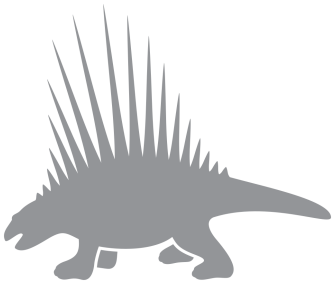

FOURTEENTH ANNUAL CONFERENCE OF THE CZECH SOCIETY FOR MASS SPECTROMETRY

Hradec Králové, November 4 – November 6, 2026

BOOK OF ABSTRACTS

Book of Abstracts from the Fourteenth Annual Conference of the Czech Society for Mass Spectrometry

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Czech Society for Mass Spectrometry

Hradec Králové 2026

Book of Abstracts from the Fourteenth Annual Conference of the Czech Society for Mass Spectrometry

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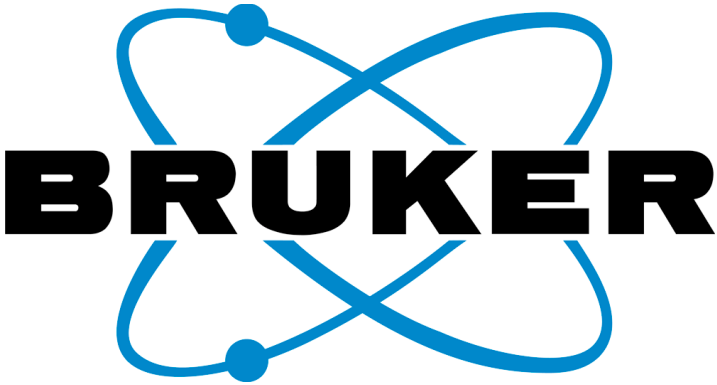

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Fourteenth Annual Conference of the Czech Society for Mass Spectrometry

Date

4th November – 6st November 2026

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Hradecká 1285

Hradec Králové

Czech Republic

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Wednesday 4th November 2026

10:00 – 18:00	Registration
12:30 – 13:30	Lunch – Canteen, Biology Centre CAS
13:45 – 14:00	Conference opening
14:00 – 14:40	**Plenary lecture I: Hamish Stewart (Thermo Fisher Scientific)** (Chairperson: Zdeněk Kukačka)
PL-01	*The development and future of the Astral analyser*
14:40 – 15:00	Karel Harant
WeO-01	*One year of Experience - Orbitrap Astral Zoom*
15:00 – 15:30	**Flash talks I** (Chairperson: Zdeněk Spáčil)
WeS-01	Svatava Polsterová: *Enhanced resolution with multi-pass mode on the Orbitrap Astral Zoom mass spectrometer*
WeS-02	Jakub Kordík: *Application of Mucorpepsin in Proteomic Clinical Studies*
WeS-03	Lada Fialová: *High-throughput singlet oxygen labeling for protein footprinting in structural mass spectrometry*
WeS-04	Tadeáš Grabic: *Structural Analysis of Diesters of 1,2-Diols Using Mass Spectrometry With UVPD*
WeS-05	Marek Masarovič: *In situ characterisation of hormonomics alterations in abiotic-stressed Lemna minor via mass spectrometry imaging*
WeS-06	Hana Kučerová: *Accelerating elemental imaging of biological tissues by quasi-random sampling and Bayesian reconstruction in the LA-ICP-MS*
15:30 – 16:00	Coffee break
16:00 – 16:40	Workshop – Pragolab (Hamish Stewart)

16:40 – 18:00	**Nucleic acids** (Chairperson: Anton Škríba)
16:40 – 17:00	Alexandr Muck
WeO-02	*Innovative High-Resolution Mass Spectrometry Workflows for Characterisation of Olionucleotides*
17:00 – 17:20	Marek Ondruš
WeO-03	*LC–MS-within Development of Chemically Modified DNA Aptamers for Continuous Biomarker Monitoring*
17:20 – 17:40	Zuzana Vosáhlová
WeO-04	*Challenges in the analysis of DNA and RNA oligonucleotides*
17:40 – 18:00	Jan Fučík
WeO-05	*Improving the Robustness of Oligonucleotide LC-UV-MS Analysis*
18:00 – 18:20	Petr Daněk
WeO-06	*Investigation of Metabolic Processing of Damaged DNA by LC-MS*
18:20 – 19:00	**Plenary lecture II: Prof. Leoš Valášek (Institute of Microbiology of the Czech Academy of Sciences)** (Chairperson: Anton Škríba)
PL-02	*Human eIF3 and eIF5 coordinate AUG recognition to sustain energy homeostasis*
19:00 – 22:00	Welcome Reception & Poster Session (atrium)

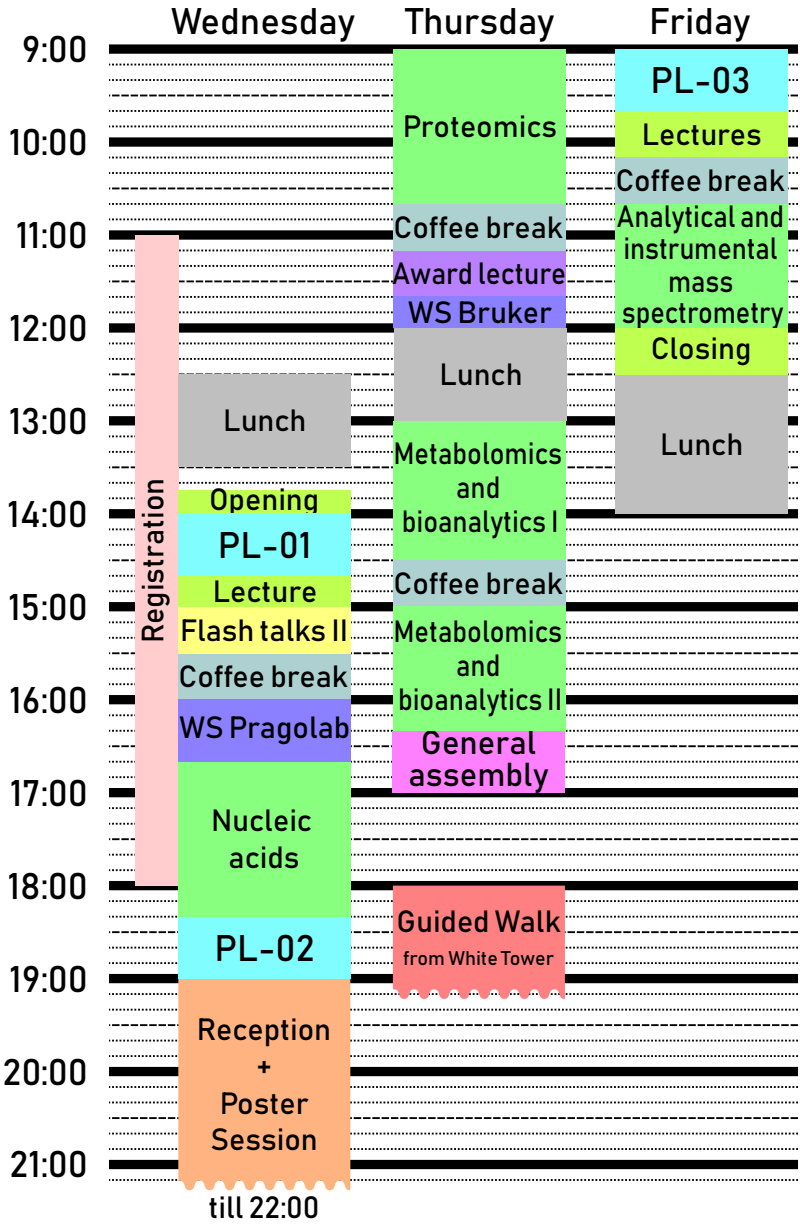
Thursday 5th November, 2026

09:00 – 10:40	**Proteomics** (Chairperson: Kristýna Gloc-Pimková)
09:00 – 09:20	Marie Vajrychová
ThO-07	*Beyond the Genome: Unveiling the Hidden Dynamics of the Proteome in Disease*
09:20 – 09:40	Juraj Lenčo
ThO-08	*Propionic acid in the mobile phase improves peptide identification in proteomic analytical- and microflow LC–MS analyses*
09:40 – 10:00	Vjačeslav Treťjačenko
ThO-09	*Encoded and non-genetic protein variants expand human functional proteome*
10:00 – 10:20	Michaela Procházka
ThO-10	*Spatial proteomics reveals therapy-induced remodeling of protein subcellular localization in acute myeloid leukemia*
10:20 – 10:40	Daniel Vláčil
ThO-11	*4D Proteomics: From Molecular Porgiles to Actionable Insights*
10:40 – 11:10	Coffee break
11:10 – 11:40	**prof. Zdeněk Herman award lecture: Barbora Sedmidubská** (Chairperson: Pavel Bouchal)
ThO-12	*The role of the low-energy electrons in the process of radiosensitization*
11:40 – 12:00	Workshop – Bruker (Daniel Vláčil)
	Expanding the Power of timsTOF: New Workflows with timsOMNI, timsMETABO and timsMRM
12:00 – 13:00	Lunch

13:00 – 14:30	**Metabolomics and bioanalytics I** (Chairperson: Miroslav Lísa
13:00 – 13:30	Tomáš Čajka
ThO-13	*LC–MS metabolomics and lipidomics: Yes, but…*
13:30 – 13:50	Noel Fitzgerald
ThO-14	*DIA Made for Metabolomics: 1 Da Sliding Scan Window DIA with ZT Scan 3.0*
13:50 – 14:10	Federico Brigante
ThO-15	*Mass Spectral Library Network (MERLIN) at 193,000 Compounds: Community-Driven Expansion of the Open MS^n Spectral Library*
14:10 – 14:30	Michal Adámek
ThO-16	*Ion Mobility Separation of Isomers for Microbial Metabolomics*
14:30 – 15:00	Coffee break
15:00 – 16:20	**Metabolomics and bioanalytics II** (Chairperson: Tomáš Čajka)
15:00 – 15:20	Iva Karlínová
ThO-17	*Heptafluorobutyl Chloroformate Derivatization Enables Molecular-Ion-Centric Metabolite Screening and Fluorine-Assisted Elemental-Composition Assignment by GC−APCI−HRMS*
15:20 – 15:40	Petr Vodrážka
ThO-18	*Chiral Analysis of Amino Acids in Biological and Complex Samples by GC–MS*
15:40 – 16:00	Karel Chalupský
ThO-19	*Hemin as a Quantitative Biomarker for Residual Blood Contamination in CNS Drug Delivery Profiling*
16:00 – 16:20	Anna Walke
ThO-20	*From Sample to Insight: Automated, End-to-End Workflows for Multi-Omics*
16:20 – 17:00	General Assembly of the Czech Society for Mass Spectrometry (in Czech)
18:00	Optional guided Walk-through historic center of Hradec Králové – start at the White Tower (main square)

Friday 6st November, 2026

09:00 – 09:40	**Plenary lecture III: prof. Helen Cooper (University of Birmingham)** (Chairperson: Jana Havlíková)
PL-03	*Native ambient mass spectrometry imaging of proteins and their complexes: Latest developments and applications*
09:40 – 10:00	Alan Kádek
FrO-21	*Native mass spectrometry: from patients to lasers and beyond*
10:00 – 10:10	Kristina Spustova
FrO-22	*Clean Background, Sharp Peaks: The Critical Impact of Water and Consumables on LC-MS Sensitivity*
10:10 – 10:40	Coffee break
10:40 – 12:00	**Analytical and instrumental mass spectrometry** (Chairperson: Alan Kádek)
10:40 – 11:00	Steven Daly
FrO-23	*How to Jazz Up Your Mass Spectrometer: Why and How to Modify Your Old Instruments*
11:00 – 11:20	Jana Knytlová
FrO-24	*Toward Improved Efficiency of the HESI Ion Source and Beyond*
11:20 – 11:40	Dominika Luptáková
FrO-25	*Multimodal Imaging Reveals Spatial Host-Pathogen Microenvironments and Neurochemical Remodelling in Escherichia coli Meningoencephalitis*
11:40 – 12:00	Maria van Agthoven
FrO-26	*Structural characterization of lignocellulosic-based pyrolysis oil by two-dimensional mass spectrometry on the new 18 T timsMRMS*
13:00 – 13:10	Closing Remarks and Awards
13:10 – 14:00	Lunch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21:00
Registration
Lunch
Opening
PL-01
Lecture
Flash talks II
Coffee break
WS Pragolab
Nucleic acids
PL-02
Reception + Poster Session
till 22:00
Proteomics
Coffee break
Award lecture
WS Bruker
Lunch
Metabolomics and bioanalytics I
Coffee break
Metabolomics and bioanalytics II
General assembly
Guided Walk
from White Tower
PL-03
Lectures
Coffee break
Analytical and instrumental mass spectrometry
Closing
Lunch

PL-01: The development and future of the Astral analyser

Hamish Stewart [1 *]

1. Thermo Fisher Scientific

Mass spectrometer performance has improved by orders of magnitude over the past decades, an advance reminiscent of Moore's law, and the sum of many individual developments. The invention of the ion mirror, for example, enabled time-of-flight (ToF) analyzers to reach far higher resolving power. Multi-reflection analysers, extend the flight path of ions by passing them repeatedly between ion mirrors, further enhance resolving power and relieve the painful focal constraints of prior analyser designs.

The Thermo Scientific™ Astral™ analyser is the most important mass analyser development in twenty years, delivering a generational leap in sensitivity and speed, with extremely high ion transmission and destructive ion detection to produce hundreds of high resolution, single ion sensitive MS/MS spectra per cycle. It was built upon decades of hidden technological developments, including several distinct, discarded, analyser concepts. The principles of the main constituent technologies, the history and process of the analyser's development are explained in the context of advances in the high-resolution mass spectrometry field.

The impact of the final Thermo Scientific™ Orbitrap™ Astral™ mass spectrometer on the major application spaces of mass spectrometry is considered, with instruments operating in labs around the world and the publication of over a thousand scientific articles. To meet the growing ambitions of analytical scientists, further improvements are described and envisaged, from those already implemented on the instrument's more advanced successor, the Thermo Scientific™ Orbitrap™ Astral™ Zoom mass spectrometer, to the hardware and methods of the future.

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PL-02: Human eIF3 and eIF5 coordinate AUG recognition to sustain energy homeostasis

Leoš Valášek [1 *]

1. Institute of Microbiology of the Czech Academy of Sciences

Precise translation initiation is essential for efficient production of proteins; missing or mis-selecting the canonical start site can have detrimental consequences. Extensive yeast research identified key initiation factors governing start codon selection accuracy, but the increasing complexity of higher eukaryotes have limited similar research in mammalian cells. We present an in cellulo study of knock-in mutants of the eIF3c subunit of the multitasking eIF3 complex that disrupt eIF3's interaction with eIF5, thereby preventing functional binding of eIF5 to pre-initiation complexes. This selectively relaxes start site selection stringency and reduces initiation sensitivity, leading to altered translation efficiency for nearly 600 proteins. Proteins with N-terminal targeting signals were among those especially affected, resulting in impaired mitochondrial function. These findings establish the first direct causal link between an initiation factor, stringent start site recognition, and impaired mitochondrial respiration and glycolytic activity, implicating the eIF3–eIF5 interaction as a key regulator of energy homeostasis.

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PL-03: Native ambient mass spectrometry imaging of proteins and their complexes: Latest developments and applications

Helen Cooper [1 *]

1. University of Birmingham

Native ambient mass spectrometry (NAMS) combines native mass spectrometry, an established technique in structural biology, and ambient mass spectrometry, in which biological substrates such as thin tissue sections are analysed directly with little or no sample preparation. The combined benefits of NAMS for analysis of protein assemblies and protein-ligand complexes include measurement of accurate mass and stoichiometry, identification of both protein and non-covalently bound ligands, together with information on spatial distribution. Recent efforts using nanospray desorption electrospray ionization (nano-DESI), have advanced NAMS for the analysis of fresh frozen issue, allowing the spatial distribution of protein assemblies to be mapped. Endogenous protein assemblies and their constituents (including small molecule ligands) can be identified by top-down dissection of assemblies in the gas phase, potentially allowing the discovery of new protein-ligand interactions. In this presentation, recent developments in native ambient mass spectrometry imaging will be discussed, together with applications in drug discovery and molecular pathology associated with neurodegenerative diseases.

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WeO-01: One year of Experience - Orbitrap Astral Zoom

Karel Harant [1 *]

1. Institute of Organic chemistry and Biochemistry of the CAS, Prague

One year with the Orbitrap Astral Zoom in our core facility. The instrument is fast — sometimes faster than the rest of the laboratory.

In this lecture, I will share practical experience from the first year of routine operation: what works well, what had to change, and how to keep pace with an instrument that can generate data at an impressive rate.

I will focus on approaches for reliable and scalable sample preparation, how to prepare enough samples to keep the instrument busy, and how we gradually turned the laboratory into a permanent production line — fortunately, still with only one shift. I will also discuss practical LC-MS strategies, organization of routine measurements, and some of the lessons learned from everyday core-facility operation.

And then comes the data. Fast acquisition means fast-growing datasets, so I will also cover how I handle data processing, transfer, storage, and the challenge of keeping the computational side of the workflow moving at the same pace as the mass spectrometer. The main message is simple: a fast instrument is only useful if the whole workflow around it can keep up. This talk is about how we tried to make that happen.

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WeO-02: Innovative High-Resolution Mass Spectrometry Workflows for Characterisation of Olionucleotides

Alexandr Muck [1 *]

1. Waters Corporation, Stamford Avenue, Altrincham Road, Wilmslow, SK9 4AX,U.K.

Synthetic oligonucleotides belong to a broad range of biologically active substances used in molecular biology research and as new biopharmaceutical therapeutics. The analyis of such molecules has seen an ever increasing interest[1]. Our development is concerned with the needs of laboratories involved in oligonucleotide characterization, monitoring and testing. Our development has been focusing on creating nucleic acid analysis solutions by utilising the advantages of high specificity and sensitivity of modern ultraperformance liquid chromatography coupled with high-resolution mass spectrometric analysis (UPLC-HRMS)[2] and designing from scratch new software workflows.

In this presentation, the author will provide an overarching view of these solutions, discuss the utility of UPLC-HRMS for analysis of oligonucleotides and assess the available range of instrumentation from the simpler single stage time-of-flight devices (TOF) up to the advantages of hybrid ion mobility coupled mass spectrometers [3]. Advantages and challenges encountered in these applications will be mentioned, along with potential solutions. The focus will be on shorter diagnostic or therapeutic oligonuleotide sequencing that lead to the release of the waters_connect Confirm Sequence application, however, an introduction to the new oligo mapping workflow, able of analysing much longer sequences, will be given.

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2. *Stoll D.R; Gilar M., Challenges and Solutions in Oligonucleotide Analysis, Part III: LC–MS Methods, LCGCInt 2026, 3, 3, 10-14.*
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WeO-03: LC-MS-within Development of Chemically Modified DNA Aptamers for Continuous Biomarker Monitoring

Marek Ondruš [1 *]

1. Institute of Organic chemistry and Biochemistry of the CAS, Prague

DNA aptamers are emerging as promising molecular recognition elements for next-generation biosensors, offering an attractive alternative to antibodies due to their chemical stability, synthetic accessibility, and compatibility with electrochemical sensing platforms. In particular, chemically modified DNA aptamers incorporating noncanonical nucleotides can provide enhanced molecular recognition properties, expanding their potential for continuous monitoring of clinically relevant biomarkers.

Our research focuses on the development of chemically diversified DNA libraries containing hydrophobic modifications and their application in the selection of high-affinity aptamers against various biomarkers. A critical aspect of this approach is the efficient enzymatic synthesis and accurate characterization of modified oligonucleotides. The incorporation of noncanonical nucleotides can present challenges associated with incomplete primer extension, variable polymerase efficiency, and the formation of shorter or longer oligonucleotide products than intended.

To address these challenges, we employ liquid chromatography–mass spectrometry (LC–MS) as a rapid analytical tool for characterizing enzymatically synthesized oligonucleotides. Compared with conventional polyacrylamide gel electrophoresis (PAGE), LC–MS provides direct molecular mass information, enabling the identification of desired products and the detection of truncated or extended DNA species. This approach facilitates the evaluation of enzymatic reaction outcomes, optimization of synthesis conditions, and verification of successful incorporation of chemically modified nucleotides.

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WeO-04: Challenges in the analysis of DNA and RNA oligonucleotides

Zuzana Vosáhlová [1 *], Sylwia Studzińska [2], Květa Kalíková [3], Martin Gilar [4], Michal Holčapek [1]

1. Department of Analytical Chemistry, Faculty of Chemical Technology, University of Pardubice
2. Department of Environmental Chemistry and Bioanalysis, Nicolaus Copernicus University
3. Department of Physical and Macromolecular Chemistry, Faculty of Science, Charles University
4. Waters Corporation, Milford, USA

The importance of oligonucleotide (ON) analysis has increased rapidly in recent years, driven primarily by the growing development and clinical application of nucleic acid-based therapeutics. This trend highlights the need for robust LC–MS methodologies suitable not only for the characterization of therapeutic ONs, but also for the comprehensive profiling of their impurities, metabolites, and other DNA- and RNA-based species. The analysis of chemically modified therapeutic ONs, particularly phosphorothioated ONs, presents additional challenges, since it requires suppression of diastereomeric separation.

Two major chromatographic approaches are currently used for ONs analysis: i) hydrophilic interaction liquid chromatography (HILIC), and ii) ion-pairing reversed-phase liquid chromatography (IPRPLC). Although IPRPLC provides effective separation of ONs, the use of ion-pairing agents can cause MS contamination. HILIC offers an alternative with improved compatibility with electrospray ionization. However, reduced sensitivity may limit the detection of low-abundance impurities and metabolites. Mixed-mode chromatography (MMC), combining multiple retention mechanisms, represents another promising approach, particularly for the separation of shorter Ons.

This contribution summarizes analytical strategies for the characterization ONs, with particular emphasis on application-specific requirements. A practical demonstration is provided through the development of a comprehensive LC–MS workflow for the analysis of plasma from pediatric patients treated with the therapeutic ON nusinersen for spinal muscular atrophy (SMA). The information obtained from the analysis of SMA patient plasma samples can be correlated with clinical status, enabling timely evaluation of treatment efficacy.

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3. *Kadlecová Z. et al.: J. Chromatogr. A 1676, 463201 (2022).*

WeO-05: Improving the Robustness of Oligonucleotide LC-UV-MS Analysis: Controlling Metal Adducts, Mobile Phase Quality, and MS Source Conditions

Jan Fučík [1 *]

1. Synthon, s.r.o.

Ion-pair reversed-phase LC-UV-MS is widely used to characterize and assess the purity of therapeutic oligonucleotides, yet performance can be affected by trace metal contamination, mobile phase additive quality, and MS source conditions. In this presentation, we examine the impact of sodium contamination in mobile phases, compare HFIP from different manufacturers, and use DoE to optimize key MS source parameters. Learn how solvent and additive selection, together with systematic source optimization, can reduce sodium adduct formation, improve MS purity and UV baseline quality, and strengthen method robustness for R&D and routine GMP/QC analysis.

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WeO-06: Investigation of Metabolic Processing of Damaged DNA by LC-MS

Petr Daněk [1 *]

1. Ústav organické chemie a biochemie AV ČR

All organisms are constantly exposed to various DNA-modifying agents. The evidence suggest that chemically damaged DNA is upon excision from the genome targeted for degradation in lysosomes. However, the catalytic pathway for processing chemically damaged DNA in lysosomes is unknown and evidence that would document enzymatic capacity of lysosomes to degrade chemically altered DNA is absent. To simulate degradation of damaged DNA, we treated circular and linear DNA substrates by a palette of commonncytostatic drugs and incubated purified damaged DNA with the lysosomal extracts. Following quantification of DNA showed that damaged DNA was partially protected from degradation by lysosomal enzymes in dose-dependent manner while native DNA was fully degraded. To characterize these residual degradation-resistant DNA fragments, we assessed degradation products of DNA modified by UV irradiation and the common cytostatic drug oxaliplatin by LC-MS. We identified that residual DNA fragments harboring chemical modifications were 5-16 nt long oligonucleotides. To investigate whether these fragments can be also formed in lysosomes of living cells, we developed a system for direct delivery of modified DNA into lysosomes and assessed their content by LC-MS. While native DNA was degraded into individual deoxynucleosides, processing of damaged DNA was indeed incomplete and resulted in the formation of degradation resistant short DNA fragments. Therefore, our data provide proof-of-concept evidence for the absence of enzymatic machinery that would effectively deal with aberrant chemically altered DNA in human cells and suggest that these degradation resistant DNA fragments might accumulate in the cells.

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ThO-07: Beyond the Genome: Unveiling the Hidden Dynamics of the Proteome in Disease

Marie Vajrychova [1 *], Rudolf Kupcik [1], Barbora Salovska [2], Ivo Fabrik [1], Jakub Radocha [3], Jaroslav Stranik [3], Zdenek Hodny [4]

1. Biomedical Research Centre, University Hospital Hradec Kralove, Czech Republic
2. Yale Cancer Biology Institute, Yale University, West Haven, USA
3. Faculty of Medicine Charles University and University Hospital in Hradec Kralove, Czech Republic
4. Institute of Molecular Genetics of the Czech Academy of Science, Czech Republic

While the genome establishes the static code of cellular potential, the actual dynamics of disease pathogenesis unfold entirely beyond it. To track these processes, mass spectrometry (MS)-based proteomics serves as a real-time window, capturing the immediate and dynamic protein responses that drive a disease phenotype. For instance, stepping beyond genetic boundaries is critical to illuminate imbalances in redox signaling and oxidative stress, determine protein upregulation reflecting intra-amniotic inflammation, and track the minimal residual disease (MRD) in multiple myeloma. This keynote address will demonstrate the versatility of MS in revealing these processes by charting functional, disease-specific protein signatures. In this presentation, we will explore SILAC-iodoTMT, a workflow combining metabolic labeling for protein quantification with reporter ion labeling to determine cysteine oxidation. We will demonstrate how SILAC-iodoTMT simultaneously uncovers shifts of both the global proteome and site-specific cysteine oxidation in senescent cells, and how silencing peroxiredoxin-6 affects their redox homeostasis and viability. Furthermore, we will present specific amniotic fluid protein upregulations associated with intra-amniotic inflammation in preterm prelabor rupture of membranes. Finally, we will demonstrate the utility of targeted MS methods to track MRD in multiple myeloma. By monitoring clonotypic peptides derived from CDR3 fragments, which serve as patient-specific molecular signatures in peripheral blood, this sensitive strategy provides a non-invasive, longitudinal alternative to traditional bone marrow biopsies.

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ThO-08: Propionic acid in the mobile phase improves peptide identification in proteomic analytical- and microflow LC–MS analyses

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

1. Charles University, Faculty of Pharmacy in Hradec Králové

We evaluated propionic acid as an alternative mobile-phase acidifier, a candidate that has been overlooked in efforts to improve ESI efficiency without compromising chromatographic performance. By reducing both the ionic strength and surface tension of the mobile phase, 0.5% propionic acid markedly enhanced ESI efficiency, yielding an average 39% increase in peptide identifications compared with 0.1% formic acid and a 12% increase compared with 0.5% acetic acid. These gains were observed in datasets from two laboratories and were consistent across analytical- and microflow LC-MS configurations, different column chemistries, and varying sample complexities. Importantly, chromatographic performance remained virtually unaffected, with only a minor reduction in peptide retention. The propionic acid mobile phase was stable and instrument-compatible and produced only a negligible MS background. The beneficial effect of propionic acid was not confirmed in nanoLC-nanoESI-MS setups.[1]

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ThO-09: Encoded and non-genetic protein variants expand human functional proteome

Vjačeslav Treťjačenko [1,2 *], Tehila Leiman [2], David Morgenstern [2], Yishai Levin [2], Dvir Dahary [2], Orna Dahan [2], Omer Asraf [2], Yitzhak Pilpel [2]

1. Institute of Organic Chemistry and Biochemistry, Academy of Science of the Czech Republic, Prague, Czech Republic
2. Department of Molecular Genetics, Weizmann Institute of Science, Rehovot, Israel

Each stage of the Central Dogma contributes to proteome diversity through mechanisms such as heterozygosity, somatic mutations, transcriptional errors, and translational errors. As a result, a diverse array of protein variants can coexist within a single proteome, such as that of humans. However, until now, methods to detect, quantify, and evaluate the functional consequences of these variants have been lacking. Here we examined a large-scale proteogenomic dataset from 29 healthy human tissues and uncovered 13,910 confidently localized variants representing 7,215 unique single amino acid substitutions co-existing alongside their corresponding reference proteoforms 1.We found that the abundance of both genetic (SNP's, somatic mutations) and mistranslated protein variants mirrors their allele frequencies in the human population. Moreover, we show that non-genetic substitutions may provide a distinct route for exploring protein sequence space, circumventing the mutational constraints imposed by the genetic code. In addition, we provide experimental validation of non-genetic substitution on selected purified proteins. We demonstrate specific and recurring non-genetic variation patterns upon amino acid starvation in proteome-wide analyses of cancer-derived cell lines and identify hundreds of substituted non-genetic proteoforms that recur consistently in multiple healthy individuals or map to annotated protein functional sites. We propose that these substitutions constitute a novel class of functional protein phenotypic variants. Collectively, our findings indicate that non-genetic amino acid substitutions in human proteins provide an abundant source to expanding the functional proteome.

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ThO-10: Spatial proteomics reveals therapy-induced remodeling of protein subcellular localization in acute myeloid leukemia

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Protein subcellular localization is linked to protein function and regulation, and its dynamic remodeling is a key component of the cellular stress response. Anti-leukemic therapy may alter protein localization independently of abundance, potentially revealing adaptive cellular processes not captured by conventional proteomic analyses. Here, we investigated how azacitidine and venetoclax (AZA/VEN) affect the spatial proteome of acute myeloid leukemia (AML) cells.

SKM-1 AML cells were treated with AZA/VEN and analysed using two complementary differential centrifugation-based spatial proteomics workflows: TMT-based LOPIT-DC coupled to data-dependent acquisition and label-free DIA-LOP. Subcellular localization was predicted from protein fractionation profiles using support vector machine-based classification. Treatment-induced changes in cell viability and apoptosis were assessed by flow cytometry.

AZA/VEN induced apoptosis in 36% of cells. LOPIT-DC and DIA-LOP quantified 5,853 and 8,955 proteins, respectively, with approximately 50% assigned to specific subcellular compartments. Comparative analysis identified 146 candidate proteins displaying treatment-associated changes in subcellular fractionation profiles and predicted localization. These proteins were associated with protein folding and stabilization, DNA repair, and cell-cycle regulation. Furthermore, the experimentally predicted localization of a subset of proteins differed from their GO-annotated localization, providing new insights into protein spatial organization in AML cells.

Together, these data reveal remodeling of protein spatial organization following AZA/VEN treatment. Validation will determine whether these changes represent direct treatment effects or adaptive responses of surviving AML cells.

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ThO-11: 4D Proteomics: From Molecular Porgiles to Actionable Insights

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Driven by the latest technological advances—including trapped ion mobility spectrometry (TIMS)-LC-MS-based proteomics now delivers the reliability, robustness, depth, and throughput required to address major challenges in translational research and advance personalized medicine.

This presentation will explore how high-quality proteomic data can be translated into actionable biological insights. Through complementary perspectives spanning immunopeptidomics and the sensitive identification of endogenous neoantigens from limited clinical material, biofluid and plasma proteomics for biomarker discovery in rare diseases, and deep proteome profiling of tumor tissues—including formalin-fixed, paraffin-embedded samples—this presentation will illustrate how advanced proteomics can reveal disease mechanisms, stratify patients, and open new avenues for personalized medicine research.

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ThO-12: The role of the low-energy electrons in the process of radiosensitization

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Radiotherapy is a crucial cancer treatment modality, with ongoing efforts to increase its effectiveness while keeping toxicity to healthy cells as low as possible. One of the strategies to enhance radiotherapeutic effectiveness and alleviate side effects is radiosensitisation. Radiosensitisation is the enhancement of tumour sensitivity to ionising radiation through the administration of specific chemical agents into tumour. Upon irradiation, these compounds may undergo radiolysis, leading to the formation of reactive species capable of damaging nearby biomolecules. One of the proposed mechanisms underlying this effect involves interactions of these compounds with secondary low-energy electrons produced in large quantities during the radiolysis of irradiated tissue[1]. Compounds that increase tumour radiosensitivity are referred to as radiosensitizers.

This work focuses on radiosensitisation processes mediated by interactions between low-energy electrons and radiosensitising compounds, aiming to provide new insights for the design of novel, more effective, and less toxic radiosensitizers. The scope of the work was to investigate the radiosensitising potential of the antiviral drug favipiravir[2] and the radiosensitising mechanism of the clinically investigated radiosensitiser Nibrozetone (RRx-001)[3].

The work combines electron attachment experiments in the gas phase using mass spectrometric detection, pulse radiolysis experiments in solution using absorption spectroscopy, and microtron irradiation followed by NMR spectroscopy, providing insights into radiation-induced processes across different temporal scales.

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ThO-13: LC–MS metabolomics and lipidomics: Yes, but…

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Liquid chromatography–mass spectrometry (LC–MS) has become an essential analytical tool in metabolomics and lipidomics, enabling the comprehensive profiling of hundreds of metabolites in biological samples. However, obtaining reliable and biologically meaningful results is not always as straightforward as it may seem. Even well-established analytical workflows can be affected by unexpected challenges arising from sample preparation, chromatographic separation, mass spectrometric detection, and data processing [1].

This presentation takes a practical "Yes, but…" approach to highlight common pitfalls, misconceptions, and troubleshooting strategies in LC–MS-based metabolomics and lipidomics. Using real-world examples, we will explore how solvent and consumable contamination, chromatographic limitations, ion suppression, detector saturation, and incorrect metabolite annotations can compromise data quality. Particular attention will be given to quality control, automated data processing, and the critical evaluation of analytical results.

Rather than providing a conventional overview of LC–MS workflows, this presentation aims to share practical lessons and demonstrate why even seemingly reliable analytical results deserve a second look.

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ThO-14: DIA Made for Metabolomics: 1 Da Sliding Scan Window DIA with ZT Scan 3.0

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Data-independent acquisition (DIA) has transformed comprehensive MS/MS acquisition in several fields, yet its adoption in untargeted metabolomics and lipidomics has remained limited. Conventional DIA methods isolate and fragment multiple precursors within relatively wide, predefined mass windows. In chemically complex samples, the resulting co-fragmentation produces highly multiplexed or chimeric spectra, complicating metabolite annotation, reducing confidence in library matching and potentially affecting fragment-level quantitation.

ZT Scan DIA 3.0 introduces a different approach. Rather than stepping through a series of fixed isolation windows, the quadrupole continuously scans across the precursor mass range using an isolation window as narrow as 1 Da. The position of this sliding window is correlated with the acquired fragment-ion signals, providing an additional dimension for associating fragments with their most likely precursors. This enables comprehensive DIA acquisition while substantially improving precursor-fragment specificity and reducing chimeric spectral interference.

The resulting workflow can be considered an “MRM of everything”: broad, unbiased acquisition with close-to-targeted precursor selectivity, MS/MS-level quantitative potential and the ability to interrogate compounds retrospectively without defining every target before acquisition. ZT Scan DIA 3.0 therefore connects two familiar analytical perspectives: the selectivity and quantitative confidence valued by triple-quadrupole users, and the comprehensive detection, accurate mass information and structural insight expected by high-resolution MS users.

This presentation will describe the analytical principles behind sliding-window DIA and demonstrate how ZT Scan DIA 3.0 can support cleaner MS/MS spectra, more confident molecular annotation and quantitation-ready discovery data within a single metabolomics or lipidomics experiment.

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ThO-15: Mass Spectral Library Network (MERLIN) at 193,000 Compounds: Community-Driven Expansion of the Open MSⁿ Spectral Library

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Confident annotation in untargeted metabolomics rests on reference MS/MS spectra, yet most detected features remain unidentified. Commercial libraries close part of the gap but, apart from lacking coverage and MS depth, they impose high costs, restrictive licensing, and training of machine-learning models. Together these constraints bottleneck routine dereplication and the development of computational annotation tools. MERLIN addresses this through a community-driven, pooled MSn acquisition on Orbitrap ID-X and LUMOS instruments. Stage 1 (Jun. 22 - Nov. 24), reported in the MSnLib publication [1], delivered 47,671 compounds and 3.98 million MSn spectra. Stage 2 (Apr - Oct. 2025) added 65,582 compounds and 4.71 million spectra. We now report Stage 3 (Nov. 2025 - Oct. 2026), contributing a further 80,354 compounds and 5.58 million spectra. MERLIN now comprises 193,607 unique structures and 14,264,874 MSn spectra, of which 86.9% are MS3 or deeper. Against the combined open LC-MS/MS libraries (GNPS, MassBank and MoNA; 75,206 compounds), MERLIN provides 2.6-fold more structures while already sharing 31,501 of them. Compared with Enveda-180 (184,944 compounds, 1,175,202 spectra) [2], MERLIN provides twelvefold more spectra and 74 spectra per compound versus 6.4, together with MSn depth absent from that MS2-only, ion-mobility-enabled resource. Critically, MERLIN and Enveda-180 share only 3,048 structures (1.6%); the initiatives are therefore complementary rather than competing. Matched against COCONUT and LOTUS, MERLIN contains 9,505 natural products, fewer than the 24,572 held by the open libraries, but including 2,008 that are not present other resources. This highlights the need to continue expanding this library effort to cover unexplored areas of the chemical space.

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ThO-16: Ion Mobility Separation of Isomers for Microbial Metabolomics

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Ion mobility has emerged as a powerful complement to mass spectrometry, offering an additional dimension of separation that may distinguish isomers and isobars. However, its performance in metabolomics has been demonstrated primarily through individual case studies. We aim to address this gap through a systematic evaluation of trapped ion mobility spectrometry (TIMS) in metabolomics with a particular focus on microbially related metabolites.

To establish a representative reference library of isomers, we developed custom Python scripts to identify commercially available isomers and categorize them into isogroups. The selection was further curated by integrating bioactivity annotations retrieved from PubChem. The resulting library includes nearly 1,000 bioactive compounds organized into 393 isogroups, each containing up to 10 isomers.

The compounds are being analyzed using LC-TIMS-MS/MS to generate high-resolution tandem mass spectrometry and ion mobility data, including collision cross section (CCS) values. To evaluate and maximize the resolving power of TIMS, we are optimizing key acquisition parameters, including ramp time to balance duty cycle and separation efficiency, the number of PASEF ramps to maintain high-quality MS/MS spectra without compromising sensitivity and TIMS stepping to ensure sufficient coverage of ion mobility window and capture the full structural diversity of the library.

We achieved successful mobility separation across several challenging classes from natural compounds through semi-synthetic to synthetic compounds. Full mobility separation was observed for certain flavonoids, terpenoids, corticosteroids and glucocorticoids. Furthermore, we achieved partial separation of certain flavonoids, alkaloids and triterpenoids.

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ThO-17: Heptafluorobutyl Chloroformate Derivatization Enables Molecular-Ion-Centric Metabolite Screening and Fluorine-Assisted Elemental-Composition Assignment by GC−APCI−HRMS

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Metabolomics enables the simultaneous monitoring of hundreds to thousands of metabolites in complex biological matrices; however, confident identification of low-abundance and unknown compounds remains challenging. Gas chromatography–mass spectrometry (GC–MS) workflows based on electron ionization (EI) often provide limited molecular-ion information because of extensive fragmentation.

Here, we present a rapid screening workflow combining one-minute heptafluorobutyl chloroformate (HFBCF) derivatization of protic metabolites with concurrent liquid–liquid microextraction and gas chromatography–atmospheric pressure chemical ionization mass spectrometry (GC–APCI–MS). HFBCF derivatization produces stable heptafluorobutyl derivatives that yield abundant protonated molecular ions, predictable class-specific fragmentation, and characteristic fluorine-specific mass signatures. Compared with full-scan GC–EI–MS, GC–APCI–MS provided up to 10–1000-fold higher sensitivity, enabling metabolite screening from extremely limited sample amounts. Characteristic mass shifts and fluorine-based elemental constraints facilitated determination of the number and the type of derivatized functional groups and supported elemental-composition assignment of unknown metabolites, even at mass accuracies of several tens of ppm.

To support compound annotation, we established an openly accessible database comprising more than 620 derivatizable metabolites and experimentally characterized retention and mass-spectral data for 240 reference standards. HFBCF–GC–APCI–MS provides a sensitive, molecular-ion-centric workflow for exploratory metabolomics, enabling annotation of unknown metabolites at different confidence levels, from standard-confirmed identifications to database-supported candidate assignments.

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ThO-18: Chiral Analysis of Amino Acids in Biological and Complex Samples by GC–MS

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Chiral analysis of amino acids is important in many areas of biological and analytical research, ranging from the determination of D-amino acids in biological samples to the structural characterization of natural products and peptides. However, GC–MS analysis of amino acid enantiomers can be challenging, particularly in the case of secondary amino acids and structurally related compounds.

A derivatization approach based on heptafluorobutyl chloroformate (HFBCF) was developed for quantitative chiral GC–MS analysis of amino acids. For secondary amino acids, the procedure was extended by an additional methylamine amidation step, which significantly improved chromatographic separation. Using a Chirasil-L-Val chiral stationary phase, the method enabled the separation of a broad range of amino acid enantiomers, including eight pairs of secondary amino acids, with reliable determination of minor enantiomeric components down to 0.1% [1].

The applicability of the method has been demonstrated for different types of samples. These include biological matrices such as urine and serum, where the approach can be used for the determination of amino acid enantiomeric profiles, as well as hydrolysates of peptides and natural products. In the latter case, the method was successfully applied to the structural characterization of Nostatin A, where it enabled determination of the absolute configuration of the unusual amino acid D-trans-4-isobutylproline [2].

These examples demonstrate the versatility of HFBCF-based derivatization for chiral GC–MS analysis. The method provides a useful analytical platform for studies of D-amino acids, biological samples, and the stereochemical characterization of structurally complex natural compounds.

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ThO-19: Hemin as a Quantitative Biomarker for Residual Blood Contamination in CNS Drug Delivery Profiling

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Despite significant advancements, the clinical translation of central nervous system (CNS) therapeutics continues to lag behind patient demand, heavily constrained by complex delivery challenges and high failure rates (1). Accurately quantifying neurotherapeutic penetration into parenchymal brain tissue remains a major bottleneck. While liquid chromatography–mass spectrometry (LC-MS) serves as the benchmark for analytical detection, measuring true parenchymal drug uptake in animal models is frequently confounded by residual blood contamination in harvested brain tissue. Often assumed to be negligible or fixed at ~5%, variable blood carryover can lead to false-positive assessments of intracellular drug accumulation despite standard heart perfusion.

To address this challenge, we used an untargeted LC-HRMS metabolomics workflow using an Orbitrap ID-X mass spectrometer to screen comprehensive blood-derived component profiles. From this screening, hemin was identified as the most statistically significant and extractable indicator of blood contamination in organic solvent extractions and using C18 column separation and ESI positive mode.

We validated hemin as a reliable internal quality control metric for assessing tissue perfusion. Specifically, comparisons between non-perfused and perfused brain tissues demonstrated a 2.5-fold reduction in hemin levels within the perfused samples. Incorporating LC-MS measurement of hemin alongside drug candidate profiling provides a robust internal quality control metric. This approach eliminates false-positive readings and ensures the precise, uncompromised measurement of true intracellular CNS drug exposure.

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ThO-20: From Sample to Insight: Automated, End-to-End Workflows for Multi-Omics

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Omics laboratories face a persistent tension between targeted quantitation, which answers known questions with high precision, and untargeted profiling, which generates new hypotheses but demands greater effort in data handling and compound annotation. Agilent therefore introduces an end-to-end workflow concept in which sample preparation, separation, mass spectrometric detection, and data interpretation are standardized and connected, so that the study scope can expand without redeveloping methods. Automated sample preparation on a Bravo liquid-handling platform improves reproducibility across users and experiments, increases hands-free time and throughput, and enables 3-in-1 extraction of metabolites, lipids, and proteins from a single sample aliquot. Still, sample preparation protocols can also be performed manually. Downstream, for metabolite analysis, a robust HILIC-Z separation paired with a library of more than 500 metabolites, including retention times and spectra from authentic standards, delivers reproducible results across instruments and laboratories and supports confident, level-aware annotation. Depending on the study scope the workflow adds value to both: TQ and Q-TOF instruments. Two applications further illustrate the flexibility of the Q-TOF platform. First, a data-independent acquisition workflow for metabolomics was developed. Collecting maximum fragment information on every sample allows the same data file to be mined twice, targeted against the metabolite library for quantitation and untargeted for discovery, removing the need for sample reinjection. Second, a metabolite identification (MetID) workflow combines *in silico* prediction of Phase I and Phase II biotransformation with MS/MS-based structural scoring via SIRIUS CSI:FingerID and time-course confirmation. Applied to verapamil in liver microsomes, it allowed for library-free annotation of a small set of confidently ranked transformation products.

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FrO-21: Native mass spectrometry sheds light into the mechanism of metabolic diseases

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Native mass spectrometry has established itself over the last two decades as an indispensable tool of modern structural biology in which it provides important insights into complex biological and medical questions. While its structural resolution is limited, native MS plays an important role probing the stoichiometry as well as the structural dynamics of protein complexes. This is due namely to its ability to analyse dilute samples in complex mixtures and its unparalleled sensitivity to even small changes in mass, thus distinguishing species otherwise hidden to higher resolving structural techniques.

Using two recent successful examples, this contribution will exemplify the importance of high mass sensitivity of native MS. First, we will look at a newly discovered variant of Kleefstra syndrome – a rare genetic disorder caused by mutations in human euchromatin histone methyltransferases, which control the epigenetic regulation in organism. We will show how native MS can help elucidate the molecular mechanism of the disease and how it enables analyses of the underlying heterodimeric complexes.[1]

Second, on an example of geranylgeranyl pyrophosphate synthase complex, important medically in multiple myeloma, we will demonstrate why control of the cofactor occupancy is important for structural analyses. Also, we will see how activation of ions en route from solution into the gas phase, while generally unwanted in native MS, can offer interesting insights into the stability of protein complex species.[2]

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FrO-22: Clean Background, Sharp Peaks: The Critical Impact of Water and Consumables on LC-MS Sensitivity

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Even the most sensitive LC-MS system cannot fully compensate for poor sample preparation. Contamination, analyte losses due to adsorption, extractables from filtration membranes, and insufficient water purity can increase analytical background, cause ion suppression, and negatively affect both sensitivity and reproducibility.

Using Sartorius solutions as practical examples, this presentation will demonstrate how precise pipetting, proper selection of filtration membranes for Minisart syringe filters, and ultrapure water produced by Arium systems help minimize contamination and preserve analytical performance. The best way to improve LC-MS sensitivity may start long before the injection.

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FrO-23: How to Jazz Up Your Mass Spectrometer: Why and How to Modify Your Old Instruments

Steven Daly [1 *]

1. MSVision

Mass spectrometry is typically done on commercially available mass spectrometers. Which are very good at what they are designed to do. However, how many times have you wished that your instrument could do something else? Be able to couple to photons? Have a higher range of transmitted masses? Be able to use multiple fragmentation methods? Well, this talk will give you an idea of why and how to modify instruments, with an emphasis on the how, by looking at several instruments I have developed during my time at MS Vision.

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FrO-24: Toward Improved Efficiency of the HESI Ion Source and Beyond

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Ionization is a crucial step in mass spectrometry, as the number of generated ions directly affects the method's sensitivity. Electrospray ionization (ESI) represents the most commonly used ionization technique in currently offered LC-MS platforms. In ESI, ions are formed through multistep desolvation from multiply charged microdroplets. However, when the mobile phase flow exceeds approximately 50-100 µL/min, the process can no longer rely on the electrostatic force alone to be efficient. Externally heated ESI (HESI), combined with nitrogen nebulizer gas, are conventionally employed together to enhance the generation of smaller droplets and their evaporation. Unlike low-flow regimes where nebulizing gas is dispensable, pneumatically-assisted HESI exhibits notoriously poor overall efficiency, typically reported in the low single-digit percentages or even way below of 1%. These substantial losses are largely attributed to turbulent dispersion, inefficient transfer toward the MS sampling orifice, and droplet populations too large for complete desolvation within the transit window. Overcoming these limitations is essential for improving analytical figures of merit. To address this challenge, we developed a high-flow electrospray interface designed with the assistance of computational modelling of aerodynamic and electrostatic forces that are key for the process. The device combines point-focusing geometries with flow laminarization. The initial prototype, operated even entirely without auxiliary external heating, was evaluated against a commercial Bruker Apollo II source. Our non-heated prototype consistently outperformed the commercial source by a factor of 3 to 5 in signal response, demonstrating the high potential of computational optimization.

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FrO-25: Multimodal Imaging Reveals Spatial Host-Pathogen Microenvironments and Neurochemical Remodelling in *Escherichia coli* Meningoencephalitis

Dominika Luptáková [1 *], Tereza Hřivnová Juříková [1], Oldřich Benada [1], Gabriela Lokočová [1], Helena Marešová [1], Hynek Mácha [1], Jiří Houšť [1], Kateřina Dvořáková Bendová [2], Miroslav Popper [2], Miloš Petřík [2,3], Andrea Palyzová [1], Lukáš Kučera [4], Evgeniya Biryukova [1,5], Jiří Novák [1], Vladimír Havlíček [1,6]

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Introduction: *Escherichia coli* is a leading Gram-negative cause of bacterial meningitis, yet the tissue-level organisation of host-pathogen interplay and its impact on brain neurochemistry remain poorly resolved.

Methods: Adult female Lewis rats received intracerebral *E. coli* CCM 5172 (5×10^2 CFU) or saline; brains and cerebrospinal fluid (CSF) were collected at 24h. Coronal brain sections were analysed by histopathology, qPCR, culture, scanning electron microscopy (SEM) and MALDI mass spectrometry imaging (MSI) of siderophores, peptides, proteins and FMP-10-derivatized neurotransmitters. CSF was analysed by MALDI-MS, LC-MS and ELISA.

Results: Infection formed structured meningoencephalitis involving ventricular, meningeal and perivascular interfaces. SEM revealed biofilm-like *E. coli* aggregates within neutrophil extracellular trap-like material. MSI detected aerobactin and salmochelin-derived metabolites, but not enterobactin, partially overlapping calprotectin proteoforms in ventricular metal-conflict niches. Spatial peptidomics revealed infection-enriched antimicrobial territories dominated by rat neutrophil peptides RatNP-2–4. Neurochemical remodeling extended beyond infection foci: total proenkephalin-derived peptides decreased by 49% in basal ganglia, and dopamine increased in the striatum and septum, with unchanged tyrosine and norepinephrine, whereas the metabolites DOPAL and HVA declined (12-fold lower HVA/dopamine ratio), suggesting impaired monoamine oxidase-dependent catabolism. Siderophores, RatNPs and lipocalin-2 were detected exclusively in CSF of infected animals.

Conclusions: *E. coli* meningoencephalitis is a spatially organized process in which bacterial communities, nutritional immunity, antimicrobial peptides and neurochemical networks occupy distinct but connected CNS niches. Host-pathogen signatures are recoverable in CSF as candidate diagnostic markers, and dopaminergic and enkephalinergic reprogramming may contribute to neurological sequelae.

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FrO-26: Structural characterization of lignocellulosic-based pyrolysis oil by two-dimensional mass spectrometry on the new 18 T timsMRMS

Maria van Agthoven [1,2 *], Theo Imhoff [1,2], Julien Maillard [3,2], Maxime Sueur [3,2], Simon Ollivier [1,2], Christopher Wootton [4], Corinne Bourhis-Loutelier [1,2], Marc-André Delsuc [5], Pierre Giusti [3,2], Carlos Afonso [1,2]

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Pyrolysis oils are intermediaries in the process of manufacturing biofuel. They are complex organic mixtures composed of thousands of distinct elemental compositions. Because raw materials are varied and complex, their composition can be unpredictable and cause problems that reduce biofuel energy content. Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) enables high-confidence assignments of elemental compositions of pyrolysis oils, but cannot differentiate between isomers without fragmentation [1]. Ion isolation is not practical for such samples. Here, we use two-dimensional mass spectrometry (2D MS), in which precursor and fragment ions are correlated without isolation, for the structural characterization of pyrolysis oil. For the first time, 2D MS is applied on the new 18 T timsMRMS instrument, which combines gated trapped ion mobility spectrometry (gTIMS) with FT-ICR MS. This setup enables us to distinguish between the fragmentation patterns of isomeric compounds.

In 2D mass spectra, fragment m/z are plotted horizontally and precursor m/z are plotted vertically. Characteristic lines can be extracted from 2D mass spectra, such as the autocorrelation line, which shows the m/z of the precursor ions, fragment ion scans, which show the fragmentation pattern of each precursor, precursor ion scans, which show all the precursors of a given fragment, and neutral loss lines [2]. After internal calibration, we assigned peaks within 1 ppm accuracy in the fragment ion dimension and 10 mDa accuracy in the precursor ion dimension. We used this result to search for neutral losses in the peaklist with 10 mDa tolerance, which enabled us to assign characteristic neutral losses (e.g. C5H10O6) that shed light on chemical structures within the pyrolysis oil.

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WeS-01: Enhanced resolution with multi-pass mode on the Orbitrap Astral Zoom mass spectrometer

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Tandem mass tag (TMT) 32-plex reagents increase multiplexing capacity for quantitative proteomics, but their reporter-ion quadruplets are separated by only 3 mDa and require approximately 90,000 resolving power at low m/z for baseline resolution. Conventional single-pass operation of the Astral analyzer provides around 60,000 resolution at m/z 138, with lower values under high space-charge conditions. We developed a TMT high-resolution (HR) mode for the Thermo Scientific Orbitrap Astral Zoom mass spectrometer that uses multi-pass ion motion to increase resolving power at low m/z while retaining fast acquisition and quantitative performance.

A fast-switching relay prism deflector was used to guide ions through the Astral analyzer, then trap them for additional drift passes before transfer to the high-dynamic-range detector. In the three-pass mode, the ion path increases from approximately 30 m to 90 m. Instrument parameters were optimized with an automated genetic-search routine to maximize transmission and suppress overtones, followed by focusing-mirror voltage optimization for resolution. TMT HR methods combined Orbitrap survey scans and single-pass panoramic Astral MS2 scans for peptide identification with separate enhanced-resolution scans over a restricted reporter-ion m/z range for quantification. TMT-labeled yeast and HeLa digests were analyzed using 78-minute gradients on an Aurora Frontier XT column coupled to a Vanquish Neo LC system.

The multi-pass mode produced resolving power above 120,000 at low m/z and baseline-resolved TMT 32-plex reporter-ion quadruplets across a broad range of ion targets. Relative transmission was typically 35% of single-pass mode, while mass accuracy and shot-to-shot stability remained comparable. Fast switching enabled acquisition to be primarily limited by the selected injection time. In a TMT 32-plex HeLa digest with expected 1:4 channel ratios, median reporter abundances closely matched the expected values. The method quantified 4,299 protein groups and 22,146 peptide groups, corresponding to 94% and 78% of identified protein groups and peptides, respectively. TMT HR mode was compatible with both 11-plex and 32-plex samples and enabled multiplexed protein identification and quantification at throughput exceeding 440 samples per day.

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WeS-02: Application of Mucorpepsin in Proteomic Clinical Studies

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Proteolytic digestion determines sequence coverage in bottom-up proteomics. This study evaluates mucorpepsin for online peptide mapping of therapeutic monoclonal antibodies and in-solution proteomic profiling of antibiotic-resistant *Escherichia coli* strains.

For online digestion, mucorpepsin was immobilized on POROS 20 AL beads and packed into a 1×20 mm column to form an immobilized enzyme reactor (IMER) integrated into an LC-MS/MS workflow. Trastuzumab, bevacizumab, and ocrelizumab were digested under acidic conditions using different denaturing and reducing reagents. Peptide maps were evaluated for sequence coverage and reproducibility. Separately, *E. coli* lysates were digested in solution and analyzed by LC-MS/MS to compare proteomic profiles among strains with different antibiotic resistance characteristics.

Online IMER digestion achieved sequence coverage of up to 100% for each antibody. The best-performing conditions were 3M urea with 100mM tris(2-carboxyethyl)phosphine (TCEP), and 2M urea with 1M thiourea and 100mM TCEP. These conditions enabled comprehensive sequence coverage and reproducible peptide mapping of disulfide-rich antibodies. In-solution digestion of *E. coli* lysates supported protein identification and revealed distinct proteomic profiles among the investigated strains.

These findings demonstrate the applicability of mucorpepsin to online antibody characterization and in-solution bacterial proteomic profiling, supporting its potential for biopharmaceutical and clinical research.

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WeS-03: High-throughput singlet oxygen labeling for protein footprinting in structural mass spectrometry

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Protein footprinting is a valuable tool for structural characterization of proteins and their complexes. By probing the solvent accessibility of the protein backbone or side chains, it reveals conformational changes and ligand binding, and comparing footprints of different structural states can identify changes in surface topography. The most common techniques are hydrogen–deuterium exchange (HDX) and fast photochemical oxidation of proteins (FPOP). As an alternative, we introduced oxidative footprinting with singlet oxygen, generated *in situ* via the photodynamic effect by irradiating a dissolved photosensitizer with a diode laser.

To make this method suitable for routine structural studies, we designed and built a high-throughput labeling apparatus. A phthalocyanine photosensitizer and a carrier buffer are delivered by two syringe pumps. Samples are injected through a valve into the buffer flow, mixed with the photosensitizer in a T-junction, irradiated with a 650 nm diode laser, and then collected and, if required, quenched. Oxidized samples are analyzed by LC-MS (Evosep One, timsTOF SCP) or by direct infusion FT-ICR MS (solariX XR 15T).

To minimize cross-contamination and maximize the duty cycle, the sample collection window and the flush time between injections were optimized. The parameters were validated by consecutive injections alternating angiotensin II and ubiquitin, with no cross-contamination detected. Reproducibility of oxidation was confirmed by LC-MS across consecutive injections. Without quenching, oxidation continued after collection. However, quenching does not have to be immediate, which simplifies future automation. These results represent the first steps towards a fully automated high-throughput apparatus for singlet oxygen labeling.

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WeS-04: Structural Analysis of Diesters of 1,2-Diols Using Mass Spectrometry With UVPD

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Diesters of 1,2-diols (1,2-DDE) are a lipid class composed of a long-chain 1,2-diol and two fatty acid acyls. This lipid class has been detected on the skin surface of various mammal species, such as mice, guinea pigs or rats [1]. In the skin of adult humans, 1,2-DDE are almost completely missing; however they are present in vernix caseosa. Vernix caseosa shows wound-healing and anti-infective properties, as well as the ability to hydrate the skin [2].

The complete characterization of 1,2-DDE remains a challenge. Upon CID or HCD fragmentation, 1,2-DDE exhibits a neutral loss of fatty acids, which is useful for determining the length of 1,2-diols and linked acyl groups; however the exact position of the acyl group in the chain could not be clarified [3].

An alternative method of ion fragmentation is UVPD, in which is the ion activated by UV photons. It has been shown that UVPD activation on [M+Li]+ adducts could lead to Norrish type reactions and fragmentation of double bonds. The product ions of these reactions can be used for determining double bond positions as well as chain length and acyl group position.

The goal of this study is to explore UVPD pathways of lithium adducts of 1,2-DDE and determine the applicability of UVPD for double bond and acyl position determination.

For this experiment, six standards of 1,2-DDE were used. These standards were diluted into iPrOH/MeOH/$CHCl_3$ (4:2:1) mixture containing 2 mM lithium formate at concentration of 10 µM. Each solution was infused into Orbitrap IQ-X Tribrid mass spectrometer (Thermo Fischer Scientific) using TriVersa NanoMate ion source (Advion). The lithium adducts of 1,2-DDE were isolated in the ion trap and fragmented by CID and UVPD. The product ions were detected with orbital ion trap.

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WeS-05: *In situ* characterisation of hormonomics alterations in abiotic-stressed *Lemna minor* via mass spectrometry imaging

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Phytohormones are a group of small signaling molecules that play an important role in regulating salinity stress responses by activating detoxification-related gene expression and counteracting oxidative damage. Although anti-abiotic stress regulator including ABA, JA, and ethylene, as well as their metabolites, have been well studied in Arabidopsis, their metabolomic interactions in aquatic plants remain unknown. Application of conventional analytical methods to aquatic plant samples remains challenging because metabolic labelling via liquid medium is technically difficult, and homogenising whole samples obscures tissue-specific distribution, leading to convoluted results. On the other hand, mass spectrometry imaging as a label-free analytical method has been widely applied for the in situ analysis of metabolites and other biomolecules, which demonstrates unique advantages in visualising heterogeneous tissue samples. Previously, we have applied MSI to detect multiple phytohormone species as well as synthetic derivatives from various plant tissues, preserving important spatial information [1]. Here, matrix-assisted laser desorption/ionisation mass spectrometry imaging was performed on Lemna minor under severe magnesium salt stress to map the unique spatial patterns of phytohormones and associated small metabolites. Our results revealed distinct spatial patterns of phytohormone species from Lemna minor leaves, while highlighting other metabolite signatures unique to anti-salinity stress. These findings demonstrate that MALDI-MSI provides a powerful solution for spatially resolved hormonomics and metabolomics differences in unique aquatic plant samples, while preserving intact tissue morphology, and offers new insights into plant abiotic stress regulation networks.

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WeS-06: Accelerating elemental imaging of biological tissues by quasi-random sampling and Bayesian reconstruction in the LA-ICP-MS

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Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) is a powerful technique for spatially resolved elemental analysis in biological tissues. However, conventional imaging relies on dense raster scanning, requiring a large number of ablation points and resulting in long acquisition times and increased analytical costs. To improve measurement efficiency, a strategy combining quasi-random sampling and Bayesian image reconstruction was developed. Sampling locations were generated using Halton sequences, while Gaussian process regression was employed to reconstruct elemental distribution maps from a reduced number of measurements. The method was first evaluated using simulated data representing a 1 cm ² sample area at a spatial resolution corresponding to a 100 µm laser spot size. The number of required ablation points was reduced from 10,000 to approximately 5,000 while maintaining image fidelity, reflected by a Structural Similarity Index Measure (SSIM) of 0.72. The approach was subsequently tested on mouse brain tissue sections. For a mapped area of 0.5 × 0.2 cm, the number of sampling points was reduced from 1,000 in a conventional orthogonal grid to only 117 quasi-randomly distributed points. The reconstructed image achieved an SSIM value of 0.78 relative to the reference map. These results demonstrate that sparse quasi-random sampling combined with Bayesian reconstruction can substantially reduce LA-ICP-MS acquisition times while preserving essential spatial information. The developed workflow is intended for future studies of pharmacokinetics and tissue distribution of lanthanide-tagged peptide therapeutics using LA-ICP-MS imaging.

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WeP-01: Biomarker Discovery in Minipigs Reveals Sex- and Survival-Associated Proteomic Signatures

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Radiological and nuclear incidents require reliable biomarkers to assess radiation exposure. We used label-free plasma proteomics to identify radiation-responsive proteins and examine sex- and survival-associated patterns in 27 Göttingen minipigs exposed to 2.1 Gy total-body irradiation. The samples were collected 3 days before and 1, 3 and 10 days after irradiation in three experimental series. Albumin was depleted from plasma and the remaining proteins were reduced, alkylated, and digested with trypsin. The peptides were analysed by reversed-phase nanoLC–ESI–MS/MS using a Q Exactive mass spectrometer (Thermo). Protein identification and label-free quantification were performed using MaxQuant; statistical analyses were conducted in Perseus and RStudio (t-test, $p \leq 0.05$). The analysis identified changes in plasma proteins related to irradiation, sex, and survival. The 18 proteins most strongly associated with irradiation showed reproducible changes in all three experimental series. In males, HBA/HBB increased ($\log_2$ fold change > 2), while proteins with Ig-like domains and heavy-chain immunoglobulin components decreased. In females, FGA/FGB, complement C3, and APOE increased. Lysozyme C-3, serotransferrin, and von Willebrand factor decreased in both sexes, with greater reductions in females. Non-survivors had higher periostin (the strongest signal) and lipopolysaccharide-binding protein, while survivors had higher Ig lambda chain and complement C1q subunits. These findings support Göttingen minipigs as a model for the discovery of radiation biomarkers and identify plasma proteins for further validation as indicators of radiation injury and potential predictors of clinical outcomes.

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WeP-02: Quaternary Aminooxy (QAO) Derivatization Reagents for the Determination of Reactive Aldehydes by LC-MS

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Reactive aldehydes are analytically challenging due to their high chemical reactivity and limited stability in biological matrices. Derivatization with quaternary aminooxy (QAO) reagents converts them into permanently charged oximes, enabling sensitive detection by LC–ESI–MS/MS.

In this study, eight reactive aldehydes—glyoxal, methylglyoxal, malondialdehyde, hex-2-enal, non-2-enal, 4-hydroxy-2-hexenal, 4-hydroxy-2-nonenal and 4-oxo-2-nonenal—were derivatized in situ in human serum. Three QAO reagents were initially compared; subsequent work focused on a single reagent selected on the basis of preliminary repeatability. A 20 µL aliquot of serum was treated with 100 µL of ACN. Excess QAO reagent was quenched with 2-formylbenzenesulfonic acid and removed using Ambersep 900 OH anion-exchange resin, which efficiently eliminated residual reagent and partially removed negatively charged matrix interferences, thereby reducing matrix effects. The final extract was centrifuged at 7000 rpm and the supernatant was collected.

Analyses were performed on an Agilent 6495B triple quadrupole mass spectrometer using a Scherzo SM-C18 mixed-mode column in positive-ion MRM mode (total run time 18 min). Under optimised conditions, the method allowed quantification of reactive aldehydes in serum over the range 1-1000 nmol/L, with repeatability of RSD = 2–20 % (n = 10). All target aldehydes were detected in the examined serum samples.

These results demonstrate that QAO derivatization combined with ion-exchange cleanup is suitable for the simultaneous determination of a broader panel of reactive aldehydes in human serum. Full validation, including precise LOQ determination and comprehensive assessment of matrix effects, is in progress.

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WeP-03: Development of a novel UHPLC-MS/MS method for simultaneous analysis of endogenous glucagon-like peptide-1 species and their synthetic analogues

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Glucagon-like peptide-1 (GLP-1) is an incretin hormone involved in the regulation of food intake and glucose homeostasis. Its synthetic analogues have gained significant attention due to their therapeutic efficacy in obesity, cardiovascular disease, and other metabolic disorders. Their increasing popularity has also raised concerns regarding illicit pharmaceutical products.

We developed a novel UHPLC–MS/MS method for the analysis of four physiologically relevant endogenous forms of GLP-1 and six synthetic analogues: semaglutide, liraglutide, lixisenatide, tirzepatide, retatrutide, and survodutide. The effects of ion source parameters on electrospray ionization of the analytes in positive ion mode were investigated and their settings were optimized using a design-of-experiments approach. In addition, the effect of the type and concentration of acidic mobile phase additives on ion abundance and charge-state distribution was also evaluated. Product ion spectra were characterized using high-resolution mass spectrometry, and suitable product ions were selected for selected reaction monitoring (SRM). Analyte separation was carried out using reversed-phase conditions on various stationary phases, including C18, phenyl, C8, and C4 chemistries. Hydrophilic interaction liquid chromatography (HILIC) was also investigated using bare silica and amide-modified hybrid columns.

The developed UHPLC–MS/MS method for the analysis of synthetic analogues and endogenous GLP-1 provides a robust and reliable tool for quality control of medicinal products, analysis of counterfeit drugs, investigation of novel compounds that stimulate GLP-1 secretion, and studies of the pharmacokinetic properties of these compounds.

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WeP-04: Biostatistical Monitoring of Secondary Effects on Metabolic Changes in Biological Studies

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In biological research, observed outcomes are often determined by the interplay of multiple factors, yet most studies focus primarily on the direct effects of key variables. A comprehensive understanding, however, requires consideration of secondary influences as well as careful control of pre-analytical variability. In this study, we employed a reversed-phase liquid chromatography–mass spectrometry (RP-LC/MS) platform [1] to characterize metabolic profiles in a well-defined cohort comprising both treated and control groups. Multivariate statistical methods were applied to identify key metabolic alterations distinguishing experimental groups, followed by a systematic evaluation of secondary effects and their influence on relationships among primary variables. An important aspect of the study was also the assessment of sample stability, as serum stability represents a critical pre-analytical factor that can substantially influence the reliability of metabolomic data. Given that such effects are often characterized by extremely subtle variations, their evaluation requires a carefully tailored statistical strategy, including rigorous data preprocessing, normalization, and noise reduction to ensure that minor changes can be reliably detected and are not confounded by analytical artefacts.

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WeP-05: Application of HPLC– IT TOF – MS Polaromics in the Investigation of Taurine-Related Metabolic Changes in Glioblastoma Cells

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Taurine is a sulfur-containing amino acid-like compound involved in cellular homeostasis, including osmoregulation, calcium signaling, membrane stabilization, and cellular stress responses. It can also modulate mitochondrial and intermediary metabolism, but its effects on the metabolic phenotype of glioblastoma cells remain insufficiently characterized. In this polaromic study, we investigated the effects of extracellular taurine supplementation on cultured human glioblastoma T98G cells. Cells were exposed to 10, 100, or 500 µM taurine for 48 h. Targeted LC–MS analysis determined intracellular and extracellular levels of taurine and selected amino acids. Total cellular protein content, LDH activity and release, glucose consumption, and lactate production were assessed in parallel. Taurine supplementation did not significantly affect total protein content, LDH release, or glucose consumption. In contrast, 500 µM taurine increased specific lactate release and cellular LDH specific activity. LC–MS analysis showed concentration-dependent intracellular taurine accumulation, highest at 500 µM. Analyzed amino acid levels remained unchanged, while leucine showed a decreasing trend. These findings suggest taurine accumulation without major alterations in the measured amino acid profile. Under the experimental conditions used, taurine did not exert a detectable cytotoxic or growth-promoting effect on T98G cells but selectively modified glucose-derived carbon metabolism by promoting lactate formation. Further investigation is needed to clarify the mechanisms linking taurine availability to glycolytic and mitochondrial metabolism.

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WeP-06: Characterization of cyclodextrin derivatives as active pharmaceutical ingredients

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Cyclodextrins (CDs) are versatile compounds widely used by the pharmaceutical industry as solubilizers and stabilizers. Increasing interest in cyclodextrins as pharmaceutical actives creates new analytical requirements for structural characterization, impurity profiling, purity quantification and methods for their characterization.

Our presentation will focus on sugammadex, a single-isomer API, and methylated β-cyclodextrin, a randomly substituted cyclodextrin derivative under investigation as pharmaceutical active, discussing their chromatographic behaviour on the ASTRA® C18-Hybrid column and the gained analytical information needed for structural characterization and purity quantification.

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WeP-07: MALDI-TOF mass spectrometry unraveling phlebotomine sand fly taxonomy

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Phlebotomine sand flies are known as proven vectors of various pathogens like arboviruses, bacteria and most notably parasitic protozoans of the genus *Leishmania* that cause leishmaniases in human and animal hosts, thus of great importance in human and veterinary medicine. When studying the epidemiological significance of sand fly fauna in endemic regions, powerful and rapid method for conclusive species identification is required to understand the dynamics of local transmission cycles, to assess potential emergence of particular sand fly species in new areas, and also to propose effective control measures to mitigate the pathogen transmission.

During the last decade, we successfully introduced MALDI-TOF MS-based protein profiling [1] 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, Caucasus, East and North Africa or Southeast Asia, constructing a reference database that currently comprises more than 45 different sand fly species of several genera. The developed standardized protocol outlines a complete pipeline of specimen trapping, storage and sample preparation that enables to acquire reproducible species-specific protein profiles useful in integrative taxonomy of sand flies.

The efficiency and robustness of the method will be documented on three examples from field surveys conducted in Moldova and Northern Africa. The approach suggested existence of yet undescribed cryptic species in Moldova [2], enabled formal description of novel sand fly species in Algeria [3], and supported conclusive discrimination of sibling species within the *Phlebotomus perniciosus/longicuspis* complex in Morocco.

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WeP-08: Improvement of Sensitivity and Throughput of Quantitative Middle-Up CZE-MS Analysis of Monoclonal Antibodies

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Monoclonal antibodies (mAbs) are complex biopharmaceuticals that require extensive characterization to ensure product quality, safety, and consistency. While liquid chromatography-mass spectrometry (LC-MS) remains the standard analytical approach, capillary zone electrophoresis-mass spectrometry (CZE-MS) offers an attractive alternative due to its high separation efficiency, low reagent consumption, and reduced operating costs. In this study, we further enhanced our previously developed quantitative middle-up (MU) CZE-MS workflow by implementing in-capillary pre-concentration and exploring multisegment injection (MSI) to improve sensitivity and throughput.

Analyses were performed using an Agilent 7100 capillary electrophoresis system coupled to UV detection and an Orbitrap Fusion mass spectrometer. Different sample matrix compositions and injection conditions were evaluated to maximize stacking efficiency and signal response.

Optimization studies demonstrated that 250 mM ammonium acetate combined with a 2 M acetic acid background electrolyte provided the most effective pre-concentration conditions, yielding the highest signal intensities and peak areas. An injection time of 250 s further improved sensitivity without adversely affecting separation performance. These optimized parameters form the basis for ongoing MSI development, enabling the analysis of multiple sample segments within a single CZE-MS run.

The proposed strategy improves the sensitivity of quantitative MU CZE-MS analysis and has the potential to increase analytical throughput, supporting more efficient characterization of therapeutic mAbs.

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WeP-09: Post-search validation of site-resolved N-glycopeptide assigments using complementary LC–MS/MS evidence

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Mass spectrometry-based glycoproteomics enables site-resolved characterization of protein glycosylation. However, glycan isomerism, isobaric species, analytical artifacts, and incomplete fragmentation can compromise automated identification. We developed a post-search workflow that validates and curates MS2-based candidate assignments using complementary precursor-level and chromatographic evidence.

Search-engine-derived candidates were reviewed and quantified across runs in Skyline using precursor monoisotope assignment, mass accuracy, isotopic-envelope agreement, fragment-ion evidence, extracted-ion chromatograms, retention-time consistency, and glycosite context. This review refuted inconsistent assignments, removed redundant peptide forms, added MS1-supported composition-level features, and retained LC-resolved isomeric features without assigning specific structures.

Using data from 52 human serum samples, we evaluated 3,071 candidate N-glycopeptide assignments and refuted 1,722 (56%) because they conflicted with precursor level and/or chromatographic evidence. Final curation also incorporated 22 additional MS2-derived entries, removed 255 redundant peptide forms, and added 320 features, comprising 152 MS1-supported composition-level assignments and 168 additional LC-resolved isomeric features. This resulted in 1,436 curated N-glycopeptide features spanning 148 N-glycosylation sites on 58 glycoproteins.

These results demonstrate that automated MS2 scoring alone is insufficient for reliable large-scale N-glycoproteomics. Integrating precursor, fragment-ion, retention-time, and cross-run evidence improves both assignment accuracy and analytical coverage.

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WeP-10: UHPLC-ESI-MS/MS Profiling of Phenolic Compounds and Matesaponins in Yerba Mate after Subcritical Water Extraction

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Yerba mate (Ilex paraguariensis A.St.-Hil.) leaves are a rich source of bioactive compounds, including phenolic compounds and matesaponins(1,2). The present study investigated subcritical water extraction (SWE) as a sustainable alternative to conventional water infusion (WI) and evaluated the distribution of bioactive compounds among individual SWE fractions. SWE was performed under elevated temperature and pressure using water as the extraction solvent. The resulting extracts were collected as sequential fractions, which were analyzed by UHPLC-ESI-MS/MS to characterize their chemical profiles. In total, 38 compounds including caffeine, theobromine, chlorogenic acids, quinic acids, and matesaponins were monitored throughout the extraction process, allowing changes in their distribution between individual fractions to be evaluated. The fractionation approach revealed pronounced differences in the composition and extraction efficiency of individual SWE fractions. Among the investigated fractions, the first SWE fractions obtained after 2 or 5 min of SWE showed the highest extraction yields. On the other hand, the highest proportion of the monitored compounds in extract was in 2-nd and 5-th extract of 2-min stages. These results indicate that the extraction efficiency was strongly dependent on the extraction stage. When the overall extraction performance was compared with WI, 10 min of SWE resulted in an extraction yield of ca 40% (w/w) of the original sample, whereas the WI provided a total yield of ca 25%. The results demonstrate that SWE can provide higher extraction efficiency than WI. Sequential fractionation combined with high-resolution LC-MS/MS analysis provides a useful approach for monitoring the release and distribution of individual compounds during SWE.

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WeP-11: Compound-Specific Remodeling of Recombinant Clr-b-Fc Fusion Protein N-Glycosylation by Fluorinated Monosaccharide Analogs

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Fluorinated monosaccharide analogs can alter cellular glycosylation, yet their effects on individual recombinant glycoproteins are difficult to predict from bulk-cell measurements. We assessed whether seven peracetylated fluorinated hexosamine or fucose analogs produce distinct N-glycosylation profiles on recombinant Clr-b-FcHis expressed in suspension-adapted HEK293T cells.

Cells were treated with 50 µM of each analog and compared with an untreated control from the same experiment. Purified proteins were digested and analyzed by reversed-phase LC–ESI–QTOF MS/MS. We identified and quantified 19 glycoforms sharing a peptide backbone. Principal component analysis and matched-control comparisons were used to assess profile differences and their structural basis.

Secreted protein was recovered after every treatment, although yields varied. The lowest yield was observed with 2F-Ac_3-Fuc (22% of its matched control); 4F-Ac_3-GalNAc yielded 53% and showed pronounced electrophoretic heterogeneity. Most analogs caused only mild glycoprofile changes within the range of variation among controls. By contrast, 4F-Ac_3-GalNAc and especially 2F-Ac_3-Fuc produced clear compositional shifts. 4F-Ac_3-GalNAc favored glycans with less N-acetylhexosamine and reduced fucosylation, consistent with impaired late-stage Golgi processing. 2F-Ac_3-Fuc markedly depleted fucosylated glycoforms.

These results show compound-specific remodeling of recombinant-protein N-glycosylation and support fluorinated monosaccharide analogs as flexible, conditional tools for screening glycan modulation without permanent host-cell engineering.

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WeP-12: Chiral SFC-MS for the analysis of diacylglycerol isomers

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Diacylglycerols (DGs) are important intermediates in lipid metabolism and signaling pathways, occurring in biological systems as complex mixtures of regioisomers and enantiomers. DGs containing two different fatty acyls can exist as six distinct isomers resulting from different positions of fatty acyl chains on the glycerol backbone and their stereochemical arrangement. Since individual isomers may exhibit different biological functions, the development of novel analytical platforms for their characterization is highly desirable.

In this work, we developed a chiral supercritical fluid chromatography–mass spectrometry (SFC-MS) method for the stereospecific analysis of asymmetric diacylglycerol isomers using polysaccharide-based chiral stationary phases. Representative DG standards containing different fatty acyls were synthesized and utilized for method development and optimization. The influence of key chromatographic parameters, including stationary phase chemistry, mobile phase composition, temperature, and backpressure, was systematically investigated.

The optimized chiral SFC-MS conditions enabled the characterization of all six isomers of selected asymmetric DGs within a single analysis. The developed approach demonstrates the significant potential of chiral SFC-MS for advanced stereospecific lipidomic analysis of complex glycerolipids.

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WeP-13: Clonotypic Peptide Mass Spectrometry for Monitoring M-Protein in Patients with Multiple Myeloma

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Background: Minimal residual disease assessment in multiple myeloma (MM) using bone marrow (BM)-based methods, such as next-generation flow cytometry, is limited by the invasive nature of BM sampling and the spatial heterogeneity of MM. Mass spectrometry (MS) may offer a minimally invasive alternative for monitoring monoclonal antibody (M-protein) detection in serum. Clonotypic peptide MS relies on patient-specific sequences, enabling subsequent longitudinal monitoring of the corresponding peptides in serum. We conducted an exploratory feasibility analysis of this approach in patients with MM.

Methods: Patient-specific clonotypic peptide sequences derived from next-generation sequencing (NGS) of myeloma cells isolated from baseline BM samples. Immunoglobulins were enriched from serum samples (n=10), followed by digestion using 3 proteolytic enzymes. Samples were analyzed by high-resolution MS to detect clonotypic peptides. The performance of the 3 proteolytic enzymes for generating clonotypic peptides originating from complementarity-determining regions 1-3 (CDR1-3), was compared.

Results: Clonotypic peptides were successfully identified in all baseline samples and subsequently detected in serum, supporting the feasibility of a blood-based approach for monitoring patient-specific M-protein. The use of three different proteolytic enzymes enabled assessment of their suitability for generating detectable clonotypic peptides from hypervariable immunoglobulin regions, including CDR3. These findings demonstrate the feasibility of MS-based detection of patient-specific M-protein in serum.

Conclusion: Clonotypic peptide MS enables blood-based detection and monitoring of patient-specific M-protein in MM and potentially reducing the need for repeated BM sampling.

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WeP-14: Effects of ESI Output Resistance and Grounding-Capillary Configuration on Intact Protein Analysis

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Elevated ionic strength may be required to maintain native-like conditions for some proteins and protein complexes. Ammonium acetate is often used in native electrospray ionization mass spectrometry (ESI-MS) because it is volatile and compatible with mass spectrometric analysis. [1] However, increasing salt concentration raises solvent conductivity and, consequently, ESI current. This can increase the voltage drop across the source's output resistance, reducing the actual potential at the ESI needle. High ion currents may also promote salt deposition at the needle and shorten its usable lifetime.

We investigated how increasing solvent salt concentration, ESI output resistance, and grounding-capillary configuration affect data quality in intact protein analysis. The results suggest that the optimum ESI voltage setpoint, typically within 2.8–3.5 kV, depends on the ion source's current-protection design, including active and passive components, as well as the grounding-capillary connection. These findings indicate that the grounding capillary should be selected with the ion source's current-protection limits in mind. Modifying or extending the ground-capillary connection may help maintain stable ESI conditions when using high-conductivity solvents.

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WeP-15: Proteomic Profiling of Brain Immune Cell Responses to Acute and Prolonged Microplastic Exposure

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Background: Nanoplastics and microplastics are present in drinking water, food, and air, making human exposure difficult to avoid. Increasing evidence suggests that exposure to plastic particles may induce inflammatory responses and oxidative stress, potentially affecting the central nervous system. The mechanisms by which micro- and nanoplastics modulate brain immune cells are still poorly understood.

Materials and Methods: To investigate the effects of microplastic exposure on brain immune cells, we employed a primary rat glial co-culture model. Cells were exposed to polystyrene and polyethylene terephthalate (PET) particles for 48 hours, representing acute exposure, or 2 weeks, representing prolonged exposure. Changes in cellular protein expression were assessed using mass spectrometry-based proteomics. Peptide mixtures were separated using an ACQUITY M-Class UHPLC system (Waters, USA) equipped with a nanoEase HSS T3 C18 analytical column. Peptides were analyzed using a ZenoTOF 7600+ time-of-flight mass spectrometer (SCIEX, USA). Spectra were acquired using data-independent acquisition mode.

Results: Microplastic exposure induced distinct proteomic responses depending on particle type and exposure duration. The most pronounced changes were observed after prolonged exposure and involved proteins associated with mitochondrial activity and endocytosis. Our findings suggest that polystyrene particles have a greater potential to induce inflammatory and oxidative stress-related responses, whereas PET particles were associated with proteins involved in endocytosis, including LRP1 protein. Overall, the data indicate that both the type of microplastic and the duration of exposure influence the cellular response of brain immune cells.

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WeP-16: Mapping Molecular Distributions in Whole-Body Sections of *Ixodes ricinus*: A Preliminary MALDI-MSI Study

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Introduction: Matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) enables the spatial visualization of molecular compounds directly within biological tissues while preserving their anatomical context. Although increasingly applied across biomedical and biological research, its potential for studying tick tissues remains largely unexplored. In view of the growing importance of tick-borne diseases, spatial molecular profiling may provide new insights into tick physiology and tissue organization. This study aimed to investigate molecular distributions in whole-body sections of *Ixodes ricinus* using high-resolution MALDI-MSI.

Materials and Methods: Unfed, pathogen-free female *I. ricinus* ticks were embedded in 20% gelatin, rapidly frozen at -80 °C, and cryosectioned using a Leica CM1950 cryostat. Sections were thaw-mounted onto pre-cooled indium tin oxide-coated glass slides, vacuum-dried, and coated with 1,5-diaminonaphthalene matrix using an M3+ Sprayer. MALDI-MSI measurements were performed using a Bruker solariX 12T 2ω FT-ICR mass spectrometer in positive and negative ionization modes, covering an m/z range of 0–2,000 at 10 µm spatial resolution. Data were processed in SCiLS Lab using the T-Rex² algorithm, bisecting k-means clustering, and peak annotation. Molecular distributions were compared with histologically identified anatomical structures.

Results: More than 2,000 molecular features were detected in each ionization mode, with subsequent analysis focusing primarily on the m/z range of 500–1,000. The resulting molecular profiles revealed distinct spatial patterns across whole-body sections of *Ixodes ricinus*. Bisecting k-means clustering identified spatially distinct molecular regions characterized by tissue-associated molecular signatures. Importantly, the spatial distributions of these molecular signatures showed strong agreement with the anatomical organization of individual tick organs, as demonstrated by comparison with histologically stained consecutive sections. This correspondence enabled the assignment of characteristic molecular patterns to specific anatomical structures.

Conclusions: This study presents the first MALDI-MSI protocol for whole-body analysis of *Ixodes ricinus*, enabling the characterization of molecular signatures of *I. ricinus* and, notably, distinct tissue-specific molecular patterns consistent with tick anatomy. This approach provides a valuable framework for future studies of tick biology and tick-borne pathogens, particularly in light of the increasing incidence of tick-borne diseases worldwide.

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WeP-17: Untargeted liquid chromatography-mass spectrometry metabolomics of serum and cerebrospinal fluid in multiple sclerosis patients

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Metabolomics using liquid chromatography–mass spectrometry (LC–MS) enables detailed investigation of biochemical alterations in neurodegenerative disorders such as multiple sclerosis. Although this disease is characterized by autoimmune-mediated demyelination of nerve fibers within the central nervous system, its detailed biochemical phenotype and disease-associated metabolic changes remain incompletely understood.

In this work, a previously developed LC–MS method based on a polar reversed-phase HSS T3 column, enabling efficient separation of a broad range of metabolite classes, is applied for untargeted metabolic characterization of disease-associated metabolites in serum and cerebrospinal fluid samples of multiple sclerosis patients.

Particular attention is devoted to the construction of an in-house spectral and ion mobility library comprising more than 300 reference compounds from major metabolic pathways, which is utilized for level 1 identification. In addition, the retention behavior of these metabolites is subsequently used to develop models for annotating unknown features based on relationships among m/z, polarity, and retention time across chemically defined metabolite classes. These models are used to improve compound annotation in biological samples and to minimize erroneous or implausible assignments arising from misinterpretation of external database matches.

Application of this annotation strategy reveals disease-associated metabolites spanning a wide range of metabolite classes, including amino acids, acylcarnitines, and saccharides in multiple sclerosis. The same disease significant metabolites between serum and cerebrospinal fluid metabolomic profiles highlight potential of readily accessible serum for diagnostic use.

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WeP-18: Characterization of an Ambient Ion Soft Landing Apparatus and Its Application to Nanomaterial Surface Modification

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Ambient ion soft landing (AISL) is an established surface modification technique. It can be used to directly modify non-reactive surfaces (e.g., metals or metal oxides) in open air without any reactive interlayer. This facilitates modifications that are difficult to access via wet chemistry methods. In the presented work, we characterize our lab-made AISL apparatus and test its capabilities in surface modifications. To generate gas-phase ions, an electrospray ionization source made of a Swagelok T-piece and fused silica capillary as an emitter was used. The ion source was mounted to an AISL apparatus with preheated nitrogen flow and an electronically controlled heated desolvation tube. A charge-sensing particle detector was employed to study the charge state and degree of desolvation of the generated charged species under different heating and nitrogen flow conditions. The range of molecules used to probe successful surface modification includes crystal violet, Alexa Fluor 488-Streptavidin conjugate and trypsin. The molecules were deposited on various substrates, such as chemically etched silver, indium tin oxide glass or tungsten-based nanomaterials. Surface-enhanced Raman spectroscopy, fluorescence spectroscopy and mass spectrometry were employed to detect the surface-tethered molecules or to test their surface activity. The ultimate goal of this project is to modify various nanomaterials by protein and enzyme ions to yield materials with unique combinations of properties. These novel materials could potentially open a new avenue for sensors, sensor arrays and molecular electronics.

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WeP-19: GC-MS determination of nerve agent A-232 (Novichok) from water containing samples

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The objective of this study was to validate a GC-MS method for determining the new-generation nerve agent A-232 (one of the so-called Novichoks), using triethyl phosphate as an internal standard and water samples diluted 1:100 with 2-propanol for direct injection. The procedure enables the analysis of A-232 in NaOH-based decontamination mixtures for the purpose of conducting decontamination studies.

A Focus 2 gas chromatograph with single quadrupole MS detector DSQ II, equipped with an autosampler AS3000 (Thermo Electron Corporation, USA), was used together with a 30m x 0.32mm i.d. column (ZB-1) coated with dimethylpolysiloxane with a film thickness 0.25µm (Phenomenex, USA). Helium at the flow rate of 1.0 ml/min (constant flow) was the carrier gas. The inlet temperature was set to 225 °C. Splitless injection (1 µl) was used with a splitless injection liner. The transfer line temperature was 280°C. The initial column temperature was 60°C. After 5 min at 60°C, the temperature was gradually raised to 250°C at the rate of 10°C/min and maintained for 1 min. The MS detector parameters were set as follows: ion source temperature 230°C, scan time 1.22 s, scan period 0.817 s, scan rate (ammu/s) 500.0, mass range 5 - 560 (positive ions).

The calibration curve was linear from 5 to 300 µmol . L^{-1} with $r^2 > 0.99$. The limits of detection and quantitation were determined to be 3 µmol.L^{-1} and 10 µmol.L^{-1} respectively. The intraday precision was < 20% R.S.D. Accuracy ranged from 101% to 78 % at the lowest concentration level. These method parameters are sufficient to perform the decontamination studies.

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WeP-20: 50 Shades of Black: Unravelling the Complex Chemistry of 19th-century Costumes

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Symbolism of black garments has changed markedly over time, from signifying wealth during the Renaissance and religious austerity in Protestant communities to the strict social codes of the Victorian era and the fashion revolution of Coco Chanel's Little Black Dress in the 20th century. Historically, dyeing black required large amounts of tannin-rich dyestuffs combined with iron mordants, a laborious process that damaged costly silk and wool. Industrial Revolution expanded the palette with new classes of colorants. Nevertheless, black synthetic dyes remained scarce among 400 dyes developed by the end of 19th century.

This study focuses on the identification of dyes in 19th-century aristocratic garments from Český Krumlov. Mild extraction was performed in two steps (DMSO and aqueous oxalic acid/methanol/acetone) coupled with a broad-scope UHPLC-PDA-MS method. Automated screening against our database (>250 natural and synthetic dye components) was complemented by data-independent all-precursor fragmentation and ion mobility separation (HDMSE) for structural elucidation of unknowns. Although essentially destructive, the method requires only a single thread about 2 mm long owing to its high sensitivity (LOD <1 ng/mL).

The analyses revealed unexpectedly complex dyeing strategies. Black textiles frequently combined traditional natural colorants, including ellagitannins, logwood, and quercitron, with mixtures of newly introduced synthetic dyes. Even fabrics from a single garment that appeared uniformly black, such as jacket CK09264, were dyed using markedly different formulations. In jacket CK09266, a deep black shade was achieved using more than thirteen dyes, while nearly 50 natural and synthetic dye components were identified in a single thread.

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WeP-21: Distinguishing metal ions from ultrasmall nanostructures in complex media: Comprehensive nanostructures characterization and *in-situ* monitoring of nanoparticle formation

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Metal-containing nanostructures frequently coexist with free metal ions originating from their synthesis, dissolution, or transformations in complex environments [1] . Distinguishing these species while simultaneously characterizing the nanostructured fraction remains a major analytical challenge. Here, we introduce capillary electrophoresis–Taylor dispersion analysis combined with inductively coupled plasma mass spectrometry (CE-TDA-ICP-MS), which separates ionic and nanostructured forms of metals and subsequently provides element-specific detection and advance characterization of the nanostructured fraction from a single injection. Using Ag nanoparticles, we demonstrate that physical separation of coexisting Ag^{+} ions is essential for accurate determination of nanoparticle concentration and particle number concentration, while preserving reliable hydrodynamic size determination and zeta-potential estimation. The approach further enables characterization of ultrasmall Au-BSA and Au-BSA-Fe nanoparticles in complex biological matrices, revealing matrix-dependent changes in their hydrodynamic behavior. In urine, CE-TDA-ICP-MS directly monitor the formation of new Fe based nanoparticles following Fe^{3+} addition to Au-BSA nanoparticles, independently supported by high-resolution transmission electron microscopy. CE-TDA-ICP-MS thus establishes a direct analytical link between metal speciation and quantitative nanostructure chracterization, providing new opportunities to investigate the behavior and transformations of metal-containing nanostructures in complex environments from synthesis to the real applications.

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WeP-22: Enhancing Digestion of Proline-Rich Targets: *An*PEP Columns for HDX and Beyond

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Hydrogen-deuterium exchange mass spectrometry (HDX-MS) is a key tool for studying protein structure and dynamics. Its spatial resolution depends on fast and efficient enzymatic digestion at low pH and temperature. Although pepsin remains the gold-standard enzyme for HDX-MS analysis, its performance is often insufficient to achieve comprehensive sequence coverage and high spatial resolution, driving the search for novel, highly efficient acidic proteases. Recently, the acidic protease *An*PEP, a prolyl endopeptidase from *Aspergillus niger*, has been introduced. Its unique cleavage pattern, with a high preference for proline, alanine, and cysteine, makes it a promising enzyme for digesting proline-rich proteins. While highly specific in solution, its immobilization on a column leads to a broader, semi-specific digestion pattern, making it suitable for processing HDX samples. In our study, *An*PEP was immobilized on a column, both alone and in combination with other HDX-compatible proteases – nepenthesin-2 and pepsin. Subsequently, their performance was evaluated under various digestion conditions and tested on proline-rich proteins and challenging protein targets, such as membrane proteins and antibodies.

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WeP-23: Influence of Sample Preparation on Fatty Acid Profiles: Optimization and Application to Serum from Preterm Infants

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Fatty acid (FA) profiling is an important tool in clinical and biochemical research for investigating metabolic status and nutritional influences in plasma and serum. However, FA profiles may vary with sample preparation, as analytical steps can affect the recovery of lipid classes and fatty acid pools.

We investigated how extraction, fractionation, and derivatization strategies influence FA profiles. Lipids were extracted using a modified Folch procedure with chloroform–methanol, followed by liquid–liquid partitioning into polar and non-polar phases. Further fractionation used solid-phase extraction (SPE) on an aminopropyl sorbent to assess fatty acid distribution among lipid fractions. Two derivatization approaches were compared: base-catalyzed transesterification [1], primarily targeting esterified fatty acids, and acid-catalyzed methanolysis [2], enabling determination of total fatty acids, including free fatty acids.

The results showed that the analytical workflow considerably affected the measured FA composition. Fatty acids expected to partition into the polar phase were also detected in the non-polar fraction, indicating a more complex distribution than anticipated. SPE fractionation revealed differences in the recovery of individual phospholipid classes. The two derivatization procedures also yielded distinct FA profiles, reflecting differences in the fatty acid pools accessible to each method.

Following this evaluation, the selected workflow was applied to serum samples from preterm infants receiving omega-3 fatty acid supplementation, demonstrating its applicability in a clinical setting.

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WeP-24: Understanding Kleefstra-like syndrome – a rare congenital disease using native mass spectrometry

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Epigenetic control of gene expression in humans relies heavily on the activity of histone methyltransferases, specifically the GLP/G9a (EHMT1/EHMT2) complexes. These complexes work by transferring a methyl group from S-adenosylmethionine (SAM) cofactor to the histone H3 tail. When this process fails, it can cause neurodevelopmental disorders such as Kleefstra syndrome, which has been associated with EHMT1 gene mutations. Recently, novel de novo EHMT2 variants were identified in patients presenting with rare congenital diseases that clinically resemble Kleefstra syndrome.

To understand the molecular cause of this disease, we used native mass spectrometry (native MS) to study the structural stability and binding affinities of the mutated G9a proteins with their cofactors. Native MS showed that the G9a variants maintain stable, folded dimers as well as GLP-G9a heterodimers. However, the mutations are located at the G9a substrate interface, leading to defective interactions. Specifically, native MS demonstrated that the mutations significantly reduce the binding affinity either towards the histone H3 peptide or the SAM cofactor. This inability to properly interact with essential cofactors renders the GLP-G9a heterodimers catalytically incompetent, which subsequently disrupts normal epigenetic regulation and lead to the pathogenesis of the rare disease.

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WeP-25: SFC/MS determination of bis(monoacylglycero)phosphates in biological matrices

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Bis(monoacylglycero)phosphates (BMPs) are key phospholipids involved in lysosomal integrity, cholesterol transport and membrane homeostasis. Altered BMP levels have been associated with various pathological conditions, including neurodegenerative diseases and lysosomal storage disorders, making them promising biomarkers for lipidomic and clinical studies. However, BMP analysis in biological samples remains challenging due to their structural complexity and the presence of structurally related lipids, such as phosphatidylglycerols, which may interfere with their reliable identification and determination. The principal aim of this work is the development of a selective supercritical fluid chromatography/mass spectrometry (SFC/MS) method for BMP analysis in biological matrices. Currently, LC/MS, especially reversed-phase (RP) and hydrophilic interaction liquid chromatography (HILIC), is the most commonly used approach for BMP determination. Nevertheless, separation of BMPs from structurally similar lipids in complex biological matrices remains challenging. Compared with conventional LC methods, SFC offers high separation efficiency, rapid analysis and lower solvent consumption, making it a valuable alternative for lipidomic applications. Different chromatographic conditions, including stationary phase, mobile phase composition, modifier and back pressure gradients, flow rate and column temperature, were investigated to improve selectivity and detection sensitivity. Tandem mass spectrometry and ion mobility spectrometry experiments were performed to enable structural characterization and discrimination of BMPs from related lipids. The developed SFC/MS approach provides a reliable analytical tool convenient for BMP analysis in complex biological matrices in clinical studies.

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WeP-26: Ambient Ion Soft-Landing Affinity Chips for MALDI-TOF MS and LC-MS Detection of Apolipoprotein A-IV in Serum

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Apolipoprotein A-IV (ApoA4) has recently been identified as a protein responsible for Autosomal Dominant Tubulointerstitial Kidney Disease (ADTKD-APOA4). This condition is characterized by amyloid deposition confined to the renal medulla which often progresses to kidney failure. Diagnosis currently relies on immunohistology and proteomic analysis of formalin-fixed, paraffin-embedded tissue, which are time consuming and require invasive biopsies. To overcome these limitations, we developed a MALDI-TOF mass spectrometry assay for ApoA4 detection in serum based on ambient ion soft-landing (AISL).

Using AISL, recombinant protein G was deposited onto indium–tin oxide–coated glass slides to form MALDI-compatible affinity chips. The protein G surface was further functionalized with anti-ApoA4 antibodies to form a sandwich-type affinity surface. Human serum samples spiked with ApoA4 were incubated on the chip under humidity-controlled conditions. After washing to remove salts and high abundance serum proteins, a MALDI matrix was applied and intact ApoA4 was analyzed by MALDI-TOF MS (Bruker Autoflex). Proteins eluted from the chip surface were analyzed by bottom-up LC-MS/MS using a Bruker timsTOF instrument.

Protein G retained its binding affinity during the deposition process, enabling subsequent coupling with anti-ApoA4 antibodies. Such sandwich-type arrays were used for selective capture of ApoA4 from complex biological matrices. Enrichment was confirmed by MALDI-MS detection of intact ApoA4. This affinity-based workflow reduced spectral interference from albumin, other high-abundance proteins, and serum salts. Moreover, eluted ApoA4 was analyzed by bottom-up LC-MS/MS, providing complementary sequence information for detection of potential amyloidogenic mutations.

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WeP-27: Correlation of $^{13}C_6$-Glucose Labeling in Mouse Liver Using MALDI Mass Spectrometry Imaging and LC-MS

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Stable isotope labeling has become popular approach in biological research, providing valuable insights into metabolic pathways and their dynamics. In this study, we designed a simple experiment in which mice were injected intraperitoneally with $^{13}C_6$-glucose, and liver tissues were collected at different time points for analysis using Fourier transform ion cyclotron resonance MALDI mass spectrometry imaging (FT-ICR-MALDI-MSI). This approach enabled us to visualize the spatial distribution of labeled intermediates of glycolysis and the tricarboxylic acid (TCA) cycle within liver tissue. The MALDI-MSI data were subsequently correlated with LC–MS results obtained from an adjacent liver lobe of the same animal. Additionally, we present preliminary results from whole-body MALDI-MSI, demonstrating the potential for extending our stable isotope labeling approach to broader metabolic investigations.

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