the mapping of tryptic peptides with released, RapiFluor-MS-labeled N-glycans in a single HILIC-FLD-MS run, utilizing convenient sequential injection of peptide and glycan fractions. The approach separates peptides in the initial part of the chromatogram, followed by glycans in later retention windows, with fluorescence labeling enhancing glycan detection. Coupled with tandem mass spectrometry, this approach provides full protein sequence coverage and superior resolution of isomeric glycoforms, deamidated peptides, and isoAsp residues versus RPLC workflows. Analysis of released, labeled glycans further outperforms glycopeptide separation, both by RPLC and HILIC, in preserving terminal sialic acids that are prone to in-column cleavage at acidic pH and high column temperatures.

This integrated HILIC workflow could simplify biopharmaceutical quality control by enabling the simultaneous monitoring of multiple CQAs, which now require distinct methods, thereby improving efficiency, reliability, and the cost per sample.

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ACKNOWLEDGEMENT:

This study was supported by the project of the Charles University Grant Agency (GA UK, No. 356325), the SVV Project No. 260782, and the ATEBIO project (Advanced Techniques for Biomedical Diagnostics, Reg. No. CZ.02.01.01/00/23_020/0008535) co-funded by the European Union.

WeO-04: G-quadruplex – ligand interaction biophysics explored by native mass spectrometry and trapped ion mobility spectrometry

Aleksandr Melikov [1 *], Frédéric Rosu [1], Anton Granzhan [1], Valérie Gabelica [1]

1. University of Geneva

Guanine-rich nucleic acid sequences are able to form G-quadruplexes (G4), both in vivo and in vitro. Due to their involvement in many important biological processes, such as gene expression and telomere maintenance, G-quadruplexes became a prominent target for anticancer therapy by small G4-binding molecules, or G4 ligands. Mass spectrometry combined with electrospray ionization source (ESI-MS) provides a great tool to relatively quickly asses the in-solution binding properties of G4 ligands, as was determined by previous studies . Coupling ESI-MS with the trapped ion mobility (timsTOF SCP) and the in-house made variable-temperature nano-electrospray source (vt-nESI) allows to obtain a detailed biophysical characterization of G4-ligand interactions.

In native conditions (room temperature), all the tested compounds showed very high affinity for parallel folds (both intra and intermolecular), as well as telomeric variants. For the telomeric hybrid topologies, the displacement of 1 of the 2 ammonium cations initially complexed by the G4 by the ligand suggests a similar binding mode as PhenDC3, that is, intercalation between G-quartets. The melting experiments conducted with the vt-nESI source allowed us to monitor temperature-dependent G4 unfolding, extrapolate binding constants to room temperature, as well as to estimate thermodynamic parameters of ligand binding by van't Hoff equation fit. In addition, the effect of coordinated cation (ammonium vs potassium) and the annealing of G4 in the temperature-controlled environment was tackled by these techniques for several human telomeric sequence variants. Future work will be devoted to the influence of ligands on protein binding to G-quadruplexes.

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ACKNOWLEDGEMENT:

We would like to acknowledge Bruker Daltonics for provided instrument upgrades and University of Geneva for funding this research.

WeO-05: Insights into CyRepA2 primase-helicase-DNA interactions using structural mass spectrometry

Tomáš Smrčka [1,2], Paulina Duhita Anindita [3], Jasmína Mária Portašíková [1,2], Roman Sobotka [3], Alan Kádek [1], Petr Man [1 *]

1. Mikrobiologický ústav AV ČR, v.v.i.
2. Přírodovědecká fakulta, Univerzita Karlova v Praze
3. Centrum Algatech, Mikrobiologický ústav AV ČR, v.v.i.

Native mass spectrometry and H/D exchange are well-established structural mass spectrometry methods. Here we show how their combination could provide a complementary information of a dynamic protein-DNA interaction. Specifically, we examined the DNA binding of cyanobacterial primase-helicase CyRepA2.

Using native mass spectrometry, the oligomeric state and binding modes of CyRepA2 was examined in complex with several DNA ligands. With H/D exchange, the sites of DNA-induced structural changes were identified and a primase/helicase domain interface was probed. Furthermore, the role of ATP and AMP-PNP binding was also extensively studied providing insights into the dynamic nature of the enzyme's function.

Overall, our work proves how the native mass spectrometry data together with the H/D exchange data nicely complement each other and together provide a comprehensive structural and functional insight into a highly dynamic protein complex.

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WeO-06: Breaking the Unbreakable: Structural Elucidation of the Hypermodified RiPP Nostatin A by IRMPD Mass Spectrometry

Jan Hájek [1 *]

1. Mikrobiologický ústav AVČR, centrum Algatech Třeboň

Ribosomally synthesized and post-translationally modified peptides (RiPPs) represent a diverse and biologically significant class of natural products. However, their extensive post-translational modifications often pose major challenges for structural elucidation. Nostatin A (NosA, ~2.5 kDa) is a highly modified RiPP belonging to the nitrile hydratase–like leader peptide family (proteusins). Its 30-amino-acid core undergoes extensive structural modification, including the formation of nine thiazole/oxazole rings, eight dehydroalanine/dehydrothreonine residues, and an additional unique sactipeptide bridge. These modifications result in an extremely rigid and compact structure that resists conventional ESI ionization and collision-induced dissociation. Furthermore, NMR spectroscopy failed to provide sufficient data for complete structural elucidation. To overcome these limitations, infrared multiphoton dissociation (IRMPD) spectroscopy was employed, enabling controlled fragmentation and the observation of interpretable fragments across the m/z range of 200–2000. Interestingly, NosA predominantly fragmented into stable tripeptidic subunits composed of amino acid–oxa/thiazole–Dha/Dhb motifs, which resisted further cleavage. A novel approach combining empirical formula analysis of these smaller fragments allowed the determination of the complete molecular formula of Nostatin A.

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ACKNOWLEDGEMENT:

The authors gratefully acknowledge Alan Kádek and the BIOCEV research center for performing the IRMPD measurements on the SolariX FT-ICR mass spectrometer

WeO-07: Unraveling Oligomerization-Mediated Inhibition in GGPPS by Structural Mass Spectrometry

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Geranylgeranyl diphosphate synthase (GGPPS) is a trans-prenyltransferase that catalyzes the production of the geranylgeranyl moiety in living organisms. In cells, the geranylgeranyl group can be attached to proteins, such as Rab or Rho proteins, enabling their interaction with membranes. Dysfunction in protein prenylation associated with GGPPS mutations has been proven responsible for the development of multiple myeloma and other malignant transformations. Therefore, GGPPS is a valuable target for anti-cancer drugs.

Oligomeric assemblies of GGPPS vary across organisms. In lower-level species, GGPPS assembles in a dimeric organization, whereas in higher-level species, such as humans, GGPPS forms a hexamer composed of three dimers. The inter-dimeric interaction is facilitated by hydrophobic interactions and hydrogen bonds.

In the past, the R235C mutation was discovered in human GGPPS of multiple myeloma patients. This mutation is localized to the active site lid region at the inter-dimeric interface. Using native MS experiments, we uncovered lower stability of the hexameric organization due to destabilized inter-dimeric interactions in the R235C mutant. Subsequently, we performed HDX-MS experiments, which showed reduced lid dynamics in the wild-type compared to the R235C mutant, thereby blocking the pathway for product release. These results indicate that faster product release in the R235C mutant leads to higher catalytic activity of the mutant protein due to decreased product inhibition. Additionally, experiments on the dimeric Y246D mutant showed even faster product release than the R235C mutant. Therefore, we conclude that these functional alterations are directly influenced by the oligomeric assembly of the protein.

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ACKNOWLEDGEMENT:

Financial support from the MEYS/EU OP JAK project PHOTOMACHINES - CZ.02.01.01/00/22_008/0004624 is gratefully acknowledged.

ThO-08: Low-input proteomics using the Orbitrap Astral

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Proteomics has undergone a significant advancement in recent years, both in terms of sensitivity and the number of identified proteins. This progress has been driven by the widespread implementation of data-independent acquisition (DIA) and the emergence of new generations of mass spectrometers. The Orbitrap Astral represents a recent innovation within the Orbitrap platform, featuring a novel Astral analyzer characterized by exceptionally high sensitivity, rapid acquisition speed, and high resolving power. The introduction of this technology has markedly expanded the limits of detection in proteomic analyses, enabling the characterization of low-abundance protein populations that frequently play crucial roles in diverse cellular processes. Consequently, it substantially reduces the amount of sample material required for comprehensive analysis, which is particularly advantageous in single-cell proteomics.

At our facility, we have optimized a workflow for the analysis of sub-nanogram sample quantities, based on nano-flow HPLC coupled to the Orbitrap Astral mass spectrometer. Under these conditions, we are able to identify over 4,300 proteins from as little as 125 pg of cellular lysate, making single-cell proteomics experiments accessible to our users.

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ThO-09: Integrated multiomics classification of triple negative breast cancer

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Triple-negative breast cancer (TNBC) is the most aggressive and heterogeneous BC subtype [1] primarily treated with systemic chemotherapy. To identify novel therapeutic targets, we performed a multiomics study using LC-diaPASEF-MS/MS-based proteomics, RNA sequencing, and whole-exome sequencing, generating an integrated dataset for 96 samples. A hybrid proteomics assay library contained 244,464 precursors and 11,564 protein groups (FDR=1%) [2]. Hierarchical clustering utilizing the proteomics data for 1,223 mostly correlated transcript–protein pairs stratified TNBC into 7 clusters designated as (i) mesenchymal stem like, (ii) basal/mesenchymal, (iii) cell cycle, (iv) immunomodulatory, (v) luminal androgen receptor, (vi) ER+metabolism and (vii) immunomodulatory+cell cycle. Among 225001 germinal mutations identified, high impact ones included those in known tumor suppressor genes (BRCA1, BRCA2, BARD1, BRIP1, PALB2, and CHEK1), distributed mainly in Clusters 7 and 2. Out of 12070 somatic mutations, TP53 was the mostly mutated gene (in 67.71%), with the highest frequency in cluster 7 (86.36 %, p=0.038). Protein quantitative trait locus (pQTL) analysis identified gene variants asssociated with changes in protein levels. Analysis of proteomics data using alteredPQR tool assigned alterations of protein complexes involved in hormonal signaling and lipid metabolism to cluster 5, immune response and interferon signaling to clusters 4 and 7, extracellular matrix organization to cluster 2, cell cycle regulation and DNA repair to cluster 3, and protein phosphorylation and fibrinolysis to cluster 6. In a summary, our study represents the most complete proteomics-driven multiomics study of the set of TNBC tissues, providing highly relevant molecular classification of the patients.

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ACKNOWLEDGEMENT:

This work was supported by Ministry of Health of the Czech Republic (project NU22-08-00230), all rights reserved. Supported by the project National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102)—Funded by the European Union—Next Generation EU.

ThO-10: Proteomics of human breast milk

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Breast milk is a complex mixture of proteins, fats, carbohydrates, minerals, vitamins, ions and hormones produced by the mammary glands of mammals, including humans. The dominant protein of breast milk is casein, the main “nutritional” protein; less abundant are alpha lactalbumin that regulates lactose production; lactoferin, an iron transporter; lysosyme, an antibacterial enzyme; and secretory immunoglobulins, aiding the newborn’s immunity. In the early stages of development, it is the sole source of nutrients for the newborn. In cases, where mother’s milk is not available, such as pre-term births, there is a need for breast milk supplementation or usage of donated milk from other mothers. Donated milk can be stored frozen for up to 3 months, however, milk quality, stability and sterility is a concern. In this study, we investigated protein composition of breast milk that has been sterilised by heat (pasteurisation) or pressure (pascalisation). The preliminary results show that there are no significant differences between the sterilisation methods regarding protein content and quantity, however, they suggest some changes in structure in some less abundant proteins.

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ThO-11: Development-dependent molecular strategies of stress adaptation in plants

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Understanding how plants navigate environmental adversity requires peering into the molecular choreography of stress adaptation, a view now achievable through mass spectrometry-based omics. By integrating proteomics, lipidomics, and metabolomics as complementary molecular lenses, we unveiled the hidden architecture of cold acclimation and drought resilience in Arabidopsis thaliana. Thousands of responsive proteins emerged from the data, revealing development-dependent survival strategies in which tissue age plays a decisive role in shaping stress mitigation and resilience. Young and aging leaves express distinct molecular dialects under stress, suggesting an intergenerational compromise within the plant body. In total, 2,064 early-response proteins were identified during cold treatment, reflecting extensive proteome reprogramming that underpins acclimation. Under drought conditions, genotypes with contrasting resilience revealed age-dependent molecular mechanisms that bolster plant survival, identifying several hundred candidate targets for enhancing drought tolerance. This omics-driven narrative reframes plant stress biology—from static gene inventories to dynamic molecular ecosystems—charting pathways toward crops engineered not merely to survive, but to thrive under environmental duress.

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ACKNOWLEDGEMENT:

Funding for this study was provided by the Czech Science Foundation (grant number 23-07376S)

ThO-12: DECIPHERING ADAPTIVE SHIFTS IN THE REDOX PROTEOME OF WHEAT SEEDLINGS UNDER DROUGHT

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Global warming increases the frequency of extreme drought events. Limited water availability for crops negatively affects yields in many regions of the planet, including Europe, thereby compromising food security. Bread wheat (Triticum aestivum) is vital for human nutrition. Exploring responses of contrasting plant genotypes enables a complex understanding of phenotypes challenged by drought and, eventually, facilitates the design of resilient crops.

Drought affects crops on molecular, biochemical, physiological, and morphological levels. Moreover, plant responses to water shortage stress are frequently associated with posttranslational modifications (PTMs) of versatile proteins. Such modifications, including reversible oxidation of cysteine thiols (redox), regulate protein functions: Activity, stability, subcellular localization, and signal perception/transduction. Redox proteome dynamics in plant leaves under stress conditions is a novel emerging field of study.

The redox state of protein cysteines regulates plant resilience during water shortage and recovery. We explore the variable impact of water deprivation and recovery on the redox proteome dynamics in leaves of wheat cultivars contrasting in drought tolerance using resin-assisted capture. The study started elucidating drought- and recovery-responsive components of the wheat redox proteome, yielding candidate markers and pathways of resilience, supported by the spatial distribution of reactive oxygen species (ROS) and expression dynamics of redox-regulating genes, thereby linking biochemical, cellular, and molecular changes. New knowledge will facilitate maintaining a stable yield under an extreme climate.

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ACKNOWLEDGEMENT:

This study was supported by the Slovak Research and Development Agency grant APVV-23-0407 and the Slovak Academy of Sciences project National Academy of Sciences project VEGA 2/0106/22.

ThO-13: No Mark, No Problem: Alternative Epigenetic Silencing in Algae

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Precise timing of gene expression is crucial for transitions between developmental stages in eukaryotic organisms. Polycomb Repressive Complexes (PRCs) play a key role in specifying cell identity and act as central epigenetic regulators in multicellular development. Among them, PRC2 is a multi-subunit histone methyltransferase that catalyzes the trimethylation of lysine 27 on histone H3 (H3K27me3), a conserved mark of facultative heterochromatin. In plants, PRC2 loss leads to severe developmental defects, while in humans, its dysregulation is linked to various diseases, including cancer, neurodegenerative disorders, and developmental defects. These observations support the long-standing view that PRC2 is both essential and evolutionarily conserved across eukaryotes.

However, our recent findings challenge this assumption. Using mass spectrometry-based proteomics—including data-dependent acquisition and parallel reaction monitoring—we examined post-translational modifications in histone extracts and pull-down samples of selected algal species. Surprisingly, H3K27me3 was not detected in certain algae, suggesting the existence of alternative mechanisms for facultative heterochromatin formation that differ from those in land plants.

These results reveal previously hidden evolutionary divergence in PRC2 composition and function, particularly within the diverse algal lineage, and raise new questions about the molecular mechanisms underlying epigenetic silencing in these species.

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ACKNOWLEDGEMENT:

This research was supported by Czech Science Foundation (project No. 25-17578S). CIISB, Instruct-CZ Centre of Instruct-ERIC EU consortium, funded by MEYS CR infrastructure project LM2023042 and European Regional Development Fund-Project "Innovation of Czech Infrastructure for Integrative Structural Biology "(No. CZ.02.01.01/00/23_015/0008175), is gratefully acknowledged for the financial support of the measurements at the CEITEC Proteomics Core Facility. Computational resources were provided by the e-INFRA CZ project (ID:90254), supported by MEYS CR.

FrO-15: Per- and polyfluorinated compounds (PFAS) in wild common bream

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Per- and polyfluorinated compounds (PFAS) are persistent and, therefore, ubiquitous in the aquatic environment. The new legislation at the European Union level (Commission Regulation (EU) 2022/2388) adjusts the concentration of four selected PFAS, namely perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA) and perfluorohexane sulfonic acid (PFHxS), in fish fillet used for human consumption. The sum of those four PFAS should be lower than 4 ng/g ww (wet weight). The study aimed to develop an analytical LC-HRMS method for monitoring the concentration of nearly 40 PFAS in wild common bream (Abramis brama) caught at ten localities in the Czech Republic. In the first step, a robust and multiresidual analytical method was developed to detect sub ng/g concentrations. In the fish fillet, 24 different PFAS were found at total concentrations up to 43 ng/g ww. Water reservoirs were more polluted than rivers. PFOS was the PFAS with the highest concentration. The limits of the four PFAS were exceeded at most of the sampled localities.

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ACKNOWLEDGEMENT:

This study was financially supported by the Ministry of Agriculture of the Czech Republic, project NAZV " Occurrence of perfluorinated and polyfluorinated compounds in the meat of fish farmed and wild in the Czech Republic" (No. QL25020015).

FrO-16: Spatial mapping of 15N-labeled ammonia with MALDI-FTICR imaging in regenerating liver tissue: Re-directing of toxic byproduct into anabolic pathways

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Liver is endowed with high regenerative activity, such that the tissue will regrow in the mouse after partial hepatectomy within days. We reasoned that this requires de novo pyrimidine synthesis to support rapid progression via the cell cycle. With MALDI imaging technique we revealed that suppression of de novo pyrimidine synthesis prevents proliferation in regenerating liver, suppressing liver regrowth. Tracing studies and spatial metabolomics revealed a metabolic shift such that ammonia, normally detoxified to urea in the periportal region under homeostasis, is redirected for generating aspartate and carbamoyl phosphate periportally, and glutamine pericentrally, and these products are utilized as precursors by the de novo pyrimidine synthesis pathway. This study uncovers a metabolic reprogramming leading to utilization of a toxic byproduct for anabolic pathways that are essential for liver regeneration.

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FrO-17: LC-ICP-MS analysis of anti-obesity peptides and their metabolites labeled with lanthanide mass-tag

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Neuropeptide prolactin-releasing peptide analogs (PrRP31) show promise for treating obesity and related diseases. Lipidization with fatty acids (palm^{11}-PrRP31) improves peptide stability and reduces food intake and body weight in rodents, but its mechanism remains unclear. To track palm^{11}-PrRP31 in vivo using the Wistar rats, we labeled it with a ClickZip lanthanide mass tag and quantified it using ICP-MS. The tag's exceptional stability allowed precise detection in biological tissues[1]. To properly identify the metabolites of the administered palm^{11}-PrRP31, we developed and validated a reversed-phase LC-ICP-MS method, overcoming the incompatibility of organic solvents with ICP-MS by using 1,2-hexanediole. The optimized method met the EURACHEM validation guidelines and enabled the reliable determination of ClickZip-tagged anti-obesity lipopeptide and its metabolites *in vivo*[2].

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FrO-18: Advancing Gut Microbiome Metabolomics: A Derivatization-Based Approach

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Microbiota-associated metabolites play a crucial role in host physiology and disease by mediating host-microbiota communication, providing essential nutrients, and regulating metabolism and immune functions. Understanding these interactions requires comprehensive chemical analysis of the metabolites. However, their low abundance, diverse physicochemical properties, and analysis in complex matrices present significant challenges, leaving us with no universal method to analyze them all. Chemical derivatization is a powerful approach, particularly for polar metabolites. Modifying the analyte structure decreases their polarity and makes them compatible with conventional reverse-phase liquid chromatography.

3-nitrophenylhydrazine (3NPH) is a widely used derivatization agent, which was previously employed in studies targeting well-known microbial metabolites such as Short Chain Fatty Acids (SCFA).[1] However, the potential of its applicability can be extended and go beyond to larger datasets, covering the majority of polar primary metabolites as well as known products of microbial metabolism.

Here, we present a large-scale analysis of nearly 600 gut microbiome-related chemical standards, derivatized using 3NPH. Standards were systematically classified based on structural similarities, biological relevance, and metabolic pathway associations using a Python-based automated categorization pipeline and public metabolomic databases. For streamlined identification, the recently introduced in-silico Derivatization Tool in MetaboScape by Bruker was employed for an automated targeted search.

To validate the applicability of this derivatization strategy, we utilized the library of derivatized standards for the targeted analysis of microbiota-associated metabolites in stool samples from mice with different statuses of microbial colonization, including germ-free models. Our results demonstrate the utility of this approach in providing valuable insights into host-microbiota metabolic interactions.

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FrO-19: Mass Spectrometry in Drug Development: From Screening to Stability and Pharmacokinetics

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The continuous emergence of new diseases, antimicrobial resistance, and unmet therapeutic needs highlight the urgent demand for novel drugs with improved safety and efficacy. Drug development is a complex and resource-intensive process requiring rapid, accurate, and sensitive analytical tools to guide decision-making from early discovery to clinical evaluation. Mass spectrometry (MS) has become a cornerstone technology across all stages of this pipeline. In the screening phase, MS-based assays enable high-throughput identification of bioactive compounds by directly monitoring enzymatic reactions, binding events, or metabolite formation with minimal labeling requirements. This accelerates hit validation and structure–activity relationship studies. During lead optimization and preclinical testing, MS provides precise molecular characterization and quantification of drug candidates and their metabolites. In stability studies, MS facilitates the detection of degradation products. Furthermore, MS plays a central role in pharmacokinetic (PK) and ADME (Absorption, Distribution, Metabolism, and Excretion) investigations, allowing sensitive quantification of drugs in biological matrices and elucidation of metabolic pathways that determine bioavailability and clearance. Coupled with liquid chromatography or implemented in miniaturized, high-throughput formats such as acoustic or droplet-based (ECHO-MS), it delivers unparalleled analytical power for both discovery and development. Altogether, mass spectrometry integrates chemical precision with biological insight, making it indispensable for the rational design and comprehensive evaluation of new therapeutic agents in modern drug development.

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ACKNOWLEDGEMENT:

Biochemical Pharmacology Group Helena Mertlíková Kaiserová Anna Janovská Alexandra Dvořáková Jaroslav Kozák Eva Tloušťová

FrO-20: Mass spectrometry of human body odor: Signatures of psychophysiological states

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Human body odor carries rich information about our genetic background as well as our physiological and psychophysiological states, including various diseases and pathologies. This seemingly simple statement is well supported by both everyday experience and extensive research — most of which has relied on olfactory perception studies using human raters or trained dogs. However, empirical knowledge about the chemical foundations of body odor differences remains limited, primarily due to past technical challenges in detecting and separating the hundreds of small molecules emitted by the human body.

With the recent advances in gas-phase metabolomics, many previously inaccessible questions regarding human body odor have become realistic research targets. In this study, we combined olfactory perception ratings with comprehensive two-dimensional gas chromatography coupled with mass spectrometry (GC×GC-TOFMS) to investigate axillary body odor patterns associated with different psychophysiological states in healthy male donors. Specifically, we examined odor changes following physical exercise, exposure to psychosocial stress, and sexual arousal stimuli. In my presentation, I will demonstrate that both perception ratings and chemical analyses effectively distinguished control samples from those collected after exercise and stress exposure. In contrast, we did not observe a systematic shift in body odor patterns in response to sexual arousal. I will also present candidate analytes that appear to underline these body odor differences across psychophysiological states.

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ACKNOWLEDGEMENT:

Funding: Pathfinder Open SMELLODI (101046369)

FrO-21: Population-scale human chemical exposomics by automated liquid-liquid extraction coupled to gas chromatography – Orbitrap mass spectrometry

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Profiling chemical exposure in large-scale human population studies is analytically challenging in terms of robustness and reproducibility, as well as laboriousness of the entire procedure. Few academic settings conduct routine analysis of studies comprising > 1000 samples. Manual sample preparation is time consuming, requires high solvent consumption, and contributes to sample-to-sample variation. Sequential on-line sample preparation offers a solution, but adoption is currently lacking in routine application.

Within the framework of ERIENE-CZ, we have developed a fully automated sample preparation workflow coupled to gas chromatography - Orbitrap mass spectrometry (GC-Orbitrap MS) for large-scale screening of chemical exposure agents in blood serum and plasma. A fully automated liquid-liquid extraction of blood (100 µL) has been developed using a cartesian autosampler coupled to GC-Orbitrap MS. In brief, analytes are separated on a Rxi-5Sil MS column with ~14 min temperature gradient (80 °C to 330 °C), electron ionisation at 70eV, and mass spectra recorded from 70-700 m/z at 60K resolving power. Quality assurance and quality control (QA/QC) includes the analysis of certified mixtures of alkanes and polychlorinated biphenyls, alongside the standard reference materials (SRM) NIST 1950, NIST 1957, NIST 1958 in each analytical batch.

We shall present i) a critical appraisal of the implemented method, including the interventional maintenance schedule and systematically recorded errors, such as vial drop rates; ii) updates on the status of multi-country, multi-site method standardisation and harmonisation; and iii) report on application to multiple cohort studies, each comprising > 1000 samples.

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1. *https://doi.org/10.5281/zenodo.10612856*
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ACKNOWLEDGEMENT:

The research has been conducted within the European Infrastructure for Human Exposome Research (EIRENE RI), in cooperation with the Network of Exposomics in the US (NEXUS) and within the collaborative framework of the International Human Exposome Network (IHEN; HEU programme grant agreement 101137317). The authors acknowledge the EU's H2020 grant agreements 857560 (CETOCOEN Excellence), 874583 (ATHLETE) and 874627 (EXPANSE); the HEU grant agreements 101079789 (EIRENE PPP) and 101096888 (DISCERN); and the RECETOX Research Infrastructure (LM2023069) financed by the MEYS (CZ.02.1.01/0.0/0.0/17_043/0009632). The views expressed are those of the author(s) and do not necessarily reflect those of the EU or REA.

WeS-01: Quantification of Oxoacids of the Human Metabolome using Liquid Chromatography-Mass Spectrometry

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Detection and quantification of metabolites serves to understand how various organisms function, as it can unveil new metabolic pathways and provide insight into their regulation. Liquid chromatography with mass detection is one of the leading analytical techniques in metabolomics. However, LC-MS detection is limited when analytes have poor stability or poor ionization efficiency. To overcome these hurdles, analytes can be derivatized to enhance sensitivity by conferring a permanent charge and generating a stable derivative [1]. Quaternary Aminooxy (QAO) reagents provide an opportunity to assess a range of metabolites with oxo groups by generating stable, positively charged oxime derivatives at acidic pH [2].

To explore the potential of QAO reagents, a method for the quantification of oxoacids in the human metabolome using QAO derivatization and LC-MS detection was developed and tested on human urine, serum, and HEK293 cells. 2-(aminooxy)-N,N,N-trimethylethylammonium iodide (2QMA) was the derivatization agent of choice and, combined with HILIC separation on a BEH-Amide column, resulted in good separation. Internal standards were generated using isotope-coded derivatization, and cross-derivatization upon mixing the standard and internal standard solutions was prevented by quenching the reaction with acetone. The method was validated according to US FDA guidelines for LLOQ, ULOQ, linearity, and carryover using deionized water as a surrogate matrix.

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ACKNOWLEDGEMENT:

This work was supported by the Czech Science Foundation (project GAČR, No. 23-06600S).

WeS-02: Analyses of human scent samples

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The main role in the identification of a person based on a scent trail currently play specially trained dogs. The currently used method is very subjective and the result depends on many factors, but above all on the trainer's knowledge of the location of the target scent can.

Our research is focused on finding an objective method for analysing human body scent. The currently used sorbent for scent sampling in Czech Republic is a non-woven fabric with the trade name Aratex®. Due to the presented impurities, it is absolutely unsuitable for instrumental analysis of human odor (olfacttronic analysis). Therefor, a material, which would be sufficiently clean (or cleanable), was searched for. Resistant materials such as glass or teflon are offered, or currently widely used nanotextiles or non-woven textiles.

Human transdermal scent collected on sorbent was extracted with ethanol. Extraction was supported by shaking and ultrasound. The extract was evaporated to dryness and the residue was dissolved in ethanol and analysed using a GCxGC-MS. Due to the possible losses during extraction, direct injection using thermal desorption was also tested. Liquid injection enables the use of sorbents, which decompose at higher temperatures (nanotextiles and non-woven textile materials). Of these materials, the sterile cover gauze squares appear to be the most promising. However, the cleanest material with the lowest amount of impurities, is glass and Teflon. However, both materials are, unlike textile materials, reusable. Both, glass and Teflon, are thermally stabile and can be directly desorb by high temperature. Thermal desorption not only reduces sample losses, but also shortens the time required for sample preparation and solvent consumption.

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ACKNOWLEDGEMENT:

This work is supported by Ministry of Interior of Czech Republic by the project No. VJ01010123.

WeS-03: rDUVLAESCI-MS/MSI: a novel approach for direct analysis of biological surfaces

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Ambient mass spectrometry has significantly enhanced the insight into the molecular composition of complex biological surfaces under native conditions, requiring minimal sample preparation [1]. Advancements in laser-based designs, including UV and deep UV lasers, enabled non-destructive, low-fragmentation analysis of various solid samples and dried spots/layers while significantly improving spatial resolution for mass spectrometry imaging (MSI) to just a few micrometers [2]. The presented work introduces a novel, fully automated Remote Deep-Ultraviolet Laser Ablation coupled with Electrospray Ionization−Atmospheric Pressure Chemical Ionization (rDUVLAESCI) ionization source, which can be easily used for either spot analysis or MSI of a wide range of polarity and molecular weights of organic molecules. The rDUVLAESCI benefits from the coupling of a 193 nm Analyte G2 laser ablation unit (Photon Machines) with a hybrid Q-TOF mass spectrometer Synapt G2S (Waters), enabling the simultaneous acquisition of complementary ESI and APCI mass spectra in a single analytical run. Using r-DUV-LAESCI-MS/MSI, suitable ionization conditions were optimized by testing various flow rates and types of sheath liquids in the dual ion source (ESCI), as well as evaluating the effects of different surface materials on signal intensities for various analytes. The applicability of rDUVLAESCI in molecular MSI was demonstrated on analysis and imaging of molecules directly in thin mouse brain tissue sections (12-30 µm thick). The main molecules detected were cholesterol and its derivatives (dehydrocholesterol, oxocholesterol, and desmosterol), free fatty acids (palmitic acid, linolenic acid), sphingosine, diacylglycerols (mainly dipalmitoyl glycerol), phospholipids, and C18 ceramide and adenine.

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ACKNOWLEDGEMENT:

The research was funded by Internal Grant Agency, Palacky University Olomouc (IGA_PrF_2024_027).

WeS-04: INVESTIGATION OF PHENOLIC COMPOUNDS IN GRAPE POMACE THROUGH UHPLC-ESI-MS/MS

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Grape pomace is a by-product of wine production.[1] Grape pomace consists of skin and pulp residue, usually with seeds, which can be eventually separated to produce oil. Grape pomace is a rich source of bioactive substances, with a particular abundance of many phenolic compounds, including phenolic acids, tannins, and anthocyanins.[2] Therefore, phenolic composition of grape pomace from wine producer (Ludwig winery, south Moravia region) was investigated by our UHPLC-HESI-Q-Orbitrap instrument.

We analyzed grape pomace derived from four varieties of white wine and one variety of red wine. nitially the samples were analyzed using untargeted metabolomics, incorporating both positive and negative ionisation. Following positive ionisation, a total of 4,100 features were detected in the samples, while 750 features were detected after negative ionisation. Subsequently, 36 major polyphenolic compounds were selected for quantitative analysis. In white wine varieties (Hibernal, Muskat ottonel, Rulandske sede and Neuburske), the total polyphenol content was found to be ca 3.2-6.3 mg/g in mixed type grape pomace. In white wine grape pomace, the highest polyphenol content was found in seed pomace (5.0-7.6 mg/g). In the red wine variety (Svatovavrinecke rose), the highest polyphenol content was found in the grape pomace made from the skins and pulp (40 mg/g). For white wine varieties, the most abundant phenolic compounds in the grape pomace were catechin and epicatechin, which accounted for approximately 1/3 of the total polyphenol content (1-2.5 mg/g). Other relatively abundant polyphenols were hyperoside, isoquercetin, procyanidin B2, and miquelianin. The most abundant anthocyanins in the red wine variety were found to be the glycosides of petunidin, malvidin and delphinidin.

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ACKNOWLEDGEMENT:

This research study was funded by the Ministry of Agriculture of the Czech Republic through project number QL24010080 and through institutional support under project number MZE-RO0423.

WeS-05: Mass Spectral Library Network (MERLIN) To Streamline The Generation Of Public Spectral Libraries

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When solving a research question, we deal with a matrix of interest and the analytical technique will detect certain metabolites that will be identified to answer our questions. With an estimated ~10^{60} small molecules [1], assigning a spectrum to its correct structure is inherently challenging. Natural products further complicate this task, as they often occur at low concentrations and feature complex scaffolds and stereochemistry. As a result, confident identification depends on high-quality reference mass spectral libraries. However, current open libraries lack chemical coverage or provide only MS^2 fragmentation, they are high-priced; or their use is restricted for specific applications [2]. This motivates the creation of an open-source, multi-stage MS^n library in which molecules are fragmented in successive rounds, rather than in a single stage, to reveal deeper structural layers and enhance molecular annotation. Importantly, this approach remains fully compatible with existing MS^2 workflows. The first stage of the MERLIN initiative, the MSnLib project [3], introduced the workflow for data cleanup, sample preparation, acquisition using a flow injection system coupled to an Orbitrap mass analyzer, and data processing for MSn (where $2 < n \leq 5$) library generation of 30,000 compounds. These are already available for download and integration into metabolomics workflows. Currently, the second stage of the initiative involves a substantial expansion of the coverage. Using the established workflow, 163,000 compounds have been measured during 2025 and an extra 97,000 compounds will be measured by 2026, being the largest open-source MSn library up to date.

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WeS-06: A Comprehensive timsTOF MS/MS Spectral Library for Improving Metabolite Annotation in Non-Targeted Metabolomics

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Metabolite annotation, or tentative identification, remains a major bottleneck in non-targeted mass spectrometry-based metabolomics due to the limited availability of authentic standards. Spectral libraries2, which rely on matching accurate mass-to-charge ratios (m/z) and MS/MS fragmentation spectra between experimental and reference data, are therefore essential for this process. However, existing libraries encompass only a small portion of chemical space, typically allowing annotation of just a few percent of features detected in a sample.

To address this limitation, we established a high-resolution MS/MS spectral library comprising over 10,000 bioactive compounds. Compounds were analyzed using reverse-phase liquid chromatography coupled to a Bruker timsTOF HT system operated in both positive and negative ionization modes, and the resulting data were processed with MZmine1.

Through several case studies, including fecal samples from gut microbiota studies and microbial culture broths, we assess the performance of our library relative to the largest public GNPS spectral database and Bruker's commercial MetaboBASE library. These comparisons highlight the critical importance of instrument-specific spectral acquisition for improving annotation rates. The resulting dataset substantially expands the chemical and instrumental diversity represented in current spectral resources and provides a robust foundation for the development of advanced, AI-driven approaches to bioactivity prediction and natural product discovery.

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ACKNOWLEDGEMENT:

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ThS-07: Propionic acid boosts sensitivity in high-flow RPLC-MS proteomics compared to formic and acetic acid

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Formic acid has long been the default acidic additive in mobile phases for RPLC-MS-based bottom-up proteomics due to its balance between chromatographic performance and electrospray ionization (ESI) efficiency, while most efforts to improve sensitivity have rather focused on sample preparation and MS instrumentation. However, recent studies have revived interest in acetic acid, revealing that its lower ionic strength enhances ESI efficiency without sacrificing chromatographic performance. Inspired by this concept, we investigated propionic acid, a homologous compound that was overlooked as a mobile phase additive. By further weakening ionic strength and lowering mobile phase surface tension, propionic acid yielded an average 12% increase in peptide identifications over acetic acid across interlaboratory datasets using analytical- and microflow LC-MS platforms, various column chemistries, and levels of sample complexity. Importantly, chromatographic performance remained virtually unaffected, with only a modest decrease in retention. The mobile phase containing propionic acid was stable, instrument-safe, and introduced negligible MS background noise. These findings challenge the long-standing reliance on formic acid and establish propionic acid as a potent, drop-in alternative for high-flow LC-MS workflows prioritizing detection sensitivity and depth of proteome coverage.

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ACKNOWLEDGEMENT:

This study was supported by the Project of the Czech Science Foundation (GAČR No. 22-21620S), the Project of the Charles University Grant Agency (GAUK No. 370522), the SVV Project No. 260782, and the project New Technologies for Translational Research in Pharmaceutical Sciences (NETPHARM, No. CZ.02.01.01/00/22_008/0004607) co-funded by the European Union.

ThS-08: Towards Biomarker Discovery: Multiomics analysis of Endometriosis

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Endometriosis is a chronic inflammatory disease characterised by the presence of endometrial-like tissue outside the uterus. Affecting approximately 10% of reproductive-age women, its diagnosis is often delayed due to nonspecific symptoms and a lack of reliable biomarkers. In this study, we employ a multiomic approach to investigate plasma-based molecular signatures associated with endometriosis. Plasma samples from patients (Grades I–IV), suspected cases, and matched controls underwent parallel proteomic, non-targeted metabolomic, and lipidomic analysis using extraction protocols optimised for both metabolite and lipid profiling.

Proteomic analysis consisted of DIA analysis and search through the DIA-NN search instrument. Metabolomic and lipidomic data preprocessing included peak picking, quality control-based signal correction (QC-RSC), and statistical filtering using the open-source PySPRESSO pipeline. Principal Component Analysis (PCA) and supervised Partial Least Squares Discriminant Analysis (PLS-DA) revealed clear group separations, particularly between control and diagnosed cases.

In the proteomics branch, cellular oxidant detoxification and the redox-active centre were among the most influenced processes. In the lipidomics branch, lipids such as PC(16:0_20:5) showed differential abundance and fragmentation patterns between groups. These findings underscore the diagnostic potential of untargeted omics approaches in endometriosis. Multi-omic Factor Analysis (MOFA) highlighted latent factors that correlated with disease class and diagnosis. Candidate features were selected based on volcano plots and statistical metrics.

Future work will focus on expanding compound annotation, integrating proteomics, and refining predictive models for clinical translation.

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ACKNOWLEDGEMENT:

The project National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102) - Funded by the European Union – Next Generation EU. & JG_2024_026 (UPOL) & IGA LF 2025_006

ThS-09: Integrated proteogenomic analysis identifies synuclein gamma as a key target of the mesenchymal stem-like subtype of triple negative breast cancer

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Triple-negative breast cancer (TNBC) is an aggressive BC subtype that represents ~15% of cases and is primarily treated with systemic chemotherapy. To identify novel therapeutic targets, we performed a multiomics study using LC-DIA-MS/MS based proteomics, RNA sequencing, and whole-exome sequencing in a set of fresh frozen TNBC tissues, generating an integrated dataset for 96 samples. Hierarchical clustering utilizing the proteomics data for 1,223 most correlated transcript–protein pairs stratified TNBC into seven clusters. Of these, cluster 1 was designated as Mesenchymal stem-like (MSL) based on Gene set enrichment analysis and TNBCtype tool outputs. Within MSL subtype, synuclein gamma (SNCG) emerged as a key protein target, originating from significant upregulation in MSL subtype compared to others ($log_2FC=1.64$, padj=0.0023), its core pathway enrichment, and association with both poor progression-free survival of the patients (PFS; HR=2.542, p=0.005, Cox; p=0.004, log-rank test) and overall survival (OS; HR=2.425, p=0.008, Cox; p=0.006, log-rank test). Functional SNCG validation via CRISPR/Cas9 knockout in MDA-MB-231 cells showed reduced proliferation in $SNCG^{-/-}$ clones in the CCK8 proliferation assay (parental vs. G10, padj=2×10^{-13}; vs. B1, padj=9.08×10^{-4}; vs. B3, padj=4.04×10^{-9}). However, no effect on cell migration capacity has been observed in the scratch assay. This is in good agreement with a previous SNCG association with breast cancer progression and recurrence[1] and with promoting proliferation in oral squamous cell carcinoma[2]. In summary, proteomics-driven, multiomics analysis of TNBC patients identified SNCG as a key protein in the MSL subtype linked to poor PFS and OS, and its association with cancer cell proliferation and growth was functionally validated.

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ACKNOWLEDGEMENT:

Supported by the Ministry of Health of the Czech Republic in cooperation with the Czech Health Research Council under project No. NU22-08-00230. Supported by the project National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102)—Funded by the European Union—Next Generation EU.

ThS-10: Human IMPs: A tool for comparing outputs of different tools for transmembrane segment predictions in the human proteome

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More than a quarter of human genes encode integral membrane proteins (IMPs), which perform key biological functions and represent the majority of targets for currently approved drugs. IMPs are defined by the presence of transmembrane regions, typically alpha helices. Accurately identifying these regions remains challenging, as available algorithms often provide different results and require computational skills for batch processing. Meanwhile, UniProt annotation data, though easily accessible, are obtained from diverse sources and tend to differ from computational predictions.

To facilitate comparison of prediction outputs and UniProt annotations, we developed Human IMPs, a web application integrating transmembrane helix data from UniProt and predictions from four widely used algorithms: Phobius, DeepTMHMM, TMBED, and TOPCONS. The application features interactive graphical visualization and supports batch analysis of multiple proteins simultaneously. Human IMPs uses the UniProt API to fetch curated annotation data across the human proteome. Predictions are stored in an optimized database, enabling rapid retrieval and comparison. The graphical interface displays predicted transmembrane regions as colored bars aligned to protein sequences, making discrepancies immediately apparent. Batch processing allows analysis of large protein datasets with results exportable in CSV or TSV formats. We demonstrate these capabilities by comparing prediction models on IMPs identified in pheochromocytoma and paraganglioma tumor proteomes. Future development plans include integration of additional prediction algorithms and implementation of consensus transmembrane region computation combining multiple methods for more reliable predictions. Explore Human IMPs at hwllffrdd.cz/imps/.

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ACKNOWLEDGEMENT:

Supported by MZČR (NU23-01-00323), Charles University (SVV 260763) and EXCELES LX22NPO5102.

ThS-11: LC-MS/MS-based terminomics unravels role of carboxypeptidase B1 in luminal A breast cancer

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Carboxypeptidase B1 (CPB1) is a metalloprotease which cleaves arginine and lysine residues from protein C-termini and 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. To identify CPB1 protein substrates, we analyzed lymph node positive luminal A breast tumors with CPB1 (vs. CPB1 negative control) via trypsin digestion and timsTOF Pro2 LC-MS/MS system. The data were processed in Spectronaut software with semispecific search and in Fragterminomics package in R. Among 76,368 identified peptides (FDR=0.01), 17,748 peptides possessed a C-terminal non tryptic cleavage, including 23 proteins with C-terminal peptides with max. 2 arginine or lysine residues removed from the C-terminus specifically in CPB1-cleaved samples. These include PDXDC1, GANAB and NID2 proteins associated with extracellular localization and cytoskeleton. As confirmation of CPB1 role in metastatic potential of cancer cells, a 3D invasion assay showed increased volumes of spheroids formed by MCF7 cells overexpressing CPB1. To associate CPB1 with clinical-pathological parameters, CPB1 immunohistochemistry was performed for 441 breast tumors. CPB1 staining was significantly associated with lymph node status and relapse in patients of all subtypes, and specifically with relapse of luminal A tumors (n=251; p≤0.05). CPB1 histoscore was related to a shorter relapse-free (RFS) and distant metastasis free survival. Multivariable Cox analysis confirmed CPB1 as the most significant factor associated with RFS in luminal A 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.

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ACKNOWLEDGEMENT:

Supported by the National Institute for Cancer Research (Program EXCELES, ID LX22NPO5102) – Next generation EU.

ThS-12: Mass spectometry in hands of experimental biology: Impaired nucleotide salvage affects *de novo* synthesis in *Drosophila melanogaster*

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Nucleotide metabolism is essential for maintaining cellular homeostasis, with synthesis pathways often impaired in disease. However, the metabolic compensation mechanisms that occur when key enzymes of the nucleotide salvage pathway are disrupted remain poorly understood. Here, we employed LC-MS combined with ^{13}C labelled glucose to trace metabolite flux in *Drosophila melanogaster* larvae lacking ribokinase (RBKS), a key enzyme in nucleotide salvage. Our results show significantly reduced labelling of intermediate from the de novo synthesis pathway.

This methodology provides new insights into nucleotide metabolism and underscores the importance of the salvage pathway in maintaining nucleotide balance.

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ThS-13: Bioaccumulation of pharmaceuticals in herbivorous fish: The role of aquatic plants as a dietary source

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Wastewater treatment plants do not completely remove micropollutants such as pharmaceuticals; therefore, these substances are commonly found in surface waters, where they can persist and bioaccumulate in aquatic organisms. While bioaccumulation in predatory and omnivorous fish is relatively well described in the literature, there are only a very limited number of studies on herbivorous fish. However, these species may represent a key pathway for the transfer of contaminants from plants to higher trophic levels. The aim of this study was to evaluate the bioaccumulation of pharmaceuticals in the tissues of grass carp (Ctenopharyngodon idella) exposed for six months in the Čežárka biological pond (Vodňany, Czech Republic), supplied exclusively with treated wastewater. Water samples, extracts from passive samplers, grass carp tissue samples, and plant samples were analyzed using liquid chromatography with mass spectrometry detection. Of the total of 78 pharmaceuticals analyzed, ten pharmaceuticals were detected in the tissues of grass carp, most commonly antidepressants and cardiovascular drugs. The highest concentrations were found in the kidneys (the sum 33 ng/g ww), followed by the brain, liver, and plasma, while no pharmaceuticals were detected in muscles. The highest bioaccumulation factors (BAF) were observed for norsertraline (up to 5600 L/kg in the brain and 4500 L/kg in the kidneys) and sertraline (1500 L/kg in the kidneys). Pharmaceuticals found in the tissues of the grass carp were also found in aquatic plants, confirming their role as a significant reservoir and dietary source of exposure.

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ACKNOWLEDGEMENT:

This study was financially supported by the Czech Science Foundation, grant No. GACR 25-17470S.

ThS-14: LC-HRMS for large-scale chemical exposure studies

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A novel liquid chromatography - Orbitrap mass spectrometry assay for screening of chemical exposure agents, suited for large-scale population analysis e.g. biomonitoring, clinical trials and cohort studies, has been established within the framework of EIRENE-CZ.

Briefly, analytes are separated upon pentabromobenzyl column with 9-minute methanol gradient, undergo electrospray ionisation and are detected via full scan - all ion fragmentation operated at minimum 60K resolution and scanning 70-1000 m/z. The assay has been applied to extracts of various biofluids (urine, serum, plasma) and environmental matrices (dust, plants, microplastics) within the scope of multiple collaborative initiatives, and has excelled in multi-laboratory comparative non-targeted analysis performance assessments viz. detection coverage (wide physicochemical space, i.e., retention cLogP from −3.34 to 12.95; low detection limits), reliable relative quantification (high linearity and sensitivity) and increased throughput. The assay is accompanied by a five-fold cross-validated quantitative structure retention relationship model, with R2-score of 0.96 and mean absolute error of 0.27 minutes, enabling accurately predicted retention times to support suspect annotation. We shall present an overview of the iterative method development, quality control and quality assurance procedures implemented, and share the status of ongoing multi-site assay replication across numerous Orbitrap models (Fusion Tribrid, Exploris 480, Exploris 240).

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ACKNOWLEDGEMENT:

The authors acknowledge the European Union's Horizon Europe programme under the grant agreement 101130769 (FRANKIE) and 101079789 (EIRENE PPP), the European Union's Horizon 2020 research and innovation programme under grant agreement 857560 (CETOCOEN Excellence), the National Institute for Neurological Research (Programme EXCELES, ID Project No. LX22NPO5107), funded by the European Union – Next Generation EU, the RECETOX Research Infrastructure (No LM2023069) financed by the MEYS (No CZ.02.1.01/0.0/0.0/17_043/0009632) and SALVAGE (CZ.02.01.01/00/22_008/0004644) financed by MEYS, co-funded by the European Union. The views expressed are those of the author(s) and do not necessarily reflect those of the EU or REA.

ThP-01: Complexome profiling workflow elucidates mitochondrial cytochrome c oxidase biogenesis

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Complexome profiling is an experimental technique that reveals protein-protein interactions by integrating native separation of protein complexes and quantitative MS. To profile the proteins from mitochondria, the energy producing intracellular organelles, blue native polyacrylamide gel electrophoresis (BN-PAGE) separation is routinely used. Here we established and used a complexome profiling workflow to investigate the cellular model of mitochondrial cytochrome c oxidase (COX) deficiency caused by deletion of subunit COX6B1. This nuclear-encoded subunit is implicated in human pathology with three rare COX6B1 missense pathological variants identified as the cause of childhood-onset mitochondrial encephalopathy. Our complexome analysis supported by the Gaussian Interaction Profiler (GIP) data analysis algorithm revealed a total loss of COX, contrasting with the expected incorporation of COX6B1 at the very late stages of COX assembly. To get a deeper insight into the mechanisms we also expressed an alternative oxidase (AOX) in the COX6B1 KO that partially released the early assembly block. Based on complexome profiles we conclude that the COX6B1 subunit does not only contribute to the stabilization of COX in the late assembly stages, but is also indispensable for redox-sensitive early COX assembly steps. In addition, this study has demonstarated the incorporation of partially assembled COX modules directly into supercomplex structures, supporting the cooperative assembly model for the respiratory chain biogenesis. More broadly, presented MS-based complexome profiling aided by extensive data analysis can be used to study the interaction networks and thus the functional roles of (mitochondrial) proteins

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ThP-02: Amino Acid Profiling Across Diverse Biological Matrices Using the MetAmino® LC-MS Kit: Applications in Free and Peptide-Bound Amino Acid Analysis

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Amino acid analysis is fundamental to clinical diagnostics, nutritional science, pharmaceutical development, and metabolomics research, yet traditional methods suffer from lengthy sample preparation, limited matrix compatibility, and restricted analytical scope. In cooperation with Chromservis company[1], we present the MetAmino® Kit, a novel integrated LC-MS platform combining micro-solid phase extraction (MSPE) with in-situ derivatization chemistry[2] for comprehensive amino acid profiling across multiple matrices and applications. The method enables quantification of over 70 compounds including proteinogenic amino acids, biogenic amines, modified amino acids, and small peptides in a 20-minute total analysis time (8-minute sample preparation plus 12-minute LC-MS run).

The system's versatility was evaluated across several analytical applications: (1) free amino acid analysis in biological fluids (serum, urine) and in beverages products such as beer; (2) total amino acid composition determination in fish feed after acid hydrolysis.

The proprietary MSPE-based cleanup effectively removes matrix interferents from diverse samples while retaining all derivatized amino acids, eliminating the need for matrix-specific protocols. The MetAmino® Kit provides a versatile platform uniquely suited for clinical metabolomics, nutritional research, pharmaceutical quality control, agricultural applications, and protein chemistry studies. Its ability to analyze free amino acids, total amino acid composition, and peptide hydrolysates in a single methodological framework, combined with rapid analysis time and robust performance, positions this technology as a next-generation solution for comprehensive amino acid profiling in modern research laboratories.

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ACKNOWLEDGEMENT:

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ThP-03: Ambient MS for rapid screening of pharmaceutically active compounds: Off-the-shelf and lab-made ion sources

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Ambient mass spectrometry allows the analysis of samples at atmospheric pressure in their natural state with no or minimal modification. We present the application of the commercial ambient ion source Sicrit based on dielectric barrier discharge (DBDI) for the analysis of selective androgen receptor modulators (SARMs). SARMs belong to the group of substances with anabolic effects and are becoming popular compounds of abuse in sport doping, especially by amateurs and semiprofessional. For analysis using the Sicrit source, tablet and capsule samples were dissolved in methanol. Dosing was carried out using a micro syringe into the heated GC/SPME module of the Sicrit ion source. The interior of the source was purged with nitrogen to isolate the system from the external environment. The Sicrit ion source was connected to an Orbitrap Exploris 120 mass spectrometer operating in positive mode. To verify the results, the samples were also analyzed by LC-MS/MS in MRM mode (Agilent 1290 Infinity II LC coupled to an Agilent 6470A TQ). Although LC MS/MS outperformed ambient mass spectrometry on a quantitative scale, the qualitative analysis results of both techniques were in good agreement, suggesting the potential use of ambient techniques for screening dietary supplements for the presence of illegal substances with anabolic effects. As an alternative, an ambient ion source based on desorption atmospheric pressure photoionization (DAPPI) was also constructed and its function was verified on a solid sample of naphthalene and CBD, a gaseous sample of allicin, and finally it was used to analyze the calibration solution of one of the SARM representatives.

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ACKNOWLEDGEMENT:

We acknowledge support from grant: Microorganisms in Ecological Remediation – Bacteria as Cell Factories for Controlled Bioremediation of Ecosystems (CZ.02.01.01/00/23_020/0008502).

ThP-04: ADAPTIVE PROTEOME UNDERPINS DROUGHT TOLERANCE IN BREAD WHEAT DURING FLOWERING

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Water scarcity during anthesis is a critical constraint for bread wheat yield. This study compared the physiological and proteomic responses of two cultivars—drought-tolerant and drought-sensitive—under moderate transient drought during flowering. Measurements of photosynthetic efficiency, water status, and oxidative stress markers revealed that the sensitive cultivar suffered more severe declines in photosynthesis and water retention. In contrast, the tolerant cultivar activated protective mechanisms such as enhanced photorespiration, earlier increased superoxide dismutase activity, and better maintenance of photosynthesis. Proteomic analysis showed that the tolerant cultivar rapidly adjusted its protein profile early during drought, while the sensitive one exhibited delayed and minimal changes. By the end of the drought, both cultivars showed significant proteomic shifts, more extensive in the sensitive genotype. After rewatering, differences between treated and control plants diminished but remained detectable, especially in the sensitive cultivar. The results suggest that greater proteomic plasticity and early activation of stress-response pathways contribute to the superior drought resilience of the tolerant genotype. Moreover, we spotted differential accumulation of chloroplastic thiol-active protein. Future work will explore redox-related post-translational modifications and their roles in stress adaptation during drought and recovery.

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ACKNOWLEDGEMENT:

This study was supported by the Slovak Research and Development Agency project VV-MVP-24-0368 and the National Academy of Sciences of Ukraine grant 0125U001842.

ThP-05: Transition from natural dyestuffs to synthetic colorants depicted by ion mobility-aided mass spectrometry

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For millennia, natural dyestuffs were the primary sources of colorants. Advances in chemical technology in the 19th century yielded an explosive dye palette represented by numerous classes of synthetic dyes. For instance, only a handful of semi-synthetic dyes were known until the mid-1800s. In 1856 the first fully synthetic dye, mauveine, was obtained. By the end of the 19th century, about 400 synthetic dyes were available, and within the next 20 years over 1200 patented formulations displaced natural colorants. It is still debated how, when and where transition from natural to artificial colorants occurred.

To address these questions, we conducted a comprehensive study of the late 19th – early 20th century garments from Czech noble families. A two-step microextraction protocol (DMSO followed by a mixture of aqueous oxalic acid/methanol/acetone) proved effective across multiple dye classes. The developed UHPLC method ensured simultaneous separation of early synthetic and natural dyes along with their degradation products, isomers and minor components. Identification combined PDA detection with high resolution MS in positive and negative ESI modes. An extended in-house database of 250 substances facilitated automatic targeted screening. Confirmation of knowns and structure elucidation of unknowns was supplemented by the data-independent all-precursor MS fragmentation with ion mobility separation (HDMSE mode, Synapt G2-S).

Analysis of ball gowns, evening and court dresses revealed multiple dyeing scenarios: exclusive use of natural dyes (e.g. barberry); mixtures of synthetic dyes (e.g. Fuchsine, Gentian violet B, Rhodamine B, Safranine T) and combinations of natural and synthetic dyes (e.g. oak gallnuts and Azoflavine 3R, Brilliant blue, Magneson I and Metanil Yellow).

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ACKNOWLEDGEMENT:

The research was financed from the funds of the Programme for the support of applied research in the field of national and cultural identity for the years 2023 to 2030 (NAKI III) of the Ministry of Culture of the Czech Republic within the project "From the Aristocratic Court to the Theatre Stage. Historical Wardrobe in the Collections of Czech Castles and Chateaux in the European Context"(DH23P03OVV046).

ThP-06: Off-line GC-GC×GC-MS focusing on scent samples

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Scent analysis is a promising method in forensic applications, as the emission of these molecules from the human body cannot normally be prevented. The most suitable method for such analysis so far appears to be 2D GC-MS. Even with the higher separation efficiency provided by the second dimension, scent samples are so complex that several co-elutions still occur, and the chromatograms—even when supported by mass spectra—are not easy to interpret.

One possible solution is to pre-separate the sample into smaller fractions and analyze them individually. For this purpose, GC-PFC can be used, where the scent may be fractionated into up to seven fractions according to decreasing volatility. This approach may lead to a deeper understanding of the scent trace itself and the identification of a narrower group of compounds that are key to individual identification. Identifying such a group of compounds could, in the future, simplify the analysis of scent traces in police practice, allowing investigations to focus more efficiently on the compounds of interest. First and foremost, it is necessary to ensure both the effective transfer of the sample from the fraction collector for subsequent detailed analysis by two-dimensional gas chromatography with mass spectrometry, and at the same time to guarantee that the yield of the injected sample from GC-PFC is sufficient for the following analysis. Yield verification is carried out on a standardized sample.

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ACKNOWLEDGEMENT:

This work was supported from the grant of Specific university research – grant No A2_FCHI_2025_011.

ThP-07: Ambient Soft Landing Voltammetry: Tool for Electrode Modification and Molecular Redox Characterization

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In an ion soft landing (ISL) experiment ions are guided by standard or custom-specific ion optics, but instead of being detected they are non-destructively deposited on a suitable surface. If chemically or biologically active molecules are landed, they retain their activity and the surface can be modified by the intact molecules and obtain their chemical or biological properties. Originally, ISL was performed under high vacuum conditions inside mass spectrometer but more recently, focus shifted to atmospheric pressure in an experiment referred to as ambient ion soft landing [1,2].

Among various electrode types and designs, screen-printed electrodes (SPEs) have gained much attention in recent years, mainly because of their low price, ease of use and simplicity of production. In addition, the working electrode of SPE can be chemically modified to enhance electrode's selectivity and sensitivity. It was already shown that SPE surface can be modified by ISL [3]. However, the cited experiments were limited to deposition of large molecules (microperoxidase-11) under high vacuum conditions.

Here, we present a method of modification of SPE surface using ambient ISL of small molecules. The experiments were performed using custom-built apparatus based on a modified 3D printer. Soft-landed molecules included crown ethers and guanidines, receptors showing affinity for amino groups and carboxylates, respectively. Modified electrodes and respective immobilized molecules were characterized by voltammetry and mass spectrometry. The results demonstrate that ambient ISL is a promising alternative to classical methods of electrode modification from solution. Coupling of ion soft landing to voltammetry can provide an insight into molecular redox properties.

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ACKNOWLEDGEMENT:

The research was supported by Czech Science Foundation Grant No. 25-16715J.

ThP-08: Translational research aimed at improving heatstroke diagnosis

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Global warming is expected to increase the frequency of natural disasters such as wildfires and storms, and, more directly, the incidence of heat-related illnesses. Among these, heatstroke (HS) is the most severe form. HS is characterized by a core body temperature exceeding 40 °C accompanied by central nervous system (CNS) dysfunction, and is often complicated by multi-organ failure involving renal, hepatic, and hemostatic systems.

In the present study, we established an animal model of HS using male C57BL/6J mice aged 20–24 weeks. The mice were exposed to a temperature of 42 °C and constant humidity (40 ± 15 %) for 45 minutes. Following exposure, plasma and brain tissue samples were collected for proteomic analysis. Peptide samples were loaded onto a nanoEase Symmetry C18 trap column (25 mm × 180 µm, 5 µm particle size) and desalted/concentrated for 2 minutes using 1 % acetonitrile with 0.1 % formic acid. Peptides were then separated on a nanoEase HSS T3 C18 analytical column (100 mm × 75 µm, 1.8 µm particle size) using a 90-minute gradient from 5 % to 35 % acetonitrile containing 0.1 % formic acid. The separated peptides were ionized using a capillary voltage of 3.1 kV and analyzed on a Synapt G2-Si quadrupole time-of-flight mass spectrometer (Waters). Data processing was performed using Progenesis QI 4.0 (Waters).

In addition, we conducted a targeted analysis of potential biomarkers associated with inflammation and synaptic dysfunction reported in HS. Using the ultrasensitive Single Molecule Array (SIMOA) system, we quantified low-abundance proteins in plasma, including neurofilament light chain (NfL). Together, these analyses allowed us to identify proteins potentially involved in modulating molecular processes related to heatstroke pathophysiology.

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ACKNOWLEDGEMENT:

Acknowledgment: EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under the project No. 09I03-03-V03- 00086, APVV-22-313, VEGA 02/0075/24.

ThP-09: Tracing the Gravettian Stone Paths

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Flints and cherts were among the most important lithic materials for toolmaking during the Upper Palaeolithic (UP) Gravettian culture, and their study offers key insights into the mobility, resource use, and social interaction of prehistoric hunter-gatherer groups. This project focuses on reconstructing Gravettian behaviour in Moravia (Czech Republic) and Slovakia by performing provenance studies of fine-grained siliceous rocks from major sites spanning both the Middle Gravettian (Pavlovian; 31–28 ka) and Late Gravettian (28–24 ka). This research aims to explore the geological diversity of flint and chert sources in Central Europe (Moravia, Poland and west-Ukraine), as well as their distribution and potential use in Gravettian contexts. By integrating approaches from geology and geochemistry, including chemical fingerprinting through X-ray fluorescence (XRF) and laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS), the study seeks to establish frameworks for distinguishing between source regions. Advanced statistical models will be applied to these datasets to create provenance models and provide robust analytical tools for interpreting Gravettian hunter-gatherer mobility in Central Europe. While the primary emphasis lies on flints and cherts, existing datasets on radiolarites (radiolarian-rich cherts) from Central Europe will serve as an important comparative reference, helping to refine methodological strategies and strengthen interpretations of human mobility during the Late Ice Age.

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ACKNOWLEDGEMENT:

This work was supported by the Czech Science Foundation (project no. 25-15848S).

ThP-10: Leaf discs as a suitable alternative for cell cultures in plant omics analyses

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The study of plant metabolites and their responses to biotic and abiotic stresses is fundamental to understanding plant physiology. While cell suspension cultures are widely used due to their experimental homogeneity and controllability, they lack the tissue organization and spatial context characteristic of whole-plant systems (Davey and Anthony 2010).

In this contribution, leaf discs of Arabidopsis thaliana are presented as a viable and reliable alternative to cell suspension cultures for plant omics analyses. This approach preserves tissue differentiation, intercellular communication, and physiological integrity, providing a more realistic model for studying plant responses to various stimuli (Chiu et al. 2021).

The use of leaf discs offers several practical advantages. It is time-efficient, cost-effective, and compatible with proteomic and microscopic analyses (Zheng et al. 2025). Thus, leaf discs represent a robust and physiologically relevant experimental system suitable for investigating stress responses, hormone signaling, and chaperone activity under controlled conditions.

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ThP-11: Characterization of Nanoparticles in Mixtures by Taylor Dispersion Analysis Hyphenated to Inductively Coupled Plasma Mass Spectrometry

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Despite the tremendous use of nanoparticles (NPs), our understanding of their self-assembly process and unique interactions with other NPs is still limited. Currently, there is a lack of analytical techniques that can be used to simultaneously characterise freely moving NPs and their interactions in conditions of high ionic strength that mimic real-life situations in living organisms or the environment. Consequently, we have witnessed a renaissance of Taylor dispersion analysis (TDA) in connection with inductively coupled plasma mass spectrometry (ICP-MS). The sensitive element/isotope specific ICP MS detection has opened up new window to advanced characterization of nanoparticles (NPs) and nanoclusters (NC) with dimensions of 1 - 10 nm. The TDA-ICP-MS has been used to characterise of Fe3O4@COOH, silver and gold nanoparticles in their mixtures. Compared to standard DAD detection, specific ICP-MS detection provides undistorted Taylor diagrams, even for metallic NP mixtures [1]. TDA-ICP-MS enables the unique characterisation of NP parameters, including chemical composition, isotope ratio, hydrodynamic diameter, particle number concentration estimation, free metal ion concentration and surface charge.

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ACKNOWLEDGEMENT:

The research was funded by Czech Science Foundation project 25-17563S.

ThP-12: Sub-cellular interacting partners of the tick-borne encephalitis virus capsid protein

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Detailed knowledge of how individual viral and host proteins interact during the progression of disease is crucial for targeting viruses within host cells. For the tick-borne encephalitis virus (TBEV; *Orthoflavivirus encephalitidis* , *Flaviviridae*), a virus widely spread across Eurasia and responsible for tick-borne encephalitis, the specific interactions involving its structural and non-structural proteins are just beginning to be uncovered. The TBEV capsid protein (TBEV C) serves multiple functions: besides its structural role in forming the viral nucleocapsid within modified cellular membranes, it also moves into the nucleus, where its functions are not yet fully understood. To identify the precise host and viral proteins interacting with TBEV C, we conducted compartment-specific co-immunoprecipitation experiments. Mass spectrometry analysis revealed several interacting proteins, distinguishing between direct protein-protein interactions and those mediated by RNA by comparing samples treated with and without benzonase. The specificity of these protein interactions was confirmed through reverse co-immunoprecipitation and immunofluorescence localisation studies. Our findings provide new insights into the molecular mechanisms of TBEV C roles in host cells that might be critical for the viral life cycle.

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ThP-13: Mass Spectrometry-Based Proteomic Profiling of *Sugiyamaella lignohabitans* Adaptation to Xylose as Carbon Source

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Sugiyamaella lignohabitans is a yeast species capable of metabolizing pentose sugars such as xylose, with ecological roles in lignocellulosic biomass degradation. To understand its adaptive protein expression, we employed quantitative mass spectrometry to analyze proteomic differences using glucose as a control carbon source. Cultures were grown on defined media containing xylose or glucose, and both cell-associated and secreted proteins were collected for analysis.

Proteins were extracted, digested with trypsin, and subjected to liquid chromatography-tandem mass spectrometry (LC-MS/MS) using a high-resolution orbitrap mass spectrometer. Data-dependent acquisition (DDA) was employed to identify peptides and proteins, followed by label-free quantification (LFQ) to assess relative abundance differences. Protein identification and quantification were performed using state-of-the-art bioinformatics pipelines, including peptide-spectrum matching and intensity normalization.

Statistical analyses identified dysregulated proteins across different substrate conditions, focusing on carbohydrate-active enzymes (CAZymes), sugar transporters, and central metabolism proteins. Secretome analysis revealed upregulation of extracellular hydrolases on xylose compared to glucose, suggesting the capacity of xylose to induce enzymes required for hydrolysis of xylose-containing polysaccharides. Intracellular proteomes showed induction of transporter proteins and enzymes involved in xylose metabolism pathways.

This mass spectrometry-based proteomic approach reveals the molecular mechanisms underlying *Sugiyamaella lignohabitans* substrate utilization and offers insights for biotechnological exploitation of this yeast in biomass conversion and pentose sugar valorization.

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ACKNOWLEDGEMENT:

Acknowlegment: This work was supported by the Slovak Research and Development Agency under the Contract no. APVV-22-0207.

ThP-14: Proteomic Analysis of Acetylsalicylic Acid-Induced Changes in the Cellular, Secreted, and Extracellular Vesicle Proteome of Malignant Melanocytes

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Acetylsalicylic acid (ASA) is a widely used drug with established analgesic, antipyretic, anti-inflammatory, and cardioprotective effects. Emerging evidence also suggests its potential role in cancer prevention. However, the molecular mechanisms underlying these anti-cancer effects remain poorly understood.

Melanoma, a highly aggressive skin cancer originating from melanocytes, is characterized by the production of cytokines, chemokines, and growth factors that promote tumor growth and metastasis. Our previous work identified malignant melanocyte extracellular vesicles (EVs) as vehicles stimulating the tumour-promoting pro-inflammatory activity of cancer-associated fibroblasts. In this study, we investigated the impact of ASA on the proteome of human malignant melanocytes, in the search for melanoma produced factors with such a pro-inflammatory effect.

Cells were treated with 5 mM ASA, and cell lysates, conditioned media, and EVs (isolated by ultracentrifugation) were collected. A multiplex immunoassay was used to quantify levels of cytokines and growth factors. Proteomic profiling was performed by LC-MS/MS (timsTOF HT) using both dda-PASEF and dia-PASEF modes, with data analysis in Spectronaut.

We quantified over 9,500 proteins in cell lysates and more than 5,500 proteins in EVs. ASA treatment significantly altered protein expression profiles, particularly those involved in RNA metabolism and cell division. Notably, ASA led to a marked reduction in EV production. Cytokine profiling revealed reduced levels of IL-8 and MCP-1, while IL-6 levels were consistently increased following ASA treatment.

These findings provide novel insights into the cellular response of melanoma cells to ASA, highlighting its impact on vesicle biogenesis and inflammation-related signaling.

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ACKNOWLEDGEMENT:

This work was supported by the Ministry of Education, Youth and Sports of the Czech Republic projects National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102) funded by the European Union—Next Generation EU, and by the project Center for Tumor ecology (reg. n. CZ.02.1.01/0.0/0.0/16_019/0000785) supported by the Operational Program Research, Development and Education.

ThP-15: Apparatus for Protein Footprinting by Singlet Oxygen

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Protein footprinting is a powerful tool for the structural characterization of proteins and their complexes. It enables the investigation of conformational changes and ligand binding by determining the solvent accessibility of backbone or side chain. The protein "footprint" is constructed by determining reactivity of its accessible sites with different reagents. By comparing the footprint of a protein in two different structural states, changes in surface topography can be determined and interpreted in the context of complementary structural information. There are several footprinting approaches, Hydrogen deuterium exchange (HDX) and Fast photochemical oxidation of proteins (FPOP) being currently the most common. Recently, an alternative oxidative footprinting was introduced by Novak group at the Institute of Microbiology in Prague. This technique uses in-situ generated singlet oxygen as reactive specie for footprinting. Singlet oxygen is generated via the photodynamic effect by irradiating a dissolved photosensitizer with a diode laser of a suitable wavelength under controlled conditions. Our follow up work is focused on building a lab-made apparatus equipped with an injection valve that enables high-throughput sampling. In this setup, the protein sample is introduced into a continuous buffer flow and mixed with the dissolved photosensitizer inside the apparatus. The oxidized protein samples are then collected and analyzed using bottom-up and top-down proteomic workflows.

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ACKNOWLEDGEMENT:

We acknowledge support from grant: Microorganisms in Ecological Remediation – Bacteria as Cell Factories for Controlled Bioremediation of Ecosystems (CZ.02.01.01/00/23_020/0008502)

ThP-16: Insight into Hemoglobin Digestion Pathways of Sphaerospora molnari by Functional Proteomics

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Sphaerospora molnari is a blood-dwelling myxozoan parasite of common carp (Cyprinus carpio) that proliferates in the host's bloodstream, leading to severe anemia. Infected erythrocytes serve as a nutrient source for the parasite [1,2]. Heme, an essential cofactor for aerobic metabolism, can be acquired by hematophagous parasites either through endogenous biosynthesis, scavenging from the host, or a combination of both. Our unpublished data indicate de novo heme synthesis via a complete heme biosynthetic pathway in S. molnari.

In this study, we investigated host hemoglobin degradation mediated by a proteolytic cascade of S. molnari enzymes. Activity assays using S. molnari blood-stage (SMBS) lysates, in combination with class- and enzyme-specific inhibitors, revealed that cysteine (cathepsin L) and aspartic (cathepsin D) peptidases are the principal hemoglobinolytic enzymes. Inhibition of either enzyme class led to partial degradation, while combined inhibition completely blocked hemoglobin cleavage. LC–MS analysis of digestion products further highlighted the key role of cysteine peptidases in the initial steps of hemoglobin breakdown.

These findings provide new insights into the metabolic adaptations of S. molnari to its blood-parasitic lifestyle and highlight key proteases as potential targets for therapeutic intervention.

These findings provide novel insight into the proteolytic and metabolic mechanisms underlying nutrient acquisition in S. molnari and highlight key proteases as potential targets for therapeutic intervention.

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ACKNOWLEDGEMENT:

This work was supported by the Czech Science Foundation (GA ČR) and the Austrian Science Fund (FWF) under grant no. 24-13238L, as well as by GA ČR grants 21-16565S and 19-28399X.

ThP-17: High-flow HESI interface with point-focusing and enhanced desolvation

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Ionization is a crucial step in mass spectrometry as the amount of ions generated in the ion source affects the method sensitivity. Electrospray ionization (ESI) represents the most commonly used ionization techniques in LC-MS. In ESI, ions are formed by multistep desolvation from multiply charged droplets that are few µm in size. Heated ESI (HESI) design with support of nitrogen nebulizing gas is commonly used to enhance the evaporation process if the flow of the mobile phase exceeds approx. 50-100 uL/min. Unfortunately, HESI suffers from low ionization efficiency due to large losses of analyte molecules. These losses are due to the excessively large droplets from which the solvent is not able to evaporate before entering vacuum region, also due to formation of clusters or neutral molecules that can't be used by mass spectrometer. It is often estimated that only 0.1 % or less of the analyte molecules are transformed into usable ions that reach the detector. This is a very different situation from the nano-electrosprays ion sources, which use orders of magnitude lower liquid phase flow rates. Nano-electrospray ion sources are significantly more efficient because smaller droplets are formed and solvent evaporates more rapidly. No nebulizing gas is needed, which prohibits disruption of the ESI process. However, for routine LC-MS analysis, nano-sources are not practical and are very seldomly used. Therefore increasing HESI efficiency is a desirable research goal with direct impact on analytical figures of merit of the mass spectrometers. Our research is focused on designing HESI ion source in which the droplets will undergo more efficient evaporation and desolvation process and thus produce higher flux of useful ions transportable into the vacuum of the mass spectrometer.

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ACKNOWLEDGEMENT:

The research was supported by Czech Science Foundation Grant No. 25-16715J and by Ministry of Education, Youth and Sport grant Microorganisms in Ecological Remediation – Bacteria as Cell Factories for Controlled Bioremediation of Ecosystems (CZ.02.01.01/00/23_020/0008502)

ThP-18: Proteomics identifies the landmark protein expression difference among the established mutation-based clusters of human pheochromocytoma and paraganglioma. Protein profiles can verify or correct diagnostic classification.

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Pheochromocytoma and paraganglioma (PPGL), are rare neuroendocrine tumors from chromaffin cells of adrenal gland or extra-adrenal paraganglia. Mutations in more than 20 genes have been identified as drivers of PPGL tumorigenesis. Four distinct clusters of PPGL (1A, 1B, 2, and 3) are being recognized based on the mutations, differing in tumor aggressiveness and patient prognosis. Roughly 25% of tumors remain “unassigned” as no mutation in the known driver genes is found. We previously demonstrated that proteomic profiles of PPGL tumors can correctly categorize the tumors in agreement with the established mutation-based clusters. This was confirmed in our current proteomic analysis which included tumors with SDHB (Cluster 1A), VHL (Cluster 1B) and RET (Cluster 2) mutations as well as “unassigned” patients. However, there was an apparent miss-categorization of one patient. While the patient/tumor was reported as having no mutation, i.e. being characterized as “unassigned” by the clinical genetic laboratory, the tumor clustered along with Cluster 1B tumors (with VHL mutation) based on our proteomic data. We therefore initiated a re-sequencing of the sample in the genetic laboratory. It confirmed that our proteomic-based cluster assignment was correct and the patient actually had a pathogenic mutation in VHL gene. Why the mutation was initially overlooked is not known. In addition to identifying the protein expression landmarks of PPGL clusters such as MTHFD2, ARG2, PYCR1, SHMT2 (Cluster 1A), BCAT1, SLC16A3, KCNAB2 (Cluster 1B) and GDF10, GDF15 and KIF21A (Cluster 2), we demonstrated the accuracy of the proteomic PPGL profiling and the potential benefits it can deliver in clinical or diagnostic settings. Support: AZV NU23-01-00323, EXCELES LX22NPO5102 and SVV 260763.

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ThP-19: Application of LC–MS/MS and fluorescence-based assays in the evaluation of novel carbonic anhydrase inhibitors

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Carbonic anhydrase (CA) plays a crucial role in maintaining glycolytic metabolism under hypoxic or acidic conditions in glioblastoma cells. To evaluate the efficacy of newly synthesized sulfonamide-based CA inhibitors, we established a comprehensive analytical and biochemical workflow. Specifically, (i) a new fluorescence-based assay was optimized to estimate CA activity under acidic conditions; (ii) in vitro inhibition assays were performed to determine the potency of the novel compounds; (iii) MS/MS spectra of all new derivatives were acquired and structurally characterized; (iv) a targeted LC–MS/MS method was developed and validated for their quantitative determination; and (v) the metabolic and pharmacological effects of the inhibitors were assessed in cultured glioblastoma cells. Among the tested compounds, five newly synthesized sulfonamide derivatives exhibited strong inhibitory activity toward CA and demonstrated potential to alter cellular metabolism and viability of glioblastoma cells. The newly obtained MS/MS spectra of these compounds will be submitted to international spectral databases (GNPS) to support further research and compound identification efforts.

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ThP-20: Production of sialylated glycoproteins in Ixodes ricinus ticks and tick cell lines

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Sialic acid is a negatively charged monosaccharide composed of nine carbons, typically attached to the ends of glycoproteins and glycolipids. It occurs in various eukaryotes, including mammals and invertebrates, as well as in bacteria and viruses. However, it is primarily recognized as a typical glycan in mammals. The high levels of sialic acid on the cell surface and in secreted proteins indicate its significant role in cellular communication. It functions as a receptor for various signalling pathways, including cell adhesion, immune responses, neurogenesis, and processes related to pathogen invasion. For these reasons, sialic acid is considered a promising target for therapeutic interventions. The tick Ixodes ricinus is a key vector for pathogens that cause severe infections in humans and livestock. Evidence indicates that ticks contain sialylated glycoproteins, potentially acquired from the blood of their hosts. To confirm the potential of ticks to produce sialylated glycoproteins, we searched the I. ricinus genome to reveal enzymes in the metabolic pathway of sialic acid synthesis and sialyltransferases attaching sialic acid to glycans. Furthermore, we confirmed sialyltransferase expression in ticks and sialyltransferase activity in tick cells. Finally, using Click chemistry and an azide-modified sialic acid precursor, we detected sialylated glycoproteins of tick origin in tick cells and tick early life stages. Thus, we confirmed the ability of ticks to produce their own sialylated glycoproteins in addition to those originating from the host blood.

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ACKNOWLEDGEMENT:

This study was supported by the Ministry of Education, Youth and Sports of the Czech Republic INTER-ACTION project LTAUSA18040, and the Grant Agency of the Czech Republic (22-25042S and 23-06525J). This work was supported by the European Regional Development Fund-Project "Mechanisms and dynamics of macromolecular complexes: from single molecules to cells" (No. CZ.02.1.01/0.0/0.0/15_003/0000441).

ThP-21: Application of LC–MS-Based “Polaromics” in Biochemistry, Pharmacology, and Biomedicine

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“Polaromics” represents an emerging analytical concept focused on the comprehensive profiling of polar and ionic molecules — such as amino acids, nucleotides, organic acids, and drugs — that are often underrepresented in conventional metabolomics. The approach emphasizes the development of optimized chromatographic–mass spectrometric workflows, particularly those based on hydrophilic interaction liquid chromatography coupled with high-resolution mass spectrometry (HILIC–MS).

We have optimized a HILIC–MS protocol that allows quantification of nearly 200 polar and ionic metabolites and more than 50 pharmacologically active compounds in various biological matrices.

The practical implementation of LC–MS-based polaromics in cell-biochemical, pharmacological, and biomedical research has provided valuable data enabling detailed exploration of anaplerotic metabolism and amino acid catabolism in human brain cells, metabolic heterogeneity among distinct neuronal and tumor cell types, and pharmacological aspects of temozolomide, 2-deoxyglucose, metformin, and gliflozins. We use LC-MS polaromics to identify the potential of newly synthesized inhibitors of carbonic anhydrase.

The LC–MS based polaromic workflows combine minimal sample handling, high analytical sensitivity, and the ability to detect both cellular and extracellular metabolites simultaneously. Collectively, LC–MS-based polaromics represents a powerful tool bridging metabolomics and pharmacology with cellular biochemistry and physiology. Its integration into biomedical research enhances our mechanistic understanding of metabolic responses to therapy, supports the identification of novel polar biomarkers.

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ThP-22: Development of Software for LC-MS Data Processing of Oligonucleotides

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We present a Python-based software tool designed for the efficient analysis and interpretation of oligonucleotide fragmentation patterns. Initially developed for the study of oxidative cleavage induced by FPOP (Fast Photochemical Oxidation of Proteins), the software tool generates theoretical libraries of monoisotopic fragment masses across multiple charge states and matches them to experimentally detected peaks within a defined mass error tolerance.

The newly expanded functionality goes beyond radical-induced fragmentation. The tool now supports simulation of enzymatic cleavage, including products generated by RNases T1 and U2 acting on RNA, and performs automatic annotation of the corresponding fragments. Additional features include graphical output and fragment quantification, enabling a flexible and efficient workflow.

In future development phases, the software could be applied to miRNA analysis from body fluids to support early diagnostics of inflammatory or cancer-related diseases. The tool supports both qualitative and quantitative analysis and, thanks to its intuitive graphical user interface, is accessible even to users with minimal programming experience.

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ThP-23: Enhanced Bottom-Up Proteomics for Histone Peptidoform Profiling

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Histone post-translational modifications (PTMs) play a crucial role in regulating chromatin organization and gene expression, directly influencing numerous biological processes and diseases. However, the complexity of histone proteoforms, resulting from the diversity of their modifications, presents substantial challenges for reliable identification and quantification by mass spectrometry-based approaches.

In our work, we employ a bottom-up approach that involves enzymatic digestion and chemical derivatization during sample preparation prior to LC-MS/MS analysis. These procedures introduce specific issues related to incomplete digestion and properties of a particular derivatization agent. To address these challenges, we focus on optimizing and developing sample preparation protocols to improve the specificity, reproducibility, and accuracy of histone PTM characterization.

Histone preparation protocols are being optimized for specific mammalian and plant matrices. As an example of applying the methodology to clinical research, a study investigating the molecular mechanisms underlying male infertility is shown. The project involves LC-MS/MS analysis of both histone PTMs and the complete sperm proteome. The obtained data are associated with the results of sperm cell morphological and physiological analysis, with subsequent correlation to patient lifestyle information. Initial data evaluation reveals trends suggesting associations between specific histone modification patterns, proteomic alterations, and sperm quality parameters. These findings provide a foundation for further statistical validation and mechanistic exploration, aiming to identify molecular markers linked to impaired fertility and inform future diagnostic strategies.

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ACKNOWLEDGEMENT:

We gratefully acknowledge Repromeda clinic for kindly providing clinical samples for this study.

ThP-24: Detection of deoxynivalenol in grain products by LC-MS

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Mycotoxins arise as secondary metabolites produced by fungi and their presence in grain products poses a significant danger to public health. Currently there are several hundreds of these toxic substances that have been identified. They are known to be carcinogenic, cytotoxic, and neurotoxic and have potential to damage the immune system. The presence of mycotoxins in cereal crops therefore poses a permanent health risk, which is eliminated by thorough testing of edible grain products. ELISA, an immuno-based technique that utilizes interaction between the target toxin and respective antibody molecule, is commonly used in testing because of its simplicity and fieldability. However, it is known that immunoaffinity methods that target low-molecular-weight antigens often suffer from both false positive and false negative errors. This issue has also been described when utilizing ELISA for mycotoxin detection. Our work is focused on investigating the presence and behavior of a specific mycotoxin – deoxynivalenol (also known as vomitoxin) - in grain samples from the Czech Republic. Deoxynivalenol is an epoxy-sesquiterpenoid that inhibits protein synthesis. It is not known to be carcinogenic, and it is toxicologically less dangerous than some of the other mycotoxins, but it occurs relatively frequently, predominantly in grains such as wheat, barley, oats, rye, and corn. In this work we have developed an LCMS/MS method for determination of deoxynivalenol in grains and compared the results with the ELISA technique used for screening. The aim of the work is to identify samples that failed ELISA screening, examine them also by non-targeted LC-MS/MS analysis, and try to describe molecular factors that cause false positive and false negative results in ELISA test.

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ACKNOWLEDGEMENT:

We acknowledge support from grant: Microorganisms in Ecological Remediation – Bacteria as Cell Factories for Controlled Bioremediation of Ecosystems (CZ.02.01.01/00/23_020/0008502)

ThP-25: FAmOS versus Percoll: Advancing Organelle Isolation Efficiency and Proteomic Compatibility in Plant Research

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Intercellular communication and regulatory interactions between different cell types and organelles are fundamental to proper plant development, growth, plasticity, and physiological responses. These interactions enable plants to adapt dynamically to internal cues (e.g., hormonal signals) and external stimuli (e.g., environmental stresses). To advance our understanding of these intricate, cell-type-specific mechanisms, we have recently developed Fluorescence-Activated multi-Organelle Sorting (FAmOS) [1], an innovative fractionation technique based on advanced flow cytometry. Using a sequential gating strategy that exploits the individual optical characteristics and fluorescence of selected live-staining dyes, FAmOS enables the parallel separation of multiple organelles from a single sample. In a proof-of-concept study of the cellular allocation of two antagonistic phytohormone families (auxins and cytokinins), this method showed significant promise for enhancing our understanding of the role of organelles in processes vital to plant life.

Here, we benchmark the performance of FAmOS against the gold-standard method of organelle isolation, Percoll density gradient centrifugation, and test its applicability to proteomics. Using chloroplasts as a model compartment, both methods were compared in terms of yield, viability, and purity of isolates, as well as time and labor demands. The obtained data suggest that FAmOS can be easily adapted for modern omics analyses, providing deep insights into the organellar proteome from limited sample inputs. As such, it could open new avenues for studying plant physiology in its native spatial context.

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ThP-26: Quantification of monoclonal antibodies by middle-up capillary electrophoresis coupled to mass spectrometry

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Monoclonal antibodies (mAbs) are biopharmaceuticals widely used in treatment of various diseases. Due to their intrinsic complexity and post-translational modifications, therapeutic mAbs are highly heterogeneous molecules requiring extensive quality control. Quantitative analysis typically relies on liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) and the gold standard bottom-up (BU) proteomic approach. This study presents an alternative middle-up (MU) approach combined with capillary zone electrophoresis–mass spectrometry (CZE–MS). CZE has previously demonstrated suitability for mAb analysis and is recognised as a quality control technique in the pharmaceutical industry. Here, we focus on the quantitative MU CZE–MS analysis of the immunoglobulin G (IgG) mAb infliximab (IFX) in the pharmaceutical product Remicade and two other biopharmaceuticals. IFX calibration standards were prepared at six concentrations, and mAb standard solution of adalimumab (ADA) (Merck, Darmstadt, Germany) as internal standard was added to each sample. Next, samples were reduced with TCEP reducing agent, then analysed using an Agilent 7100 CE system coupled to an Agilent 6410 triple quadrupole mass spectrometer (Agilent, Santa Clara, USA). Biopharmaceutical formulations were diluted to 20 µg/mL and analysed using the same MU CZE–MS workflow. The developed method was validated in accordance with the ICH Q2(R1) guidelines for analytical method validation. It demonstrated good accuracy and precision for IFX quantification in Remicade, and was further applied to quantify bevacizumab and rituximab in their respective drug formulations. The results indicate that the optimised MU CZE–MS method is suitable for the quantitative analysis of various IgG therapeutic mAbs.

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ACKNOWLEDGEMENT:

This work was funded by the projects VEGA 1/0514/22, VEGA 1/0525/25 and by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under the project No. 09I03-03-V04-00208.

ThP-27: Integrative proteomic and hormonomic analysis of barley chloroplast mutant chlorina f2f2

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Barley (Hordeum vulgare) is a key cereal crop and a model organism for studying chloroplasts. Its value is enhanced by the availability of various mutants, notably chlorina f2f2, which lacks chlorophyll b. This mutant has been widely used to study the role of chlorophyll in photosynthesis, thylakoid organization, and chloroplast structure. Despite its significance, a detailed proteomic analysis of chlorina f2f2 has not been conducted until now. In this study, we compared chloroplasts and thylakoid membranes from wild-type barley (cv. Morex) and chlorina f2f2 using comprehensive proteomics. We found strong downregulation of proteins related to photosynthesis, chlorophyll biosynthesis, thylakoid structure, and unexpectedly, hormone biosynthesis. Subsequent phytohormone and metabolite profiling confirmed these findings. These results reveal that chlorina f2f2 is not only a model for chlorophyll b deficiency but also reflects broader changes in hormonal balance and stress signalling, making it a valuable system for exploring the links between pigment biosynthesis, photosynthetic function, and hormonal regulation.

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ACKNOWLEDGEMENT:

This research work was supported by IGA_PrF_2025_019.

ThP-28: Microplastics and the Blood–Brain Barrier: Mechanisms of Transport and Endothelial Response

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Microparticles are particles with a diameter ranging from 1 to 1000 micrometers. Microparticles are found in our environment, including drinking water, food, and air, making human exposure inevitable. The blood-brain barrier (BBB) is a highly selective barrier that protects the central nervous system (CNS) by preventing most substances in the blood from entering brain tissue. Understanding how microplastics interact with the BBB has become a critical area of research to determine the potential neurological risks associated with microplastic exposure. Although the precise mechanisms by which microplastics cross the BBB remain incompletely characterized, several pathways have been proposed, including passive diffusion, endocytosis, and transcytosis. To investigate microplastic transport, we employed a primary rat endothelial cell model of the BBB. Transport efficiency and alterations in endothelial protein expression were analyzed. To characterize changes in protein expression we used mass-spectrometry. Aliquots of purified peptide mixtures (500 ng) were separated using an Acquity M-Class UHPLC system (Waters, Milford, MA, USA). Peptides were introduced into a nanoEase HSS T3 C18 analytical column and analyzed on a ZenoTOF 7600+ time-of-flight mass spectrometer (Sciex, USA). Spectra were acquired in a data-independent acquisition mode (SWATH), and data processing was performed using PEAKS Studio 13 software.

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ACKNOWLEDGEMENT:

Interreg VI-A SK-AT, APVV-22-0313, VEGA 2/0075/24

ThP-29: Reactivity of Singlet Oxygen Toward Amino Acid Side Chains

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Covalent labelling techniques coupled with mass spectrometry are powerful tools for elucidating the structure of proteins and protein complexes, capturing conformational changes and identifying interaction sites with other biomolecules. These protein footprinting techniques rely on the irreversible modification of solvent accessible amino acid residues by a chemical probe. Singlet oxygen (1O2) is a reactive oxygen species that reacts with a broad range of biomolecules, including proteins. It exclusively oxidises the side chains of Cys, His, Met, Phe, Trp and Tyr, and is easily and cost-effectively generated, making it a promising alternative for protein footprinting. In this work, we explored the potential of 1O2 generated via red laser irradiation of phthalocyanine using short polypeptides under varying pH conditions and in the absence or presence of ammonia salts. A noteworthy increase in the extent of oxidation with pH was found for most modifications, with pH 7 and 8 identified as optimal for most of them. Multiple oxidation products of His, Met, Tyr and Trp were detected with sub-amino acid resolution, including a +50 Da mass shift on His identified in angiotensin, not yet experimentally reported. Ammonium salts showed little effect apart from the +31 Da modification on His, corresponding to ammonia hydrolysis. Overall, our findings support the premise that 1O2 is a powerful addition to the existing array of probes for protein footprinting.

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ACKNOWLEDGEMENT:

This work was supported by the Czech Science Foundation (25-18181S), MEYS - OP JAK (CZ.02.01.01/00/22_008/0004597) and MEYS - OP JAK (CZ.02.01.01/00/23_020/0008540).

ThP-30: Applied and Residual: Assessment of Pesticide Residues and Transformation Products in Irrigated Czech Agricultural Fields.

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Modern agriculture depends heavily on pesticides to secure crop yields, yet many active ingredients and their transformation products (TP) persist in soils or leach into groundwater. To distinguish legacy contamination from recent applications, we collected composite soil samples. We matched plant samples (edible parts and leaves) from carrot, potato, maize, onion, and parsley fields at the same locations in 2023 and 2024. Targeted LC–MS screening quantified 57 pesticides and their TP in the soil–leaves–edible plant tissue system ($ng \cdot g^{-1}$, dry weight). Authorized, crop-targeted pesticides dominated soil burdens (carrot fields: 59–98%; potato fields: 31–95%; with a range of 350$ng \cdot g^{-1}$ to 3000 $ng \cdot g^{-1}$ in potato fields). TP occurred more frequently and at higher abundance in soils than in plant tissues. They were occasionally detected in leaves and rarely in edible parts, where residues and their number consistently remained well below applicable MRLs (maximum residue limits set by legislation). The detection of approved pesticides not authorized for the given crop in leaves suggests potential off-label use, making leaves a practical marker of recent application. The results also confirm that soil acts as a persistent “memory” of past inputs and, under irrigation, is a potential source of groundwater contamination via leaching. Combined matrix sampling (soil, leaves, and edible tissue) can help indicate both applied and residual components, improving the assessment of application practices and environmental risk.

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ACKNOWLEDGEMENT:

Acknowledgments: The work was supported by the Ministry of Agriculture of the Czech Republic, project No. QK23020018

ThP-31: Expanding the FFAP protocol for structural characterization of membrane proteins

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Protein footprinting is a mass spectrometry based method that provides information about solvent accessible residues through covalent modification. Because the extent of modification depends on the environment surrounding the modifiable residues, this method can be used to monitor protein-protein or protein-ligand interactions, structural comparison of wild type and mutant proteins.

In this work, we applied the Fast FluoroAlkylation of Proteins (FFAP) protocol developed in our laboratory [1], to study membrane Cl-/H+ transporter (ClC-ec1). Specifically, we focused on the comparison of the wild type and the QQQ mutant, that was designed to mimic the outward facing state of the channel. The FFAP workflow involves mixing the studied protein with a Togni reagent II [2] that releases a CF3 radical upon activation by ascorbic acid. The released radicals can modify side chains of aromatic residues and cysteine. The studied protein was analysed by mass spectrometry (timsTOF Pro, Bruker Daltonics) using bottom-up approach.

The results show that we were able to modify the ClC-ec1 membrane protein using the FFAP protocol. Overall, ten residues were modified in the wild-type and the QQQ mutant, suggesting that this technique is capable of modifying membrane proteins under ambient conditions.

Funding of the Charles University Grant Agency (grant no. 448425) and of the Czech Science Foundation (grant number 25-18181S) is gratefully acknowledged.

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ThP-32: DigDig: Streamlining Digestion Analysis for Proteomics and HDX-MS

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Proteolytic digestion is a fundamental step in both classical bottom-up proteomics and structural proteomics workflows such as chemical cross-linking, radical labeling, and hydrogen/deuterium exchange (HDX) mass spectrometry. While traditional proteomics relies on specific proteases with well-defined cleavage patterns and minimal missed cleavages, HDX experiments employ non-specific or semi-specific acidic proteases that generate complex peptide mixtures with extensive overlaps. Although such complexity complicates data interpretation, it simultaneously enhances spatial resolution and increases the number of measurable data points. With the growing diversity of proteolytic columns and enzymes, reliable tools for detailed assessment and comparison of digestion efficiency are increasingly required.

To address this need, we developed DigDig, a standalone Java-based software designed for comprehensive analysis and visualization of proteolytic digestion. DigDig processes outputs from various search engines together with sequence databases to extract key digestion metrics, including sequence coverage, reproducibility, redundancy, cleavage preferences, and peptide length distributions. The software offers an array of intuitive, customizable visualization modes – coverage maps, redundancy plots, peptide length histograms, and cleavage preference profiles – allowing rapid comparison of different digestion conditions or proteases. We demonstrate how these visualizations facilitate protease characterization, optimization of HDX-MS workflows, and quality control in both targeted and complex proteomic analyses.

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ACKNOWLEDGEMENT:

Financial support from the MEYS/EU project OP JAK Photomachines (CZ.02.01.01/00/22_008/0004624) and access to the Centre of Molecular Structure (CMS), BioCeV, provided by CIISB LM2023042 and ERDF "UP CIISB" (CZ.02.1.01/0.0/0.0/18_046/0015974) is gratefully acknowledged.

ThP-33: Illuminating Proteins: Dual UV/IR Photodissociation in a custom FT-ICR system

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Top-Down mass spectrometry (TDMS) elucidates the molecular weight, characterizes proteoforms, and, in its native form, can even inform binding stoichiometry and higher-order structure of proteins and their complexes. The information gained is, however, largely dependent on the protein system in question and the dissociation technique used. Therefore, there is a great need for more efficient and informative fragmentation methods to complement continued improvements in mass analyzers. These are together essential for extending the Top-Down MS to larger and more diverse assemblies. Photodissociation approaches, such as infrared multiple photon dissociation (IRMPD) and ultraviolet photodissociation (UVPD), coupled with ultrahigh-resolution MS, offer interesting avenues for multi-modal fragmentation schemes with high information content.

In this contribution, following up on the pioneering work by Halim et al. (JASMS 2016) we explore parallel 193 nm UVPD and 10.6μm IR laser activation applied to proteins using a custom laser-coupled 15-Tesla FT-ICR mass spectrometer. A rich and balanced fragmentation array of a/x, b/y, and z ions is produced when intact ubiquitin is exposed to UV and IR lasers simultaneously or sequentially in a single MS/MS experiment. Internal fragment inclusion in data processing further increases identified fragment numbers and improves average sequence coverage for denatured proteins. Our initial results demonstrate that the use of mixed photodissociation provides benefits for comprehensive protein characterization of denatured proteoforms and offers great promise for future native TDMS, especially when combined into multi-modal fragmentation schemes with other dissociation techniques available in an FT-ICR.

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ThP-34: Decoding Therapy-Induced Protein Subcellular Relocalization in Leukemia

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Protein localization within cellular compartments is a tightly regulated process essential for proper protein functioning. Different localizations can affect protein activity, sometimes resulting in new functions. Recent studies have shown that anti-cancer treatment can induce protein relocalization, enabling them to acquire new functions that help cells survive the treatment. Such adaptive mechanisms play a major role in the development of cancer therapy resistance, which remains a challenge in the treatment of acute myeloid leukemia (AML). Our study explores how anti-leukemic therapy affects protein subcellular localization to uncover mechanisms that enable leukemic cells to withstand treatment.

To investigate this, we treated an AML cell line with the combination of hypomethylation therapy (5-azacytidine, AZA) and an inhibitor of anti-apoptotic protein BCL-2 (venetoclax, VEN). We applied the LOPIT-DC approach, which combines fractionation by differential ultracentrifugation, followed by isobaric labeling with tandem mass tags and LC-MS3 to untreated AML cells and AML cells treated with AZA and VEN. We identified 5,853 proteins and, using SVM-based prediction, we assigned 51% of proteins to specific cellular compartments. Our data revealed that AZA/VEN alters the subcellular localization of 215 proteins, identifying 12 relocalization events with high confidence (SVM score > 0.7). The proteins are crucial for protein stability, protein folding, and cellular division. Our findings highlight the potential of spatial proteomics to uncover how protein localization influences treatment response and resistance in AML.

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ThP-35: Advancing AI/ML-Based Post-Translational Modification Assignment Using a Verified FPOP Dataset

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Artificial intelligence (AI) and machine learning (ML) algorithms have recently become increasingly popular in scientific research, including studies involving omics technologies. These algorithms learn patterns directly from data and generate predictions without the need for explicit programming tailored to each task. Therefore, using carefully validated model datasets for training is essential to ensure accurate learning and reliable results.

Fast Photochemical Oxidation of Proteins (FPOP) is a sophisticated structural mass spectrometry (MS) method that introduces oxidative modifications to amino acid side chains within a protein sequence (1). The hemoglobin (Hb)–haptoglobin (Hp) complex poses a particular analytical challenge because of its structural complexity, including disulfide bonds and glycosylation sites, which demand precise sample handling. To investigate FPOP-labeled HbHp complexes, we integrated verified spectral libraries with data-independent acquisition (DIA) analyses. Initially, data-dependent acquisition (DDA) datasets were processed with FragPipe and validated in Skyline to construct a spectral library. The DIA data were then matched to the library entries, and dot product scores were computed to assess similarity. This process improved the reliability of the library by filtering out nonrepresentative identifications, resulting in 421 accepted entries for the model HbHp dataset.

Compared with conventional FPOP data processing—where search engine results are followed by general filtering—using a manually curated spectral library increases identification confidence and enhances spatial resolution (2). The final dataset contains 346 validated fragment spectra corresponding to oxidative modifications, including both mono- and dioxidations. This dataset provides a high-quality reference for AI/ML training focused on post-translational modification (PTM) identification, where single-amino-acid resolution is critical, since all entries are experimentally verified. Furthermore, it helps resolve challenges related to isobaric peptides, where oxidation occurs at the same residue but in different structural positions, such as o-, p-, and m-hydroxyphenylalanine.

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ACKNOWLEDGEMENT:

This work was financially supported by the Technology Agency of the Czech Republic (ODEEP-EU TH86010001).

ThP-36: Analysis of glycerophospholipids using the method of direct infusion into a tribrid mass spectrometer with high mass resolution

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Glycerol phospholipids are the most abundant and a key structural component of all biological membranes. The structure consists of a glycerol backbone with two fatty acids in the positions 1 and 2, and a phosphate group with a hydrophilic head attached to the position 3[1]. Their unique mass spectrometric properties enables avoiding a chromatography step and can by directly injected to the mass spectrometer – a shotgun approach[2]. Low resolution triple-quadrupole-mass spectrometers have been extensively used for the shotgun lipidomics. However, introducing new generations of high-resolution mass spectrometric instrumentation facilitating several fragmentation principles and parallel mass spectrometric experiments made the analysis faster and richer regarding the structural information[3]. A particular challenge is to determine and quantify isobaric and isomeric species.

We developed a method of direct-infusion approach using Orbitrap Fusion (Thermo Scientific) mass spectrometer in connection with noncommercial software LipidHunter2 and in-house developed software Lipidator. Furthermore, we validated using highly abundant fatty acids fragments arising from MS^2 experiment for quantification of isobaric phosphatidylcholine species.

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ThP-37: *In situ* comparison of hormonomics changes in different biotic stresses using mass spectrometry imaging

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As one of the most important signalling biomolecules, plant hormones, together with their metabolites, play critical roles in regulating plant responses to different biotic stresses, such as insect damage or microbial infection. However, direct visualisation and *in situ* identification of multiple plant hormones from their original localisations are still challenging tasks due to their very low abundance and complex molecular backgrounds inside plant tissues.

Currently, LC-MS and reporter gene assays are predominant methods for the targeted plant hormone analysis in plants, but spatial distributions of untargeted plant hormones and compounds involved in the metabolomics network cannot be measured within the same experiment. On the other hand, MS imaging (MSI) as an untargeted and label free analytical method has been widely applied for the *in situ* visualisations of plant metabolites, lipids, proteins as well as other biomolecules, and has demonstrated unique advantages in comparison with classical analytical methods [1]. Recently, we have applied desorption electrospray ionisation MSI (DESI-MSI) to visualise multiple hormone species and related derivatives from leaves and roots [2,3].

Aiming to further compare plant hormonomic alterations between different biotic stress responses, MALDI-MSI was performed on *Arabidopsis* leaves and flowers damaged via pathogenic insect and bacterial wounding to characterise major plant hormones as well as other related metabolites from their intact positions. Our results highlighted unique distributions of salicylic acid, auxins, jasmonic acid, and cytokinins from stressed tissues. Further correlation analysis reveals potential spatial connections among plant hormone species and other detected metabolites. This suggests that MALDI-MSI not only enables high throughput analysis of hormones in plants under biotic or abiotic stress, but also offers insights into hormone-hormone and hormone–metabolite interactions within heterogeneous plant tissues.

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ACKNOWLEDGEMENT:

The work was supported by the Czech Science Foundation (no. 24-11511S).

ThP-38: Large-scale metabolomics screening of understudied plant lineages

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The Plant Kingdom represents an immense source of chemical diversity. However, phytochemical research has historically focused on a small number of model species, leaving most plants chemically unexplored. We aim to uncover and characterize this hidden diversity by combining automated literature mining, standardized sample collection, and computational metabolomics.

We identify chemically understudied plant lineages by combining phylogenomic datasets with automated mining of scientific literature. Selected plant species are collected from botanical gardens following the standardized Digital Botanical Garden Initiative (DBGI) protocol, ensuring reproducible sampling and metadata capture. The samples are analyzed using LC-MS-based metabolomics, generating comprehensive chemical profiles that are processed using advanced computational algorithms to detect and prioritize previously unknown molecular structures.

This integrative workflow provides a scalable framework for systematically exploring the chemical space of plants and accelerating the discovery of novel natural products from underexplored species.

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ThP-39: Quantitative and Redox Proteomics Identify Signaling Networks Shaping Leukemia Survival and Resistance

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INTRODUCTION:

Treatment of Acute myeloid leukemia (AML) remains challenging. The standard therapy, including hypomethylating agents and anti-apoptotic protein inhibitors, often fails due to the development of resistance. Our data and recent evidence indicate that resistant AML cells undergo metabolic reprogramming that enhances antioxidant defenses. Dysregulated redox homeostasis alters oxidative signaling and protein function, promoting drug evasion.

AIMS:

To identify redox-regulatory proteins supporting leukemia cell survival and therapy resistance.

METHODS:

Therapy-sensitive and -resistant AML cells were treated with inhibitors of glutathione (GSH) metabolism, protein S-glutathionylation (P-SSG), and the thioredoxin (TXN) system. Quantitative (TMT 16-plex) and redox (iodoTMT 6-plex) proteomics were performed using an Orbitrap Ascend LC-MS^3 platform. Data was processed in Proteome Discoverer and visualized in Perseus.

RESULTS:

We quantified ~7,700 proteins and identified oxidation states of ~22,000 cysteines in ~6,800 proteins. GSH inhibition caused modest proteome changes but altered >500 cysteines in proteins regulating cell cycle, DNA repair, and proteolysis. TXN inhibition upregulated acute stress proteins (HMOX1, HSPs, FOS) and induced global oxidation.

CONCLUSION:

Complementary proteomic analyses revealed distinct pathways impacted by GSH- and TXN-mediated antioxidant systems. Inhibition of both pathways suppressed proliferation, but through different mechanisms. GSH depletion oxidized cell cycle proteins, while TXN disruption upregulated cell death–associated proteins.

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ThP-40: Deciphering phlebotomine sand fly taxonomy using MALDI-TOF MS protein profiling

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Phlebotomine sand flies (Diptera: Psychodidae) are important vectors of various pathogens like *Leishmania* protozoans, bacteria and viruses. When studying the epidemiological significance of sand flies in endemic regions, conclusive species identification helps to understand the dynamics of local transmission cycles, to assess potential emergence of sand flies in new regions and to design efficient disease control measures. During the last decade, protein profiling using MALDI-TOF mass spectrometry has been successfully employed by our group in sand fly research. The approach was proved as a method of choice for reliable, time and cost-effective species identification of large sets of field-collected specimens from various endemic regions of the Old World including the Mediterranean, East Africa or Southeast Asia, creating a reference database that currently includes more than 40 different sand fly species. Standardized protocol of specimen trapping, storage and sample preparation enables to acquire reproducible species-specific protein profiles that serve as useful tool in integrative taxonomy, supporting formal description of new species (*Phlebotomus creticus* in Greece; *Sergentomyia imihra* in Algeria), suggesting existence of yet undescribed cryptic species (*Adlerius sp.* in Moldova), discriminating among sibling species (*Phlebotomus perniciosus/longicuspis* complex in Morocco) and challenging validity of established taxons while allowing utilization of a single sand fly specimen for parallel application of complementary approaches (DNA barcoding, morphological analysis, bloodmeal identification, pathogen screening).

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ACKNOWLEDGEMENT:

Czech Science Foundation GA23-06299S

ThP-41: Metabolomics Analysis of Type 1 Diabetes Mellitus Among Qatari Children Using LC-MS

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BACKGROUND:

Type 1 diabetes mellitus (T1DM) is an autoimmune disorder with rising incidence in Qatar, yet metabolic profiling in pediatric populations remains scarce. We investigated serum metabolomic and inflammatory signatures in Qatari children with T1DM to identify potential biomarkers.

METHODS:

Serum samples from T1DM patients (n=50) and healthy controls (n=50) were analyzed using the Biocrates MxP® Quant 500 kit on an ACQUITY UPLC–Xevo TQ-S LC-MS/MS system (>600 metabolites). Parallel cytokine profiling was performed by multiplex immunoassay. Multivariate analyses (PCA, PLS-DA, regression) identified discriminatory metabolites and pathway alterations.

RESULTS:

T1DM children exhibited distinct metabolic signatures compared to controls. Key differences included reduced methionine, glutamine, and succinate, with elevations in lactate and disrupted lipid metabolism (phosphatidylcholines, sphingomyelins, acylcarnitines). Several metabolites showed strong classification potential (AUC >0.85). Integration with cytokine data revealed correlations between IL-6, TNF-α, and lipid/amino acid perturbations, suggesting interconnected metabolic–inflammatory pathways.

CONCLUSION:

This first LC-MS-based metabolomics study of pediatric T1DM in Qatar highlights novel metabolic and immune biomarkers with diagnostic and prognostic potential. These findings provide insights into disease mechanisms and support precision medicine approaches for early detection and management.

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ACKNOWLEDGEMENT:

We would like to thank Qatar University (Grant No. QUCP-CHS-2024-358) for funding this project.