16TH CENTRAL AND EASTERN EUROPEAN PROTEOMIC CONFERENCE

8TH INFORMAL PROTEOMIC MEETING

10TH CZECH MASS SPECTROMETRY CONFERENCE

Prague, September 29 – October 1, 2022

BOOK OF ABSTRACTS

Book of Abstracts from the

16th Central and Eastern European Proteomic Conference

8th Informal Proteomic Meeting

and

10th Czech Mass Spectrometry Conference

Czech Society for Mass Spectrometry

Prague 2022

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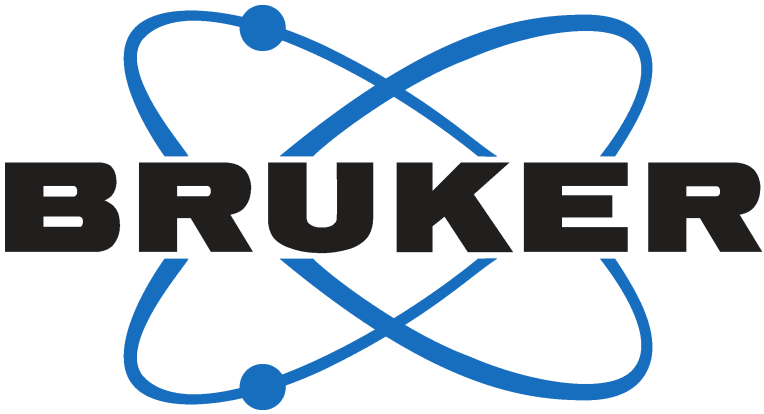

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Sixteenth Central and Eastern European Proteomic Conference, Eighth Informal Proteomic Meeting
and
Tenth Annual Conference of the Czech Society for Mass Spectrometry

Date

29th September – 1st October 2022

Venue

National Library of Technology, Prague

Technická 2710/6,

160 80 Praha 6

Czech Republic

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WELCOME ADDRESS

Dear Delegates

On behalf of the Organising committee we have great pleasure in welcoming you all to the 16th Central and Eastern European Proteomic Conference organized jointly with the 8th Informal Proteomics Meeting and the 10th Czech Mass Spectrometry Conference in Prague, Czech Republic.

We are sure that this Conference will once again immerse you all in excellent proteomics, Mass Spectrometry and 'cutting- edge' science. We are privileged to have the presence of eminent speakers from different countries, who will share their expertise each day. In keeping with the CEEPC ideology, we warmly welcome young researchers who will share their novel ideas and fascinating research for all to enjoy.

A multidisciplinary program encompassing method development, novel instrumentation, enabling software, and the role of mass spectrometry in advancing our understanding of protein functionality in biomedicine is there for all to enjoy. We welcome all participants to share their excitement and urgencies of very many diverse scientific, clinical and proteomic challenges of the day, the central aim being translation of potential findings into viable solutions and /or therapies for diseases affecting mankind.

We are sure that the academic heritage of Prague and its past will infuse a sense of pursuit of excellence over the next few days and that the historical architecture of the city and its offerings will provide a magical charm and excitement to take back home. We also hope you will enjoy the social programme, whilst actively engaging in productive interactions, networking and friendship.

We wish you a very fruitful Conference and a pleasant stay

Suresh Jivan Gadher on behalf of the organizers

THE CENTRAL AND EASTERN EUROPEAN PROTEOMIC CONFERENCE (CEEPC)

The Central and Eastern European Proteomic Conference has progressed to its present-day status and stature with an expansive network of proteomics in Central and Eastern Europe with links to international institutions worldwide to facilitate expert scientific interaction and collaborations. An informative website at http://ceepc.eu/ not only distinguishes CEEPC from other proteomic organizations but also underlines the uniqueness and individuality of its ethos and ideology. The birthplace of CEEPC together with advancing proteomics is captured by the CEEPC ***logo showing the ascending spires of the city of Prague*** outlined by the intensity of protein / peptide peaks of mass spectrometry depicting the pinnacles of excellence and cohesion of the CEEPC community.

The initial vision of a forum for enthusiastic scientists and researchers to meet and discuss their work in a relaxed manner in middle sized meetings remains unchanged to this day. Rotation of the meeting's venue each year to cultural cities of the world such as Prague, Vienna, Budapest, Jena, Poznan, Kosice, Bucharest and this time Prague, Czech Republic, adds to the intertwining of 'cutting edge' research and theexcitement that goes with it. CEEPC's success is not only due to different aspects of proteomics but also due to encompassed diverse proteomic and omics topics as well as appreciation of the hot topics of the advancing science and medicine.

Proteomic technologies have progressed over the last decade allowing in principle the comprehensive analysis of expressed proteins in time and space. Until now, quantitative proteomics has been pin-pointing minor differences in the protein levels between normal and pathological samples. There is now an urgent need for sophisticated ***'enabling technologies'*** to identify structural differences in proteins introduced by mutations or structural variations induced by post-translational modifications or protein truncation that are associated with a disease. Additionally, comprehensive characterization of the small molecule metabolites in the biological systems and biological applications of the ***Metabolome*** together with the ***Proteome*** in ***Precision Medicine*** of the patient, stands to revolutionize global health.

The complexity of the data generated has also been a stumbling block in understanding diseases because proteome analysis does not provide a simple 'yes/no' answer but rather requires deep interpretation. To this end, utilization of data and information from various 'multi-omics' studies including proteomics, metabolomics, together with software and AI in the hands of skilled researchers, can have significant societal impact.

CEEPC's careful balance between excellence and focus on **societal needs**, also holds the key to its success. CEEPC raises concerns about humanitarian issues whilst addressing the above mentioned needs, may it be mental health issues affecting the generation of today, need for effective COVID or Ebola vaccines, malnutrition in poor countries or need for clean water, sanitation and impact of pollution on future generations to come!

Suresh Jivan Gadher

THE 10TH CZECH MASS SPECTROMETRY CONFERENCE

The relatively new tradition of the Czech mass spectrometry conferences was established only 11 years ago, the very first event took place in Hradec Králové in 2011. Since then, the conference was organized every year except for 2020, the year of the pandemic. Over the years, the conference took place in Hradec Králové (4×), Prague (3×), Olomouc (2×) and České Budějovice (1×). The invited speakers included leading figures of mass spectrometry such as Günter Allmaier, Frank Tureček, Dieter Gerlich, Miloš Novotný, James Bruce, Renato Zenobi, Michael Przybylski, Kevin Schug, Andrea Sinz, Helmut Schwarz, Magnus Palmblad, David Goodlett, Aleš Svatoš, Zdeněk Lánský, Karel Lemr, Michal Holčapek and Vladimír Havlíček. This year, we are fortunate to welcome Karl Mechtler, Carlos Cordeiro and Bernd Wollscheid as invited plenary speakers. The conference has been generously supported by all major mass spectrometry vendors and many other companies, universities, and the Czech Academy of Sciences.

Since the very beginning, the Czech mass spectrometry conference put a lot of emphasis on the active participation of graduate students and postdocs. The conference is a unique opportunity for young mass spectrometry scientists from Czechia and Slovakia to present at a local event, but in an international format and in front of wider Central European audience.

This year, the 10th Czech mass spectrometry conference is co-organized together with the 16th Central and Eastern European Proteomic Conference. The joint meeting gives our modest scientific event broader international exposure and diverse European attendance. We trust that the next ten years will bring even more development and expansion and that the conference will establish itself as regional meeting in the field.

We wish you great stay in Prague and we hope to see you again in the future

Michael Volný
Institute of Microbiology, Czech Academy of Sciences

THURSDAY 29TH SEPTEMBER, 2022

8:30 – 10:00	Registration
10:00 – 10:10	Opening of the 16th CEEPC, 8th IPM and 10th CMSC
10:10 – 11:00	**Plenary lecture I: Karl Mechtler** (Chairperson: Petr Novák)
PL-01	*New insights through Single-Cell Proteomics*
11:00 – 11:20	Coffee break
11:20 – 13:00	**Session I – Method Development**
	(Chairperson: Katarina Davalieva)
11:20 – 11:40	Ondřej Vít
ThO-01	*Identification of potential cell-surface targets and druggable enzymes in human pheochromocytoma and paraganglioma using classical and membrane-targeting proteomic approaches*
11:40 – 12:00	Petr Lapčík
ThO-02	*Global SEC-PCP-SILAC mapping reveals protein complexes mediating NF-κB activation in breast cancer*
12:00 – 12:20	Haci Mehmet Kayili
ThO-03	*Determination of changes in the milk proteome in production and storage of kefir using mass spectrometry-based omics approaches*
12:20 – 12:40	Katarína Maráková
ThO-04	*Non-Immunoaffinity Extraction of Intact Proteins from Biological Fluids and Their Analysis by Liquid Chromatography – Triple Quadrupole Mass Spectrometry*
12:40 – 13:00	Adam Pruška
ThO-05	*Advancing Cyclic Ion Mobility Mass Spectrometry Methods for Studying Biomolecules: Towards the Conformational Dynamics of Mega Dalton Protein Aggregates*
13:00 – 15:30	Lunch followed by **Poster session I** (Odd-numbered posters)
15:30 – 16:30	**Company Workshop – Bruker Daltonics**
	Gary Kruppa: The latest proteomics development at Bruker
	Florian Meier: Clinical (phospho)proteomics with TIMS and PASEF
16:30 – 16:50	Coffee break

16:50 – 17:30	**Short talks** (Chairperson: Petr Man)
ThS-01	*Josef Dvořák: Detection of C. difficile Toxin B by biomolecule-modified chips and mass spectrometry*
ThS-02	*Zuzana Kalaninová: Optimized HDX-MS workflow for antibody structure monitoring by HDX-MS*
ThS-03	*Denis Naplekov: RPLC-UV and HILIC-UV characterisation of on-site produced Ramucirumab-DTPA immunoconjugate*
ThS-04	*Agnes Revesz: Collision energy setting in proteomics and glycoproteomics: From individual species to a practical perspective*
ThS-05	*Matěj Běhounek: Novel circulating biomarkers of biventricular heart failure*
ThS-06	*Dana Dobešová: Comprehensive metabolomic and lipidomic study of tauopathy and Alzheimer's disease patients*
ThS-07	*Roman Tuzhilkin: Overlooked oligomerization process in Azurin, a model metalloprotein*
17:30 – 18:00	**Josef Chmelik Award**
18:00 – 19:30	Guided tour to the conference dinner
19:30 – 23:00	Conference dinner and sightseeing boat trip

FRIDAY 30TH SEPTEMBER, 2022

8:50 – 9:40	**Plenary lecture II: Carlos Cordeiro** (Chairperson: Michael Volný)
PL-02	*An FT-ICR-MS metabolomic journey*
9:40 – 11:20	**Session II – Novel Instrumentation and approaches**
	(Chairperson: László Drahos)
9:40 – 10:00	Alan Kádek
FrO-06	*In a flash of light: Native mass spectrometry for single particle imaging with X ray free electron lasers*
10:00 – 10:20	Volker Kruft
FrO-07	*Qualitative and quantitative advancements of QTOF performance: the SCIEX ZenoTOF 7600 system*
10:20 – 10:40	Suresh Jivan Gadher
FrO-08	*Synergistic success of proteo-genomics in profiling human tear fluid for disease biomarkers using a novel high sensitivity Immunoassay*
10:40 – 11:00	Stanislav Kukla
FrO-09	*Back to Basics with Antibodies*
11:00 – 11:20	Maria van Agthoven
FrO-10	*Two-dimensional mass spectrometry for top-down analysis and structural characterization of proteins*
11:20 – 11:40	Coffee break
11:40 – 13:00	**Session III – MS and SW tools**
	(Chairperson: Eva Csosz)
11:40 – 12:00	Florian Meier
FrO-11	*Rapid ion mobility-resolved phosphoproteomics with dia-PASEF*
12:00 – 12:20	Tomáš Ječmen
FrO-12	*Prediction of Intact N-Glycopeptide Retention Time Windows in Hydrophilic Interaction Liquid Chromatography*
12:20 – 12:40	Jiří Novák
FrO-13	*Quantification of small molecules in liquid chromatography/mass spectrometry datasets by CycloBranch*

12:40 – 13:00	László Drahos
FrO-14	*Mass spectrometric analysis of intact proteins: the dark side of deconvolution*
13:00 – 15:30	Lunch followed by **Poster session II** (Even-numbered posters)
15:30 – 17:30	**Session IV – Applications in Biomedicine 1**
	(Chairperson: Maria van Agthoven)
15:30 – 15:50	Katarina Davalieva
FrO-15	*Identification of potential biomarkers for azoospermia by human testis proteomic analysis*
15:50 – 16:10	Pavel Bouchal
FrO-16	*Proteotype classification of renal cell carcinoma for prognosis and therapy response*
16:10 – 16:30	Jiří Houšť
FrO-17	*Stability Study of Triacetylfusarinine C and Gliotoxin in bodily fluids*
16:30 – 16:50	Petr Halada
FrO-18	*MALDI-TOF mass spectrometry: A powerful method for blood meal identification in insect vectors*
16:50 – 17:10	Durga Jha
FrO-19	*Characterization of glycosphingolipids in iPSC-derived cerebral organoids and its application in neurodegenerative disorders*
17:10 – 17:30	Kristýna Pimková
FrO-20	*Redox resetting by modulation of redox sensor Kelch-like ECH-associated protein 1 restores leukemic cells sensitivity to Azacytidine*
17:30 – 18:10	**Zdeněk Herman Award presented by Resonance Foundation and presentation of the winning thesis**
18:10 – 18:30	Coffee break
18:30 – 19:00	**General assembly of the CSMS**

SATURDAY 1ST OCTOBER, 2022

8:50 – 10:50	**Session V – Applications in Biomedicine 2**
	(Chairperson: Haci Mehmet Kayili)
8:50 – 9:10	Martin Hubálek
SaO-21	*Proteomic Analysis of Hepatitis B Virus*
9:10 – 9:30	Jakub Sýs
SaO-22	*The single amino acid substitutions in Mason-Pfizer Monkey Virus matrix protein modulate its proteolytic cleavage rate*
9:30 – 9:50	Viorel-Iulian Suica
SaO-23	*Cardiac alarmins as residual risk markers of atherosclerosis under lipid-lowering therapy*
9:50 – 10:10	Tomáš Oždian
SaO-24	*Cervical mucus – a non-invasive uterine biomarker source?*
10:10 – 10:30	Darshak Gadara
SaO-25	High-Throughput µLC-MS/MS Lipidomics of 3D In Vitro Disease Models to Investigate Lipid Dysregulation
10:30 – 10:50	Eva Csosz
SaO-26	*Multi-omics network analysis in atherosclerosis reveals mechanisms driving the progression towords complicated lesion*
10:50 – 11:10	Coffee break
11:10 – 12:00	**Plenary lecture III: Bernd Wollscheid** (Chairperson: Pavel Bouchal)
PL-03	*Light-mediated discovery of surfaceome nanoscale organization and inter-cellular receptor interaction networks*
12:00 – 12:20	Poster prize, Closing remarks
12:20 – 13:30	Lunch

PL-01: NEW INSIGHTS THROUGH SINGLE-CELL PROTEOMICS

Claudia Ctortecka [1], David Hartlmayr [1], Manuel Matzinger [1], Elisabeth Müller [1], Anjali Seth [2], Sasha Mendjan [3], Guilhem Tourniaire [2], Karl Mechtler [1,3] *

1. Research Institute of Molecular Pathology (IMP), Vienna BioCenter (VBC), Campus-Vienna-Biocenter 1, 1030 Vienna, Austria

2. Cellenion SASU, 60F avenue Rockefeller, 69008 Lyon, France

3. Institute of Molecular Biotechnology of the Austrian Academy of Sciences (IMBA), Vienna BioCenter (VBC), Dr. Bohr-Gasse 3, 1030 Vienna, Austria

The analysis of single cell proteomes has recently become a viable complement to transcriptomics and genomics studies. Proteins are the main driver of cellular functionality and mRNA levels are often an unreliable proxy of such. Therefore, the global analysis of the proteome is essential to study cellular identities. Both multiplexed and label-free mass spectrometry-based approaches with single cell resolution have lately attributed surprising heterogeneity to believed homogenous cell populations. Even though specialized experimental designs and instrumentation have demonstrated remarkable advances, the efficient sample preparation of single cells still lacks behind.

Here, we introduce the proteoCHIP, optimized for multiplexed single cell proteomics sample preparation at surprising sensitivity and throughput. Sample processing using the cellenONE® robot, allows to reduce final sample volumes to low nanoliters submerged in a hexadecane layer simultaneously eliminating error prone manual sample handling and overcoming evaporation. This results in around 1,500 protein groups per analytical run at remarkable reporter ion signal to noise while reducing or eliminating the carrier proteome. We identified close to 2,600 proteins across 170 multiplexed single cells from two highly similar human cell types. This dedicated loss-less workflow allows to distinguish in vitro co-differentiated cell types of self-organizing cardiac organoids based on indicative markers across 150 single cells. In-depth characterization revealed enhanced cellular motility of endothelial cells and acute myocardium sarcomere organization in cardiomyocytes.

In addition, we evolved a robust and sensitive one-pot label free single cell workflow. By working in standard 384 well plates and compatibility with both, cell sorting in the cellenONE® or using alternatives like a FACS device the need for specialized equipment is obsolete making this workflow easy to use, cheap and accessible to a broader community. By keeping the sample in a hydrated state during proteolytic digestion and addition of DMSO for storage we improved recovery of hydrophobic peptides and boosted ID numbers to more than 1000 proteins from a single cell without match between runs.

In conclusion, our versatile, and semi-automated sample preparation workflows have not only proven to be easily adoptable but are also sufficiently sensitive to drive biological applications of single cell proteomics.

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PL-02: AN FT-ICR-MS METABOLOMIC JOURNEY

Marta Sousa Silva [1], António Ferreira [1], João Luz [1], Mariana Louro [1], Rodrigo Osawa [1], Francisco Traquete [1], Henrique Silva [1], Mónica Soeiro [1], Mariana Pereira [1], Carlos Cordeiro [1 *]

1. FT-ICR and Structural MS Laboratory, MARE – Environmental an Marine Research Centre, Faculdade de Ciências, Universidade de Lisboa, Portugal

Untargeted metabolomics provides a broad survey of the instant composition of living cells or biological fluids. Dynamic range and chemical diversity of the metabolome are the main challenges for its meaningful in depth characterization. Extreme resolution and mass accuracy MS decoupled from chromatographic separation allow the simultaneous analysis of the most diverse range of metabolites in a short period of time, enabling high throughput studies. Here we show the application of FT-ICR-MS to several biological, environmental and forensic problems, highlight the role of computational methods to transform MS spectra into biological information.

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PL-03: LIGHT-MEDIATED DISCOVERY OF SURFACEOME NANOSCALE ORGANIZATION AND INTER-CELLULAR RECEPTOR INTERACTION NETWORKS

Berndt Wollscheid [1 *]

1. Institute of Translational Medicine, ETH Zurich, Switzerland

The molecular nanoscale organization of the surfaceome is a fundamental regulator of cellular signaling in health and disease. Technologies for mapping the spatial relationships of cell surface receptors and their extracellular signaling synapses would unlock theranostic opportunities to target protein communities and the possibility to engineer extracellular signaling. Here, we develop an optoproteomic technology termed LUX-MS that enables the targeted elucidation of acute protein interactions on and in between living cells using light-controlled singlet oxygen generators (SOG). By using SOG-coupled antibodies, small molecule drugs, biologics and intact viral particles, we demonstrate the ability of LUX-MS to decode ligand receptor interactions across organisms and to discover surfaceome receptor nanoscale organization with direct implications for drug action. Furthermore, by coupling SOG to antigens we achieved light-controlled molecular mapping of intercellular signaling within functional immune synapses between antigen-presenting cells and $CD8^+$ T cells providing insights into T cell activation with spatiotemporal specificity. LUX-MS based decoding of surfaceome signaling architectures thereby provides a molecular framework for the rational development of theranostic strategies.

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THO-01: IDENTIFICATION OF POTENTIAL CELL-SURFACE TARGETS AND DRUGGABLE ENZYMES IN HUMAN PHEOCHROMOCYTOMA AND PARAGANGLIOMA USING CLASSICAL AND MEMBRANE-TARGETING PROTEOMIC APPROACHES

Ondřej Vít [1 *], Zdeněk Musil [2,3], Igor Hartmann [4,5], Zdeněk Fryšák [6,5], Karel Pacák [7], Jiří Petrák [1]

1. Charles University, 1st Faculty of Medicine, BIOCEV, Vestec
2. Charles University, 1st Faculty of Medicine, Institute of Biology and Medical Genetics
3. General University Hospital in Prague
4. University Hospital Olomouc, Department of Urology
5. Palacký University, Faculty of Medicine and Dentistry, Olomouc
6. University Hospital Olomouc, Department of Internal Medicine III
7. Eunice Kennedy Shriver National Institute of Child Health and Human Development, NIH, Bethesda, USA

Integral membrane proteins (IMPs) represent optimal drug targets but are under-represented in standard proteomic analyses due to their amphipathy, lack of trypsin cleavage sites, and low expression levels. To identify tumor-upregualted IMPs and druggable enzymes in rare neuroendocrine tumors – pheochromocytoma and paraganglioma (PPGL), we combined three approaches: 1) hpTC method, where the identification of IMPs is based on their hydrophobic alpha-helices, isolated by proteolytic shaving and re-cleaved with CNBr; 2) two glycocapture methods - lectin entrapment on ultrafilters (N-glyco-FASP), and solid-phase enrichment with hydrazide chemistry (SPEG); and 3) the classical detergent-trypsin approach.

We focused mainly on the high-risk tumors belonging to the so-called cluster 1, characterized by mutations in genes related to citric acid cycle and hypoxia, such as SDHB, VHL, and EPAS1). The classical trypsin-based proteomic approach pointed us toward upregulated soluble druggable enzymes (autotaxin, SHMT2, and Arginase 2) and upregulated cell surface IMPs (including CD146 and CD171). The glycopeptide enrichment approach provided additional potential cell surface targets (CD39), and the hpTC method provided additional IMP targets (e.g., anoctamin-1).

The above-mentioned IMPs and soluble enzymes have all been previously shown to be upregulated in several human cancers and to affect tumor progression directly. Their marked upregulation was confirmed by specific antibodies. Together, this makes these molecules promising candidates for drug targets and/or proteins enabling sensitive tumor imaging.

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THO-02: GLOBAL SEC-PCP-SILAC MAPPING REVEALS PROTEIN COMPLEXES MEDIATING NF-κB ACTIVATION IN BREAST CANCER

Petr Lapčík [1 *], Greg Stacey [2], David Potěšil [3], Leonard Foster [2,4], Pavel Bouchal [1]

1. Masaryk University, Faculty of Science, Department of Biochemistry, Brno
2. University of British Columbia, Michael Smith Laboratories, Vancouver
3. Masaryk University, Central European Institute of Technology, Brno
4. University of British Columbia, Department of Biochemistry and Molecular Biology, Vancouver

NF-κB has essential role in immune response and is associated with lymph node metastasis of luminal A breast tumors [1]. Analysis of protein interactome and its changes in response to NF-κB modulation could uncover pro-metastatic mechanisms related to NF-κB. We apply metabolic isotope labeling SILAC, size exclusion chromatography (SEC) and protein correlation profiling (PCP) [2] to construct a network of interactome rearrangement in response to NF-κB modulation in MCF-7 breast cancer cells.

We generated two co-fractionation datasets consisting of 80 fractions from SILAC-labeled and 80 fractions from label-free native MCF-7 lysates with inhibited or native NF-κB activity. LC-MS/MS analysis of SEC fractions using Orbitrap Lumos and Bruker Impact II mass spectrometers quantified 3308 and 5460 protein groups in SILAC and label-free datasets, respectively (FDR = 0.01). Interactome reconstruction using PrinCE [3] detected 7568 interactions among 1520 proteins. Co-elution of subunits of known complexes, such as ribosome, proteasome and MCM, was observed. Modulation of NF-κB was linked to interactome changes of proteins involved in immune response, cell cycle and DNA replication. NF-κB factor RELA interacted with proteins co-eluting with activators of NF-κB and these interactions were modulated by NF-κB inhibition.

Our interaction network represents a complex insight into dynamics of MCF-7 protein interactome associated with NF-κB pathway and could serve as a basis for future studies characterizing NF-κB in breast cancer.

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THO-03: DETERMINATION OF CHANGES IN THE MILK PROTEOME IN PRODUCTION AND STORAGE OF KEFIR USING MASS SPECTROMETRY-BASED OMICS APPROACHES

Sena Damar [1], Hacı Mehmet Kayili [1 *], Mehmet Atakay [2], Hüseyin Avni Kirmaci [3], Bekir Salih [2]

1. Karabuk University, Faculty of Engineering, Biomedical Engineering Department
2. Hacettepe University, Faculty of Science, Chemistry Department
3. Karabuk University, Safranbolu Tourism Faculty, Gastronomy and Culinary Arts Department

Kefir is an important food source based in the Caucasus region. The beneficial effects of kefir on human health are indicated in the literature. The mechanisms underlying these critical properties of kefir have not yet been elucidated. This study aims to examine the changes in milk protein profiles during kefir production and storage by using mass spectrometry-based-omics approaches. In addition, the detection of kefir-based proteins that transfer to milk from kefir microflora was investigated within the scope of the study. First, Kefir production was carried out. During the production and storage of kefir, kefir samples were taken from the periods determined in the study, and milk proteins were extracted. Peptides were produced using Lys-C and trypsin enzymes by a classical proteomics approach. Afterward, the fractionation of the peptides was carried out. Peptide-containing samples were analyzed by nLC-QExactive-Plus mass spectrometry. The data obtained as a result of the analysis were processed with the Maxquant software, and statistical analyzes were performed. According to the results obtained, significant changes were found in the milk proteome in 3 proteins between 0-12 hours, 39 proteins between 0-24 hours, 41 proteins between 0-7 days, and finally, 42 proteins between 0-28 days. From the 24th hour, a significant change was observed in 23 proteins. In the analysis, it was determined in the study that 398 kefir-based proteins were released into the milk. It is anticipated that these results will provide valuable contributions to the literature in terms of showing the changes in the milk proteome in kefir production.

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THO-04: NON-IMMUNOAFFINITY EXTRACTION OF INTACT PROTEINS FROM BIOLOGICAL FLUIDS AND THEIR ANALYSIS BY LIQUID CHROMATOGRAPHY – TRIPLE QUADRUPOLE MASS SPECTROMETRY

Katarína Maráková [1 *], Shannon L. Thomas [2], Beatriz J. Renner [2], Kevin A. Schug [2]

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One of the crucial steps in quantitation of intact proteins from complex biological matrices is, except their reliable analysis, also sample preparation to achieve sufficient specificity and sensitivity. Commonly used immunoaffinity-based methods are characterized by their superior selectivity, although this can be a drawback if simultaneous analysis of multiple different proteins from a single sample is required. In our work, we developed non-immunoaffinity sample preparation based on a generally widely affordable microelution solid phase extraction for eleven model intact proteins (5.5 – 29 kDa) with various isoelectric points (4.5 - 11.3). Extracted intact proteins were analysed by reversed-phase liquid chromatography coupled with a triple quadrupole mass spectrometer operated in a multiple reaction monitoring (MRM) mode. Reversed-phase separations were performed on the Restek wide-pore Viva C4 column, as mobile phases served water and acetonitrile acidified by 0.1% difluoroacetic acid and 0.2% formic acid. The best recoveries for most of the selected proteins were obtained by using the HLB stationary phase. 1% trifluoroacetic acid and 0.2% Triton X-100 were used as efficient pretreatment reagents to release interactions between the proteins and biological matrix. Multiple sample loading was found out to be essential to obtain recoveries >65% in urine for all targeted proteins (up to 30kDa) and >50% in serum/plasma for most of the proteins. Limits of quantitation in biological matrices were in the range 2 - 1200 ng/mL, corresponding to 0.23 - 97.6 nM.

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THO-05: ADVANCING CYCLIC ION MOBILITY MASS SPECTROMETRY METHODS FOR STUDYING BIOMOLECULES: TOWARDS THE CONFORMATIONAL DYNAMICS OF MEGA DALTON PROTEIN AGGREGATES

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Native mass spectrometry is a powerful tool for the analysis of non-covalent complexes. When coupled with high-resolution ion mobility, this technique can be used to investigate the conformational changes induced in said complexes by different solution or gas-phase conditions. In this study, we describe how a new generation high-resolution ion mobility instrument equipped with a cyclic ion mobility cell can be utilized for the analysis of large biomolecular systems, including temperature-induced protein aggregates of masses greater than 1.5 MDa, as well as a 63 kDa oligonucleotide complex. The effects of and the interplay between the voltages applied to the different components of the cyclic ion mobility spectrometry system on ion transmission and arrival time distribution were demonstrated using biomolecules covering the m/z range 2 000 to 10 000. These data were used to establish a theoretical framework for achieving the best separation on the cyclic ion mobility system. Finally, the cyclic ion mobility mass spectrometer was coupled with a temperature-controlled electrospray ionization source to investigate high-mass protein aggregation. This analysis showed that it was possible to continuously monitor the change in abundance for several conformations of MDa aggregates with increasing temperature. This work significantly increases the range of biomolecules that can be analyzed by both cyclic ion mobility and temperature-controlled electrospray ionization mass spectrometry, providing new possibilities for high-resolution ion mobility analysis.

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FRO-06: IN A FLASH OF LIGHT: NATIVE MASS SPECTROMETRY FOR SINGLE PARTICLE IMAGING WITH X RAY FREE ELECTRON LASERS

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Native mass spectrometry enables the ionization and transfer of intact non-covalent protein complexes into the gas phase. As such, it is a great tool to study protein assemblies in a mass and conformation specific manner, probing structural transitions they undergo. Such transient states are highly interesting for structural biology, but generally cannot be purified and are very hard to access for complementary methods such as crystallography or electron microscopy.

Despite its sensitivity and selectivity, the structural resolution in native MS alone is limited. The amount of structural information could be vastly increased by its combination with powerful hard X ray free electron lasers (XFELs) such as the LCLS II in Stanford (USA) or the European XFEL, the world's brightest light source in Hamburg (Germany). One of the science drivers for these facilities has been enabling structural studies on single particles. However, these efforts have been hindered by the lack of efficient ways to introduce samples into the X-ray beam. [1] In this regard, native MS offers huge benefits in producing and transmitting molecular ions ranging in size from single proteins and non-covalent protein complexes up to multi-megadalton viral particles. Also, profiting from ion charges, MS can select and separate protein complexes based on their mass and conformation and possibly also pre-orient particles to simplify subsequent data processing and structure determination. [2,3]

This contribution will present the work and current development status of the highly interdisciplinary MS SPIDOC consortium, which works toward a native MS-based sample introduction system for use at beamlines of the European XFEL and other light sources.

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FRO-07: QUALITATIVE AND QUANTITATIVE ADVANCEMENTS OF QTOF PERFORMANCE: THE SCIEX ZENOTOF 7600 SYSTEM

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A novel hybrid collision cell is at the heart of the technological innovations introduced with the SCIEX ZenoTOF 7600 system.

In the past, QTOF mass spectrometers have suffered from duty cycle losses; that is, losses in ion transmission in the ion path. This was mainly due to the mating the continuous beam coming from the quadrupole ion path with time-of-flight (TOF) analysis, a pulsed, discontinuous measurement technique. A series of ion-staging events and reverse-mass sequential ion release, with high-capacity ion traps, have been introduced just after the CID collision cell (Q2) and before the pusher region of the TOF. This allows the duty cycle losses to be mitigated leading to MS/MS sensitivity gains of 4-20 fold [1].

The newly engineered collision cell also has the ability to perform both collision induced dissociation (CID) and electron activated dissociation (EAD) experiments for high-resolution, high sensitivity MS/MS flexibility. Electron kinetic energies can be tuned from 0-25 eV without the use of chemical transfer reagents. This precise tunability means EAD can be performed on a wide range of analytes , from multiply charged peptides to singly-charged small molecules [2].

The increase in MS/MS sensitivity, the high speed of acquisition (133Hz) and the choice of fragmentation regimes enables improvements in data quality and depth on analysis in proteomics, metabolomics and lipidomics workflows. Protein IDs from cell lines exceeds 5000 protein groups with up to 95% of these reliably quantitated (CV <20%) at minimal protein loads. Lipids can be fully characterized at an LC time scale (around 30ms EAD reaction time) including their lipid class, acyl group structure, and the location of double bond(s) [3].

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FRO-08: SYNERGISTIC SUCCESS OF PROTEO-GENOMICS IN PROFILING HUMAN TEAR FLUID FOR DISEASE BIOMARKERS USING A NOVEL HIGH SENSITIVITY IMMUNOASSAY

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Quest for key disease biomarker(s) is one of the biggest challenges of today. Human tears, with their comprehensive biomolecule repertoire, are a good source of biomarkers and offer an excellent opportunity for patient stratification in precision medicine. Tear fluid is a potential medium for biomarker discovery in ocular, metabolic and systemic diseases such as diabetic retinopathy, cystic fibrosis and cancers [1]. The proximity of the tear fluid to the brain, makes it an ideal fluid for studying neurological disorders such as multiple sclerosis, Parkinson's disease and brain tumors. Tear collection is fast and noninvasive using a Schirmer's strip [2].

Researchers studying the multifaceted etiology of various diseases require powerful investigational tools with greater sensitivity, better dynamic range, miniscule micro-litre volume consumption and robust data analysis software. ProQuantum™ High Sensitivity Immunoassays incorporating analyte specificity of high affinity antibody-antigen binding with the signal detection and amplification of real-time PCR technologies, offers a flexible and scalable qPCR assay platform enabling multidimensional analysis of DNA, RNA, and now biological proteins. It can play an integral role in screening large number of tear samples for molecular signature of human diseases at protein level in eye clinics and for establishing biomarker profiles of disease status [3].

It is anticipated that an established 'tear test' would be a very powerful prognostic and / or diagnostic test in eye clinics in the near future for systemic, neuronal and ocular diseases. ProQuantum™ Immunoassay with its unique features, can benefit precision medicine by maximizing patient's data outcome while minimizing potential health disparities.

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FRO-09: BACK TO BASICS WITH ANTIBODIES

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In this sponsor talk, a couple of selected overlapping areas between proteomics research and mass spectrometry (MS) techniques will be highlighted and shown on antibodies and their manufacturing, characterization and usage in various antibody-based techniques as examples. You will learn about enhanced antibody validation during which new strategies and recently developed technologies are being used (including MS techniques) as a concept to bring more reproducibility and predictability to Life Science. Part of the talk will be also dedicated to describing different LC-MS approaches for middle-up, intact mass, glyco-profiling and peptide mapping analysis of protein therapeutics, such as monoclonal antibodies (mAbs). All discussed topics will be illustrated by our in-house R&D data that helped develop all the new reagents and tools that will be presented.

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FRO-10: TWO-DIMENSIONAL MASS SPECTROMETRY FOR TOP-DOWN ANALYSIS AND STRUCTURAL CHARACTERIZATION OF PROTEINS

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Two-dimensional mass spectrometry (2DMS) is a method for tandem mass spectrometry that relies on ion radius modulation instead of ion isolation to correlate between precursor and fragment ion peaks. 2D mass spectra show all the fragmentation patterns of the analytes in a sample. Signal multiplexing yields high signal-to-noise ratios and therefore complete sequence coverage (e.g. for biomolecules) [1]. Modifications can easily be assigned and located visually with precursor ion scans and dissociation lines. 2DMS has also successfully been used for label-free relative quantification of modified histone peptides [2].

Acetylation is a covalent labelling method to probe tertiary and quaternary structures of proteins which has successfully been used in combination with top-down analysis to probe the structure of ubiquitin [3]. In this study, we analyse acetylated ubiquitin with 2DMS. We use the accuracy of the precursor-fragment correlation to identify and locate the acetylations in the sequence and we use fragment ion abundances for label-free relative quantification. We show that acetylation combined with 2DMS yields accurate information on ubiquitin tertiary structure.

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FRO-11: RAPID ION MOBILITY-RESOLVED PHOSPHOPROTEOMICS WITH DIA-PASEF

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Although protein phosphorylation is one of the best-studied post-translational modifications, cellular function and kinase-substrate relationships remain enigmatic for the vast majority of all identified modification sites. To decipher cellular signaling networks, functional mass spectrometry-based phosphoproteomics is an increasingly attractive strategy that benefits directly from further technological advances. Here, we explore the analytical merits of trapped ion mobility mass spectrometry and data-independent acquisition (dia-PASEF). Using an optimized data acquisition scheme, we quantified over 12,000 phosphopeptides in one hour from low sample amounts equivalent to ~20 ug protein extract per analysis without a spectral library. Strikingly, in 7 min gradients we still quantified about 80% of the class I sites with high accuracy and reproducibility. Our data shows that this is at least partly due to the ion mobility separation compensating for the increased spectral density at shorter gradients. We thus conclude that dia-PASEF offers great potential for scaling up phosphoproteomics both in terms of throughput and sensitivity.

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FRO-12: PREDICTION OF INTACT N-GLYCOPEPTIDE RETENTION TIME WINDOWS IN HYDROPHILIC INTERACTION LIQUID CHROMATOGRAPHY

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Analysis of protein glycosylation is challenging due to micro- and macro-heterogeneity of the attached glycans. Mass spectrometry is a key tool for structural characterization of intact glycopeptides. However, to achieve high-confidence identification, information-rich MS/MS spectra are needed. If such spectra are not available for all analyzed glycopeptides, the confidence of their identification can be improved by including orthogonal information, such as those derived from chromatographic parameters into the search engine algorithms. Here, we propose a simple model predicting relative retention time (RRT) windows for glycopeptides in hydrophilic interaction liquid chromatography (HILIC), which is a mode of choice for separation of this type of analytes as they are inadequately resolved by reversed phase chromatography.

We determined chromatographic parameters for 6 differently glycosylated tryptic peptides of 3 plasma proteins – haptoglobin, hemopexin, and sex hormone-binding globulin – separated by HILIC. For all of them, we calculated retention times of different glycoforms attached to the same peptide relative to the respective bi-antennary form, which is typically found in high yield. We showed that the RRT differences between the glycoforms do not depend greatly on the character of the peptide moiety, and based on the observed variance of the RRTs of individual glycoforms we derived an easy-to-use mathematical model. To prove the concept, we used it to accurately predict the retention time windows, within which fetuin glycopeptides are eluted. The model could therefore be included into post-search filtering of the glycopeptide assignments when HILIC-MS approach is selected for site-specific protein glycosylation characterization. [1]

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FRO-13: QUANTIFICATION OF SMALL MOLECULES IN LIQUID CHROMATOGRAPHY/MASS SPECTROMETRY DATASETS BY CYCLOBRANCH

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CycloBranch (https://ms.biomed.cas.cz/cyclobranch/) is our open-source, cross-platform, and stand-alone tool originally dedicated to the analysis of accurate tandem mass spectra of cyclic and branched peptides [1]. Recently, the tool was extended to support the dereplication and de novo molecular formula determination of compounds in high-resolution conventional mass spectra, liquid chromatography/mass spectrometry (LC/MS) datasets, and imaging mass spectrometry datasets [2, 3]. Here, we show how the tool can be used for the quantification of small molecules in LC/MS data. For this purpose, the latest version of CycloBranch processes multiple LC/MS data files in a batch. Shapes of peaks in automatically constructed extracted ion chromatograms are approximated with Gaussian and exponentially modified Gaussian functions. If an input calibration dataset is available, CycloBranch calculates concentrations of compounds from the areas under chromatographic peaks upon an automated calibration curve construction. The tool supports the community standard mzML file format as well as several vendors' native file formats. A custom database of compounds and a list of ion types to be found can be defined in a user-friendly graphical interface.

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FRO-14: MASS SPECTROMETRIC ANALYSIS OF INTACT PROTEINS: THE DARK SIDE OF DECONVOLUTION

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One of the most commonly used technique for the study of intact proteins, including monoclonal antibodies (MABs), is the HPLC-MS. Study of pure, large amounts of protein is fairly easy, but for mixtures the situation is more complicated. However, the real challenge is to study small amounts of protein mixtures having large molecular mass. In this case, automatic evaluation (e.g. deconvolution of mass spectra) can give incorrect results and HPLC separation of the mixture is becoming increasingly important.

In this presentation, I would like to illustrate the process, problems and difficulties of evaluation from simple proteins to the study of complex antibody-drug conjugates. In the latter case, when the mixture is too complex, a reliable result can only be obtained using a detailed manual evaluation of the ion chromatograms and mass spectra.

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FRO-15: IDENTIFICATION OF POTENTIAL BIOMARKERS FOR AZOOSPERMIA BY HUMAN TESTIS PROTEOMIC ANALYSIS

Katarina Davalieva [1 *], Aleksandar Rusevski [1], Milan Velkov [1], Predrag Noveski [1], Katerina Kubelka-Sabit [2], Vanja Filipovski [2], Toso Plaseski [3], Aleksandar Dimovski [1,4], Dijana Plaseska-Karanfilska [1]

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Azoospermia, as the most severe form of male infertility, no longer indicates sterility due to medical advancements. As the current diagnosis is based on testicular biopsy, there is a high need for non-invasive testing. The key point here is the identification of testis-specific proteins that could accurately pinpoint the stage of spermatogenesis failure.

The aim of this study was the identification of proteome differences in human testicular tissues among obstructive azoospermia (OA) and non-obstructive (NOA) subtypes hypospermatogenesis (Hyp) and Sertoli cell-only syndrome (SCO).

We have analyzed 27 FFPE testicular tissues using highly efficient extraction/digestion procedure [1] and label-free data-independent LC-MS/MS acquisition coupled with ion mobility. Validation was done on additional 49 FFPE testicular tissues using qPCR.

Out of 2044 proteins identified based on ≥2 peptides, 61 proteins had the power to quantitatively discriminate OA from NOA and 30 to quantitatively discriminate SCO from Hyp and OA. Among these, H1-6, RANBP1 and TKTL2 showed superior potential for quantitative discrimination among OA, Hyp and SCO. Bioinformatics enrichment analysis revealed an association with several GO annotations and pathways. Comparison with 2 transcriptome datasets revealed 278 and 55 co-differentially expressed proteins/genes with statistically significant positive correlation. Gene expression analysis by qPCR of 6 genes with the highest discriminatory power on protein level and the same regulation trend with transcriptomic datasets, confirmed the proteomics results.

Data from our study provides deep insights into the proteins involved in spermatogenesis failure and gives a number of potential candidates for discrimination between OA and NOA.

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FRO-16: PROTEOTYPE CLASSIFICATION OF RENAL CELL CARCINOMA FOR PROGNOSIS AND THERAPY RESPONSE

Jan Šimoník [1], Richard Štefaník [1], Pavla Bouchalová [1], Petr Lapčík [1], David Potěšil [2], Ján Podhorec [3,4], Milan Hora [5], Alexandr Poprach [3,4], Ondřej Fiala [6], Pavel Bouchal [1 *]

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Renal cell carcinoma (RCC) represents a serious oncological disease with one of the highest incidences in the Czech Republic across the world. Reliable molecular prognostic and predictive biomarkers for RCC are mostly unavailable, namely at protein level. To quantify proteins associated with pro-tumorigenic and pro-metastatic mechanisms in RCC, we first generated a comprehensive RCC-specific spectral library of targeted proteomic assays for 7960 protein groups (FDR=1%) [1]. Second, we have applied data independent acquisition mass spectrometry (DIA-MS) on QExactive HF-X LC-MS system to analyze a well-characterized set of initially localized RCC tumors (n=86) of which a half exhibited a relapse in <5 years after diagnosis. We have identified a single potential biomarker and two protein classifiers able to predict the relapse, for which we have developed selected reaction monitoring assay for further validation and routine quantification. CRISPR/Cas9 knockdown confirmed the role of the key protein in cell migration in 786-0 cells, supporting its role in metastatic potential of RCC. Third, we have analyzed a well-characterized set of metastatic RCC tumors (training set n=53, validation set n=22) and adjacent non-cancerous tissues (n=17) a part of which responded and a part did not respond to tyrosine kinase inhibitor (TKI) treatment. We have identified and validated a single protein biomarker and one classifier associated with a poor response to TKI but not with tumor grade and lymph node status. Functional assays using CRISPR/Cas9 knockdown confirmed its role in metastatic potential of 786-0 cells. In a summary, next generation proteomics based on DIA-MS is a powerful tool to classify RCC tissues, to identify prognostic biomarkers and alternative therapeutic targets.

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FRO-17: STABILITY STUDY OF TRIACETYLFUSARININE C AND GLIOTOXIN IN BODILY FLUIDS

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Introduction: During invasive pulmonary aspergillosis (IPA), Aspergillus fumigatus (Af) produces secondary metabolites supporting proliferation within a neutropenic host. Of note, extracellular siderophore triacetylfusarinine C (TafC) captures ferric cation [1], and mycotoxin gliotoxin (Gtx) fights host immunity [2]. Detection of these specific biomarkers in bodily fluids can improve early IPA diagnosis [3]. Methods: We tested the stability of TafC/Gtx in human urine (pH 6.44) and serum and rat urine (pH 7.86) at 37 °C in a one-week due course. We collected all data with Dionex UltiMate 3000 HPLC system coupled to a SolariX 12T FTICR and evaluated with DataAnalysis 5.0 and OriginPro 2021b. We calculated the TafC/Gtx half-life (t1/2) after data fitting with linear and exponential decay functions. Results: In human urine, TafC decay followed the zero-order kinetics with a t1/2 = 29.8 days. On the other hand, TafC decomposed 58.4x (t1/2 = 12.2 h) and 48.9x (t1/2 = 14.6 h) faster in the basic pH rat urine and human serum, respectively. The TafC degraded to triacetylfusarinine B and N2-acetyl-N5-anhydromevalonyl-N5-hydroxy-L-ornithine residues, which are simultaneously detected in bodily fluids for diagnostic purposes. Similarly, the most stable matrix for Gtx was human urine (t1/2 = 5.8 days) compared to rat urine and human serum, where 80 and 94% of Gtx decomposed within one day into unknown metabolites, respectively. Conclusion: A slightly acidic human urine represents a suitable matrix for preserving the clinically valuable Af virulence factors secreted during proliferation in a host. The simultaneous detection of secreted secondary metabolites and their degradation products in urine could play an essential role in IPA diagnostics even in the early stages of infection.

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FRO-18: MALDI-TOF MASS SPECTROMETRY: A POWERFUL METHOD FOR BLOOD MEAL IDENTIFICATION IN INSECT VECTORS

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Determination of blood meal sources of hematophagous arthropods is crucial for understanding transmission cycles of vector-borne diseases in endemic areas. Most of currently used methods are nevertheless laborious and challenged by tiny volumes of rapidly degraded host blood. A promising approach towards blood meal identification was recently developed for sand flies employing MALDI-TOF MS of host-specific haemoglobin peptides generated by trypsin digestion of the engorged blood [1].

The method was first tested on lab-reared sand flies, allowing correct host identification of 100% females until 36h post blood meal (PBM) and for 80% of samples even 48h PBM and thus providing longer reliable blood source determination than other nowadays used methods. Blind study using sand flies collected during a field survey in Greece yielded unambiguous host identification for 96% of females. Moreover, the method successfully determined blood meals of engorged females collected in Bosnia & Herzegovina and Croatia and stored frozen in ethanol for several years prior to the analysis. The approach also works on blood meals spotted on a filter paper that represents a simple and low-cost alternative of sample storage enabling easy shipment at ambient temperatures from regions of collection to MS facilities for the analysis. Furthermore, it allows correct identification of mixed blood meals as was demonstrated on both experimentally fed and field-collected sand flies and reliable differentiation of closely related host species within the same genus.

MALDI-TOF MS was shown as an accurate method for blood meal identification with a minimal sample input. Besides sand flies, it was applied also on mosquitoes and may be universally applicable to various hematophagous arthropods.

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FRO-19: CHARACTERIZATION OF GLYCOSPHINGOLIPIDS IN IPSC-DERIVED CEREBRAL ORGANOIDS AND ITS APPLICATION IN NEURODEGENERATIVE DISORDERS

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Gangliosides are sialylated glycosphingolipids, highly abundant in the neuronal lipid membrane. Utilizing 3D cerebral models, like cerebral organoids (COs), can provide an opportunity to understand the role of these lipids in aging and neurodegenerative diseases. The study aimed to develop a novel UHPLC/ SRM method for identifying eight sub-classes of gangliosides with information on the composition of fatty acid composition and the sphingoid bases for each sub-class. The method was applied to understand the effect of known risk factors of Alzheimer's disease on these lipids, such as apolipoprotein E and secretases. More than 70 gangliosides were identified in the cerebral organoids. In the organoids, fatty acids were characterized by a prevalence of chain lengths in sizes from C14 to C22. Overall the profiles of different chain length lipids were similar within the same sub-class of gangliosides.

Interestingly the C16 and C18 fatty acids were present in the highest concentrations in the organoids, in contrast to C18 and C20, which are more abundant in an aging human brain. There was a global upregulation in the level of gangliosides in the presence of ApoE4. A similar effect was observed in the treatment of these organoids with secretases. This method can be helpful for the analysis of healthy and neuropathological changes associated with aging and neurodegenerative diseases in the cerebral organoids.

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FRO-20: REDOX RESETTING BY MODULATION OF REDOX SENSOR KELCH-LIKE ECH-ASSOCIATED PROTEIN 1 RESTORES LEUKEMIC CELLS SENSITIVITY TO AZACYTIDINE

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A hypomethylating drug 5-azacytidine (AZA) is used to treat patients with myeloid malignancies who are in a high risk of progression to leukemia. Although AZA significantly prolongs patient survival, the efficacy of treatment is often hampered by the early development of resistance. The mechanisms by which leukemic cells overcome AZA toxicity are not yet fully understood, but there is considerable evidence that the molecular events leading to the loss of response to AZA are driven by redox mechanisms. In this study, we focused on investigating the role of redox homeostasis in leukemic cell resistance to AZA.

We used a quantitative mass spectrometry-based proteomics approach to identify protein targets of oxidative modifications in a leukemia cell model of AZA resistance developed in our laboratory. Analysis showed that treatment of AZA-sensitive cells (AZA-S) resulted in altered cysteine oxidation in 20% of proteins (578 of 2853). We identified key cysteine sites of proteins that regulate apoptosis. We hypothesize that AZA-induced oxidative stress is a key factor in its cytotoxic effect. AZA resistance was associated with changes in the oxidative state of 14% of cysteine proteins involved in glutathione metabolism and the antioxidant defense system, such as the key redox sensor Kelch-like ECH-associated protein 1 (KEAP1). The data suggest that AZA-R are under chronic oxidative stress and have adapted to redox stressors. We show that inhibition of KEAP1 blocks redox adaptation and restores AZA-R cells' sensitivity to AZA.

In conclusion, we demonstrated that the mechanism of AZA resistance involves cellular adaptation to oxidative stress and that modulation of the KEAP1 cellular antioxidant response pathway can re-sensitize AZA-resistant cells in vitro.

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SAO-21: PROTEOMIC ANALYSIS OF HEPATITIS B VIRUS

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According to the World Health Organisation, an estimated 296 million people worldwide are infected with Hepatitis B virus (HBV). HBV is a small enveloped DNA virus from Hepadnaviridae family that persists in the infected hepatocytes by establishing an episomal covalently closed circular double-stranded DNA (cccDNA) genome containing four open reading frames (C, P, S and X). The ORFs largely overlap and encode multiple proteins using different in-frame start codons. The S ORF encodes three forms of surface envelope glycoproteins S-, M-, and L- HBs). The HBV preC-C gene gives rise to two different products translated from distinct mRNAs – core protein (HBc) and precore protein (HBe). Despite their high sequence similarity, these proteins exhibit different functions and subcellular localizations. The P ORF encodes viral polymerase, and the X ORF encodes regulatory HBx protein.

Mass Spectrometry department of IOCB has established a collaboration with the groups of Iva Pichova and Jan Weber from IOCB. This collaboration on proteomics experiments lead to the discovery of several important biological and biochemical findings including identification of interacting partners of HBc, HBx and HBe proteins or posttranslational modifications of HBc protein. The collaboration resulted in several publications. The proteomic analysis of individually expressed viral proteins has also captured out-of-frame products suggesting the possibility of frameshift or alternative internal initiation of translation. The presentation will highlight the proteomic analyses of HBV at IOCB

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SAO-22: THE SINGLE AMINO ACID SUBSTITUTIONS IN MASON-PFIZER MONKEY VIRUS MATRIX PROTEIN MODULATE ITS PROTEOLYTIC CLEAVAGE RATE

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N-terminal domain of polyprotein Gag of Mason-Pfizer Monkey Virus (M-PMV) Matrix protein (MA) is naturally myristoylated on its N-terminus. When MA is a part of polyprotein Gag, the myristate moiety is buried inside hydrophobic pocket and exposed probably upon interaction with host cell plasma membrane (PM). This mechanism called myristoyl switch does not occur in M-PMV as readily as it does in HIV-1, suggesting that it may have an important role in M-PMV maturation by regulating the MA cleavage from Gag polyprotein.

To address this hypothesis, we have treated the myristoylated (myrMAPPHis) and nonmyristoylated MAPPHis (nonmyrMAPPHis) M PMV construct, bearing the cleavage site for M-PMV viral protease (Pr13), on artificial liposomes mimicking PM by Pr13 for several time points. In contrast to rapidly degraded nonmyrMAPPHis even without liposomes, the myrMAPPHis, surprisingly, become cleaved more frequently only after addition of liposomes indicating the possible exposure of myristate upon interaction with PM.

To support our findings, we have designed and examined also four mutants of MAPPHis M PMV with single amino acid substitutions with expectation to block or, conversely, facilitate the myristoyl switch by stabilization (A79V, A79L) or destabilization (I51A, I86A) of MA M PMV hydrophobic pocket. The mutants A79V and A79L were cleaved even less effectively than myrMAPPHis as well as the degradation of mutants I51A and I86A was faster compared to nonmyrMAPPHis. The different cleavage rates of proteins were confirmed also on structural level by using method of hydrogen-deuterium exchange coupled with mass spectrometric detection. It shows that the protease cleavage site has different dynamics in proteins as a result of disruption/stabilisation of hydrophobic pocket.

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SAO-23: CARDIAC ALARMINS AS RESIDUAL RISK MARKERS OF ATHEROSCLEROSIS UNDER LIPID-LOWERING THERAPY

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Background: The major cause in the initiation and progression of atherosclerosis are the high levels of low-density lipoproteins. Although statin treatment can effectively lower these levels, there is still a residual risk of cardiovascular events. We hypothesize that a specific panel of alarmins, a family of stress-sensing molecules, could indicate the persistence of silent atherosclerosis residual risk.

Methods: New Zeeland White rabbits were divided into: the control group (C) with standard diet, a group which received a high-fat diet for 12 weeks (Au) and a treated hyperlipidemic group, with a lipid diet for 8 weeks followed by standard diet and hypolipidemic treatment (atorvastatin and PCSK9 siRNA-inhibitor) for 4 weeks (Asi). We used the LTQ Orbitrap Velos Pro mass spectrometer to analyse the left ventricle lysates. The experiments were complemented by immunologic and genomic assays to corroborate the data.

Results: The hyperlipidemic diet determined a general alarmin up-regulation tendency over C group. A significant spectral abundance increase was measured for specific heat shock proteins, S100 family members, HMGB1 and Annexin A1. The hypolipidemic treatment demonstrated a reversed regulation trend, with non-significant spectral alteration over the C group for some identified alarmins.

Conclusion: Our study highlights the discriminating potential of alarmins in hyperlipidemia or following a hypolipidemic treatment.

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SAO-24: CERVICAL MUCUS – A NON-INVASIVE UTERINE BIOMARKER SOURCE?

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Cervical mucus (CM) is a viscous fluid produced by cervical glands located in the myometrium of the uterine cervix. Usually, CM forms a plug between vagina and uterus. During the ovulation, CM starts to be more fluidic to allow the sperm to go through. During the ovulation, there is a good window for non-invasive sampling of the CM, originating from uterine derived tissues. The main hypothesis for CM proteomic study is to develop a method for CM sampling, processing and to answer if there are enough proteins in the CM for potential biomarker search. The resulting protocol consists of mucus dissolution, multi-enzyme digestion, and LC-MS proteomic analysis. The optimized approach allows to identify more than thousand proteins from every sample tested. The bioinformatic analysis reveals that the identified proteins are both intra and extra cellular and discovers the proteins with enriched expression in the broader female reproductive tract. To our best knowledge, we provide the most extensive proteomic characterization available.

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SAO-25: HIGH-THROUGHPUT MLC-MS/MS LIPIDOMICS OF 3D IN VITRO DISEASE MODELS TO INVESTIGATE LIPID DYSREGULATION

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Microflow liquid chromatography interfaced with mass spectrometry is increasingly applied for the high-throughput profiling of biological samples, as it demonstrated an acceptable tradeoff between sensitivity and reproducibility. However, a limited number of applications developed in lipidomics. This study introduces a robust, sensitive, and high-throughput µLC-MS/MS lipidomics workflow, applied in a differential lipidome study of APOE 3 and 4 phenotypes in iPSC-derived cerebral organoids (CO). First, we optimized the microbore column gradient conditions, injecting a mixture of 15 lipid standards on the UHPLC system coupled to a 6469 QQQ. Our µLC-MS/MS method allows quantitation of 351 lipid species from the single CO. The microflow lipidomics shows an average 3.7-fold increase in response compared to a conventional high-flow method. To benchmark the quantitative performance, 303 samples were acquired over 75 hours. The median coefficient of variation (CV) of 351 lipid species was 12.95%, only 26 lipid species show CV higher than 30%. We demonstrated that microflow separation coupled to three-stage quadruple MS substantially increases the sensitivity and allows for robust multiplexed lipid profiling (including cholesterol determination) in a single cerebral organoid. As a proof of concept, lipidomics analysis was performed for the lipid extract of APOE 3/3 and APOE 4/4 (n=10) CO samples. To our knowledge, this is the first systematic report to demonstrate the microflow LC-MS/MS allows for sensitive, high-throughput, and robust measurement of global lipidome, opening an avenue for routine application of microflow separation in the field of lipidomics.

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SAO-26: MULTI-OMICS NETWORK ANALYSIS IN ATHEROSCLEROSIS REVEALS MECHANISMS DRIVING THE PROGRESSION TOWORDS COMPLICATED LESION

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From a biological point of view, it is crucial to understand the mechanisms laying behind atherosclerosis and its complicated forms. In the process of atherosclerosis, first, an atheromatous plaque builds up, which in some conditions can lead to the appearance of complicated lesions. The complicated lesion is characterized by ruptures on the atherosclerotic plaque surface and/or hemorrhage into the plaque. According to the WHO, hemorrhaged lesions, contribute to a considerable extent of deaths worldwide.

The main goal of our project was to perform a multi-omics study involving transcriptomics and proteomics data to get more insights into the pathophysiological processes dominating the complicated lesions.

Transcriptomic (RNAseq) and proteomic (label-free quantification) data were obtained by the analysis of artery samples originating from healthy arteries, atheromatous lesions, and complicated lesions. Both data types were examined separately by Ingenuity Pathway Analysis and later an overlay of the networks was achieved.

120 differentially expressed (DE) proteins and more than 4000 DE genes were detected. The deep analysis of the data could identify the proteins and transcripts characteristic of the complicated lesions and could reveal the pathways specific to atheroma and complicated lesions, respectively. The DE genes were arranged into 25 networks, while the DE proteins were into 12 networks. The lower number of DE proteins compared to the number of DE genes did not result in loss of information, indicating the power of the proteomics experiments. In spite of providing a lower number of DE items, the biological information achievable from proteomics experiments has equal value compared to transcriptomics data.

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THS-01: DETECTION OF *C. DIFFICILE* TOXIN B BY BIOMOLECULE-MODIFIED CHIPS AND MASS SPECTROMETRY

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Clostridium difficile is an important human pathogen responsible for almost 224 000 hospitalized cases and 13 000 deaths annually in the USA. This anaerobic bacterium causes diarrhea and inflammation or can even cause life-threatening pseudomembranous colitis. *C. difficile* uses two toxins – Toxin A and B. Toxin B catalyzes RhoA glucosylation of threonine 37, therefore inactivating its GTPase activity. The inactivation of RhoA may lead to the disruption of the intestine cytoskeleton.

This study presents a novel approach of MALDI-TOF MS-based Toxin B detection using neutravidin chips prepared by ambient ion soft-landing technology for surface modification with biomolecules. The RhoA protein with a biotin tag was prepared by recombinant expression and successfully used as a substrate in enzymatic reaction with commercially available Toxin B under previously published conditions. The biotinylation allows in-situ enrichment of RhoA protein from complex samples by neutravidin chips compatible with MALDI MS. Therefore, the in-situ enriched RhoA modified by Toxin B can be detected using MALDI TOF mass spectrometry. This study's main aim is to prepare a simple kit that allows the detection of Toxin B presence in complex samples of patients suffering *C. difficile* infection.

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THS-02: OPTIMIZED HDX-MS WORKFLOW FOR ANTIBODY STRUCTURE MONITORING BY HDX-MS

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Hydrogen/deuterium exchange mass spectrometry (HDX-MS) is one of the most prominent and versatile techniques providing unique structural information. The main advantages of this method are no limitations in protein-size and compatibility with any experimental conditions such as pH, temperature, protein concentration, or buffer composition. HDX-MS is also officially approved by legal authorities as a suitable tool for validation of protein-based biotherapeutic molecules, including monoclonal antibodies. However, antibodies are challenging analytical targets due to their N-glycosylation and compact structure stabilized by disulfide bonds.

In this study, we systematically evaluated crucial steps in the initial phase of the HDX experiment. This included testing of various quench conditions - disulphide bond reduction and subsequent proteolysis where different proteases, alone or in combination were utilized. We also developed detailed data processing workflow that helps in complete understanding of digest parametrization and easy cross-comparison. The final, fully optimized conditions provided full sequence coverage, optimal peptide length and reasonable redundancy and thus improved spatial resolution.

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THS-03: RPLC-UV AND HILIC-UV CHARACTERISATION OF *ON-SITE* PRODUCED RAMUCIRUMAB-DTPA IMMUNOCONJUGATE

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Conjugates of antibodies with chelating linkers, such as diethylenetriamine pentaacetate (DTPA), represent a specific class of biopharmaceuticals often produced on-site by research groups for scientific purposes. The conjugation reaction is usually carried out within a long period of time, leading to the stochastic linkage of the chelating agent and highly heterogeneous products [1]. In turn, these conjugates represent a real challenge for characterization by liquid chromatography because the separation of heterogeneous products is imperative for monitoring the linker attachment sites and their quantification. Reversed-phase liquid chromatography (RPLC) and hydrophilic interaction liquid chromatography (HILIC) have demonstrated the potency to suffice a need of this kind at intact, subunit, and peptide levels [2]. In turn, RPLC and HILIC analyses are still complicated for free and chiefly on-site conjugated mAbs. Yet, such separation methods must be fast and simple but reliable. We found optimal conditions for analyses of ramucirumab and its DTPA-conjugated variant at intact, reduced, subunit, and peptide levels. The conjugation efficiency has been confirmed by the differences between the resulting peak of ramucirumab-DTPA against the peak of free ramucirumab using both RPLC and HILIC. However, none of those modes could baseline separate the mixture of conjugated ramucirumab molecules. Our pilot data suggest that conjugation reaction can be monitored online in RPLC-UV mode to select the optimal reaction time, which could limit the attachment of the chelating linker to desired amino acid residues only.

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THS-04: COLLISION ENERGY SETTING IN PROTEOMICS AND GLYCOPROTEOMICS: FROM INDIVIDUAL SPECIES TO A PRACTICAL PERSPECTIVE

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In bottom-up proteomics, the choice of collision energy in tandem mass spectrometric experiments has outstanding role since it fundamentally influences the obtained fragmentation pattern. A few years ago, we were the first to examine a direct connection between the collision energy setting and the identification confidence for a large number of individual peptides.[1] The unprecedented amount of peptide-level information, processed by our in-house developed program Serac, has provided new insights into the factors behind the overall performance of bottom-up methodologies. It allowed us to design optimized workflows, and to suggest simple approaches to quickly optimize methods on other instruments.

Recently, we have focused to the more challenging glycopeptides, where we mapped the Byonic and pGlyco search engine scores of almost 200 individual N-glycopeptides from standards as a function of collision energy settings. We found that the peptide sequence heavily influences the energy for the highest score, on top of an expected general linear trend with m/z. Our optimized workflow based on the results led to 10-50% more identified N-glycopeptides on HeLa, blood plasma, and monoclonal antibody samples.

We also investigated how the two most widely employed MS instruments (QTof and Orbitrap) should be set up such that they deliver spectra with comparable information content. To this end, energy dependent studies were performed for a large number of peptides on different instruments and similarity indices were determined between the obtained MS/MS spectra.[2]

Last, but not least, we have started a systematic investigation to unravel the impact of the ion mobility cell on the optimum collision energy at the individual peptide level.

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THS-05: NOVEL CIRCULATING BIOMARKERS OF BIVENTRICULAR HEART FAILURE

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Chronic heart failure is characterized by insufficient supply of blood to body organs. Heart failure typically affects left ventricle (LVHF), in some patients the dysfunction may also affect the right ventricle, leading to biventricular heart failure (BiVHF). BiVHF patients have worse prognosis and respond differently to therapies. The molecular mechanisms responsible for HF are still poorly understood and reliable diagnostic markers distinguishing BiVHF from LVHF are missing.The aim of the study is to identify proteins differentially expressed in BiVHF compared to LVHF. We are interested in proteins whose differential expression in heart would be reflected in patient plasma, proteins that could serve as diagnostic biomarkers. We performed LFQ proteomic analysis of human hearts solubilized by Triton X100 to get rid of a large part of the myofibril proteins. The proteomic analysis of both ventricles from 10 LVHF, 10 BiVHF patients and 10 healthy donors identified roughly 3000 proteins. Among the differentially expressed were proteins associated with myofibril, cell adhesion and extracellular matrix, cell metabolism, calcium homeostasis, endothelial cell repair and protein degradation. We confirmed differential expression of APOA4, AHSG, FBLN5, HMGCS2, MFAP4, and UCHL1 by western blots, and tested whether their abundance in circulation reflects the differential expression in the heart tissue. ELISA showed significantly decreased plasma concentration of APOA4 in both types of HF compared to healthy individuals and confirmed significantly elevated concentrations of fibulin-5 (FBLN5) in BiVHF patients compared to healthy controls. These putative circulating markers of general HF and BiVHF, respectively, will be tested for their specificity and selectivity in a larger study.

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THS-06: COMPREHENSIVE METABOLOMIC AND LIPIDOMIC STUDY OF TAUOPATHY AND ALZHEIMER'S DISEASE PATIENTS

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Alzheimer's disease (AD) belongs to the group of tauopathies, which are classified as neurodegenerative disorders. AD is manifested by dementia, cognitive loss, and other neurological impairments. Currently, more than 55 million people worldwide have dementia and 60-70% of them have been diagnosed with AD. One of the causes of AD development is the accumulation of structurally disrupted tau protein, which aggregates into insoluble neurofibrillary tangles that disrupt neurons.

The aim of this study was to describe the pathological processes in AD patients and five cohorts of tauopathy and to compare them with the profile of healthy subjects. The tauopathy cohorts were as follows: Progressive supranuclear palsy (PSP), Corticobasal degeneration (CBD), Behavioral variant of frontotemporal dementia (bvFTD) and Semantic variant/ Non-fluent agrammatic variant of primary progressive aphasia (svPPA/ nfaPPA). Cerebrospinal fluid samples were obtained from patients and controls. A combination of targeted metabolomic and lipidomic approach was chosen for an objective assessment of the study.

The metabolic and lipid profile revealed differences predominantly in tauopathies compared to controls. Elevated levels dominated in several amino acids, acylcarnitines, lysophosphatidylcholines, ceramides and sphingomyelins. These observations are probably related to the anti-inflammatory processes [1] or the loosening of phospholipid membranes due to neurodegeneration [2]. Subsequently, this may have resulted from impaired mitochondrial function and the breakdown of other cellular components [3]. The findings significantly contribute to the understanding of the pathobiochemistry of AD and tauopathies and also affect the frontiers of diagnosis and treatment.

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THS-07: OVERLOOKED OLIGOMERIZATION PROCESS IN AZURIN, A MODEL METALLOPROTEIN

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Pseudomonas aeruginosa azurin is a small blue copper protein commonly used as a model in electron transfer (ET) experiments due to its characteristic physiological function and UV-VIS spectrum changes during ET activity. Its lesser-known characteristic is formation of oligomers which can influence ET in solution even under concentrations close to physiological. In this study we have employed chemical cross-linking of different azurin forms with lysine-reactive chemicals (DSS or DSG) paired with SDS-PAGE visualisation and MS identification to map the interactions in aforementioned oligomers. Additionally, we have employed a novel purification approach to prepare apo-azurin – a protein deprived of the central copper ion which is crucial both structurally and functionally. The same approach was also applied to prepare zinc metallised form to substitute the central copper ion. Acquired data correlates with our previous results and shows important role of C-terminal β-sheet in the interaction. Based on MS data and site directed mutagenesis, we predict the cross-link is formed between K122 residues of two azurin monomer units. The mutant protein with K122 substitution produces no oligomers covalently linked by DSS or DSG on SDS-PAGE, even though other experiments revealed generally higher oligomerization rate for this mutant. More than that, the nature of the metal cofactor or its absence influences DSS/DSG visualisation of azurin oligomerization through altering exposure of C-terminal β-sheet (Cu > Zn > Apo). To sum up, we have resolved the structure of largely overlooked azurin oligomers and uncovered abnormal oligomerization behaviour occurring in non-conventional azurin metal forms.

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THP-01: A MIXTURE OF INNATE CRYOPROTECTANTS IS KEY FOR FREEZE TOLERANCE AND CRYOPRESERVATION OF A DROSOPHILID FLY LARVA

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Insects that naturally tolerate internal freezing produce complex mixtures of multiple cryoprotectants (CPs). Better knowledge on composition of these mixtures, and on the mechanisms of individual CP interactions, could inspire development of laboratory CP formulations optimized for cryopreservation of cells and other biological material. Here, we identify and quantify (using high resolution mass spectrometry) a range of putative CPs in larval tissues of a subarctic fly, Chymomyza costata, which survives long-term cryopreservation in liquid nitrogen. The CPs proline, trehalose, glutamine, asparagine, glycine betaine, glycerophosphoethanolamine, glycerophosphocholine and sarcosine accumulate in hemolymph in a ratio of 313:108:55:26:6:4:2.9:0.5 $mmol \cdot l^{-1}$. Using calorimetry, we show that artificial mixtures, mimicking the concentrations of major CPs in hemolymph of freeze-tolerant larvae, suppress the melting point of water and significantly reduce the ice fraction. We demonstrate in a bioassay that mixtures of CPs administered through the diet act synergistically rather than additively to enable cryopreservation of otherwise freeze-sensitive larvae. Using matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI), we show that during slow extracellular freezing trehalose becomes concentrated in partially dehydrated hemolymph where it stimulates transition to the amorphous glass phase. In contrast, proline moves to the boundary between extracellular ice and dehydrated hemolymph and tissues where it probably forms a layer of dense viscoelastic liquid. We propose that amorphous glass and viscoelastic liquids may protect macromolecules and cells from thermomechanical shocks associated with freezing and transfer into and out of liquid nitrogen.

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FRP-02: DEVELOPMENT OF ON-LINE PRETREATMENT METHODS FOR GROWTH FACTOR ANALYSIS USING CZE-MS

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Growth factors are a heterogeneous group of proteins that are secreted by various tissues in the body. Their presence in biological samples may indicate the development of various diseases, so there is a growing need to develop new high-performance analytical methods and procedures for their identification and quantification. The developed methods could subsequently be used either in medicine and diagnostics, or in therapeutic monitoring and anti-doping. The aim of this work is the development of on-line pretreatment methods aimed at concentrating the sample before the analysis itself using an on-line combination of capillary zone electrophoresis (CZE) and mass spectrometry (MS) [1,2]. Optimization and comparison of two pretreatment methods, transient isotachophoresis (tITP) and dynamic pH junction (DPJ), was performed on a mixture of standards of three selected intact growth factors, namely IGF-1 (insulin-like growth factor-1), EGF (epidermal growth factor), and TGF-α (transforming growth factor- α). The lowest detection limits of 66 ng/mL for IGF-1 and EGF and 40 ng/mL for TGF- α were reached using the transient isotachophoresis (tITP) method and using 200 mmol/L ammonium formate (pH 4.0) as the leading electrolyte. The benefit of such a method is a prerequisite for its use in the analysis of growth factors as potential biomarkers of diseases in biological samples, where these substances are found at very low concentration levels.

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THP-03: LC-MS/MS ANALYSIS OF SHORT-CHAIN FATTY ACIDS AND THEIR DERIVATIVES IN THE PLASMA OF WOMEN WITH GESTATIONAL *DIABETES MELLITUS*

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Gestational diabetes mellitus (GDM) represents a heterogeneous group of hyperglycemic metabolic disorders. Currently, the diagnosis of GDM is based on repeated measurements of elevated fasting plasma glucose (FPG) or on results showing elevated postprandial plasma glucose (PPG). However, recent research has focused on studying changes in the gut microbiome of women during pregnancy. Metabolic changes may cause gut dysbiosis in pregnant women and thus the development of GDM. The products of gut bacteria, short-chain fatty acids (SCFA) and their derivatives, may play a vital role in the diagnosis of GDM. Current studies use the classification of women with GDM mainly according to the week/trimester of pregnancy. However, this study compares groups of women with GDM according to the method of diagnosis of the disease. For the analysis of SCFA and their derivatives, the LC-MS/MS method was optimized according to the validated protocols by Jaochico et al. (2019) and Shafaei et al. (2021). SCFA and their derivatives were derivatized and separated by reversed-phase liquid chromatography (RPLC) and detected by triple quadrupole mass spectrometer (QqQ). Fatty acids were evaluated along with baseline body composition characteristics and biochemical parameters in women with three different GDM phenotypes, healthy pregnant and non-pregnant women. 3-hydroxybutyrate (3-OH-BA), 4-methylvalerate (4-MVA) and isovalerate (IVA) were assessed as statistically significant within the groups of women measured. 3-OH-BA was elevated in all three groups of women with different GDM phenotypes. Increased/decreased concentrations of 4 MVA/IVA were found in all groups of pregnant women. In comparison with previously published studies, we found new potential biomarkers for GDM, 4-MVA and IVA.

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FRP-04: DETERMINATION OF ANIMAL ORIGIN OF RECENT AND ARCHAEOLOGICAL BONES BY MASS SPECTROMETRY

Alena Meledina [1 *]

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The skeletal remains of vertebrates or more often their fragments are among the most common findings at archaeological sites. The determination of the animal origin of the bones is therefore necessary in the field of archeology and paleontology, as well as in the field of food safety (control of compliance with marketing standards for meat-bone meal) and forensic analysis.

The aim of this work is to develop a new proteomic methodology suitable for the differentiation of animal origin of bones based on their protein composition.

This work is based on proteomic analysis of bone samples using two mass spectrometry (MS) techniques – MALDI-TOF (Matrix-Assisted Laser Desorption/Ionization – Time of Flight) and LC-ESI-Q-TOF (Liquid Chromatography – Electrospray Ionization – Quadrupole – Time of Flight). The mass spectra obtained from MALDI–TOF MS were searched for unique markers in the form of m/z values for each species. Characteristic markers represent a reliable tool enabling a rapid identification of the animal origin of bone samples. Due to the data from LC-ESI-Q-TOF, specific proteins and their peptide fragments were identified in the form of amino acid sequences, from which specific markers for each animal were selected.

The obtained results demonstrate that using these two MS techniques, the animal origin of the bone samples can be determined based on the differences in their protein composition. This information can be used to determine the animal species which the found archaeological bone material comes from. Based on the found characteristic markers, it is possible to create a database that will enable the identification of the animal origin of unknown samples faster and more reliably than currently used techniques (e.g. DNA analysis).

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THP-05: COMPLEXOME PROFILING OF MITOCHONDRIAL OXPHOS KNOCKOUT MODELS

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Proteins are key biomolecules that mostly exert their function thru interaction with other macromolecules. Several techniques to elucidate the protein-protein interactions (PPIs) are available and they frequently employ the mass spectrometry detection. One such technique is complexome profiling that quantifies the proteins separated by the native, non-denaturing, electrophoresis. Correlative analysis of migration patterns is used to infer the multiprotein assemblies. Here we used the blue native polyacrylamide gel electrophoresis (BN-PAGE) followed by 96 well plate-based processing to analyze the HEK293 cellular CRISPR/Cas9 knockout models of mitochondrial oxidative phosphorylation (OXPHOS) defects. Isolated mitochondria of cytochrome c oxidase COX4I1+4I2 or COX6B1 knockouts allowed us to study interdependency between the mitochondrial Complex I and Complex IV (COX). We observed accumulation of Complex I assembly intermediates, indicating that its biogenesis, rather than stability, was affected. Complexome profiling was also used to characterize the knockout disease model of inner mitochondrial membrane protein TMEM70, an assembly factor of ATP synthase. We observed an aberrant ATP synthase biogenesis with accumulation of sub-assemblies and also characterized two novel ATP synthase subunit c interacting proteins (TMEM242, c15orf61). Recently proposed standards "The Minimum Information About a Complexome profiling Experiment" (MIACE) published within a CEDAR (ComplexomE profiling DAta Resource) project (PMID: 33722514) were followed. The technique of complexome profiling is a reproducible method that provides deeper understanding of cellular disease models.

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FRP-06: OPTIMISATION OF MIDDLE-UP HPLC-MS AND CE-DAD APPROACHES FOR INFLIXIMAB QUANTIFICATION IN THERAPEUTIC DRUG MONITORING

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Infliximab (IFX) is a chimeric mouse-human monoclonal antibody (mAb) commonly used in treatment of inflammatory bowel disease (IBD). During the therapy, some patients completely lack response to IFX treatment, while for other patients the treatment becomes ineffective over time. Therapeutic drug monitoring is therefore of high importance. This work focuses on optimisation of middle-up workflow for IFX quantification by using high performance liquid chromatography mass spectrometry (HPLC-MS) and capillary electrophoresis coupled to diode-array detector (CE-DAD). Stock solutions of IFX reference standard (Sigma Aldrich, Missouri, US) were prepared. IFX disulfide bonds were reduced by using tris(2-carboxyethyl)phosphine hydrochloride (TCEP), dithiothreitol (DTT) and DTT combined with iodoacetamide. Samples containing reduced IFX were analysed by HPLC system coupled to an 6520 qTOF mass spectrometer and CE 7100 system with DAD detection (all Agilent). The three reducing conditions (producing two different mAb subunits – heavy and light chains) were tested by HPLC-MS. So far, only a partial chromatographic separation of heavy and light chains was achieved. Subsequent deconvolution of the acquired mass spectra showed that the observed peaks correspond to IFX light and heavy chain subunits. CE-DAD experiments were carried out to confirm the presence of both heavy and light chains in the reduced IFX samples. For each reducing agent used, two separate peaks were detected in the electropherograms of the reduced IFX sample. CE-DAD was shown as a promising approach for middle-up analysis of reduced IFX with the option of coupling to MS detection in future experiments.

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THP-07: WOLFFIA AUSTRALIANA - A NEW PERSPECTIVE MODEL FOR PLANT PROTEOMICS?

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Duckweeds are the fastest-growing angiosperms capable of rapid biomass accumulation under optimal conditions. With the shortest recorded doubling time for Wolffia spp. in less than 30 hours, these plants are perspective for both biotechnology and for analyzing molecular mechanisms underlying plant growth and development. Wolffia is rich in protein content (20 to 30% of the lyophilized mass), but that could represent an issue for proteomics analysis. It has been demonstrated that the composition of tissues with a high protein content is usually dominated by only a few protein families. Here, we analyzed the proteome composition of australiana, employing two complementary strategies for proteome fractionation using off-line C18 peptides fractionation and an online FAIMS-based fractionation technique. We show the benefits of proteome fractionation and present first insights into the proteome composition of this perspective organism.

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FRP-08: DEVELOPMENT AND OPTIMIZATION OF CZE-MS METHOD FOR THE ANALYSIS OF SELECTED GROWTH FACTORS

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Growth factors have many physiologically beneficial functions in the body. However, in various diseases, an imbalance between stimulatory and inhibitory signals can occur, which can result in abnormal expression of growth factors [1]. These qualitative and/or quantitative changes can occur much earlier than the disease manifests itself, and the size of the abnormality may indicate disease progression or treatment success. Analysing these changes is still a challenge today and requires the development of new high-efficiency separation and detection methods.

In this work, we optimized the capillary zone electrophoresis (CZE) hyphenated with mass spectrometry (MS) for top-down analysis of insulin-like growth factor-1, transforming growth factor-α and epidermal growth factor.

First, we identified the charge state distribution of each protein and optimized the fragmentor voltage and dwell time for selected ions (m/z). Suitable background electrolyte (BGE) was composed of 500 mM HFo with 5% ACN, and the optimum separation voltage was set at 20 kV. Next, we compared bare fused-silica capillary with permanently coated polyvinylalcohol capillary and linear polyacrylamide capillary. The optimal sheath liquid consisted of 50% MeOH with 0.1% HFo. The drying gas was set at a temperature of 300 °C and a flow rate of 6 L/min. Nebulizing gas pressure was set at 4 psi and capillary voltage at 4 kV.

With optimized parameters, we reached preliminary LOD of 250 µg/L for all three analytes. Further optimization is needed before the method will be suitable for the analysis of growth factors also in biological samples.

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THP-09: TANDEM MASS SPECTROMETRY OF DNA CODONS: FROM CATIONS TO CATION RADICALS

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Hydrogen-adducted radicals are important highly reactive intermediates formed by fast solvation following secondary electron capture in DNA. Unlike in the condensed phase, cation radicals generated in the gas phase by reductive electron transfer allow convenient isolation and characterization using tandem mass spectrometry. By studying the fragmentation patterns of cations and cation radicals for 64 DNA codons in the gas phase, we were able to create complete a map useful for the interpretation of oligonucleotide fragmentations. Nucleobase eliminations, as well as backbone cleavages were determined for different sequences in cations and cation radicals with the help of selectively N-15 labeled codons.

CID-MS2 spectra of monocations and dications showed neutral and protonated nucleobase eliminations following the trend: cytosine > guanine > adenine >> thymine and 5' > 3' > middle position. The most abundant backbone cleavage was forming w2 ions. Formation of d2 ions was not observed. To generate cation radicals while avoiding contamination by hydrogen-loss product isotope peaks, dication complexes of dibenzo crown ether and trinucleotides were formed and isolated for ETD-MS2. In ETD-CID-MS3 of cation radicals, nucleobase eliminations followed the same trend as with cations, involving both neutral bases and hydrogen-adducted base radicals. Surprisingly, abundant d2 fragments as well as w2 fragments were observed from cation radicals. The formation of w2, w2+H and w2+2H indicated that the radical was located on either the w2 or complementary a type moieties. In contrast, only even-electron d2 and d2+2H ions were produced in high abundance, indicating that the radicals were located on the complementary z fragments.

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FRP-10: THE USE OF MOBILE PHASE WITH A LOW FORMIC ACID CONCENTRATION IN LC-MS PROTEOMIC ANALYSIS LEADS TO ENHANCED ELECTROSPRAY IONIZATION AND IMPROVED SENSITIVITY

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When liquid chromatography is hyphenated to mass spectrometry for analysis of protein samples, the use of a mobile phase with high ionic strength is not recommended as it causes inefficient electrospray ionization [1]. Formic acid at a concentration of 0.1% has been a popular choice as an acidifier for its decent ion-pairing ability and relatively low MS signal suppression. In this study, analytical columns packed with a charged surface hybrid (CSH) stationary phase, designed to function efficiently with mobile phases of low ionic strength [2], were used to evaluate MS sensitivity gain and extent of peptide identification at a varied concentration of formic acid in the mobile phase (0.1% to 0.01%). Well-characterized peptides, a digested monoclonal antibody, and a complex bacterial sample were included in this study to evaluate the effect on varying sample complexity. About 40% to 50% gain in the MS sensitivity was observed using mobile phases with reduced formic acid. Furthermore, the number of identified peptides was increased when using the mobile phase with lower formic acid concentration. CSH stationary phases have opened doors for proteomic LC-MS analysis to use mobile phases with a minimal concentration of formic acid as an acidifier and exploit the advantages of the enhanced MS detection with no peak distortion.

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THP-11: MAXIMIZING PEPTIDE YIELD IN BIOLOGICAL SAMPLE PREPARATION FOR LC-MS ANALYSIS

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The mass spectrometric (MS) analysis of peptides and proteins has become an important tool for understanding biological processes. Due to variability in the chemical nature of the peptides, its accurate and sensitive quantitation is not routine. In this work, we report the development and optimization of peptide extraction protocol using solid-phase extraction (SPE) for liquid chromatography (LC)-MS analysis. A set of four peptides with different structures in the 1–3.5 kDa range (concentration of 100 ng/mL) were selected and analyzed using C18-LC connected to Fourier transform ion cyclotron resonance MS. Initially, suitable sample solution and acid modifiers were tuned; after which 40% acetonitrile with 1% formic acid was selected as an initial sample solvent. Based on it, a suitable SPE protocol for peptides extraction was optimized. Later, we compared the peptide extraction by HLB and Sep-Pak SPEs and molecular weight cut-off filters (MWCO) (Amicon, Microcon). MWCO returned recovery from 0 % to 10 %, whereas SPE (Sep-Pak) with developed protocol had recovery from 60 % to 95 %. Further, we used 6M urea with 4% TFA (1:1) in a protein denaturation step for human cell culture samples. The application of the pre-treatment step had a beneficial effect in reducing the non-specific binding of peptides. Selected conditions were applied on cell culture samples wherein recoveries between 60 % to 550 % were obtained for spiked peptides. Our results showed significantly different peptide recovery for organic and acid modifiers and extraction approaches tested, which was in contrast to routinely used protocols. The optimized peptide extraction protocol will now be used in mapping the antimicrobial peptides in human neutrophils.

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FRP-12: PEA SEED METABOLITE PROFILING USING COMBINED GC/MS AND PYGC-MS ANALYSIS

Jana Nádvorníková [1 *], Petra Krejčí [1], Zbyněk Žingor [1], Štěpán Dostál [1], Dominika Vysloužilová [1], Petr Bednář [1], Petr Barták [1]

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A new method of metabolite profiling of hard-to process seed microsamples using combined GS/MS and PyGC-MS analysis is presented. Certain types of sturdy plant microsamples frequently pose a significant challenge for metabolite profiling analysis. [1] Generally, that is due to their resistance against standard sample preparation and metabolite extraction methods and due to the incomplete coverage of metabolite types the usable methods can provide. [2] The advantage of the described method is the capitalization on the combined sample preparation protocol and the complementary information from both GC methods applied to different matrices produced during the extraction process and thus provide a more complete metabolic profile. The method is demonstrated on analysis of seed coat and hilum of two pea genotypes. It enabled the detection of numerous metabolite products both non-polar (wide range of short- and long-chain fatty acids and several phytosterols, namely β-sitosterol, campesterol, isofucosterol etc.) and polar (sugars, sugar alcohols, organic acids, amino acids, and few phenolic compounds such as epigallocatechin) from one piece of pea seed microsample. This protocol involves one-step reaction for preparing fatty acid methyl esters using rapid methanolysis/methylation procedure, which also substitutes the often costly or complex conventional additional derivatization steps otherwise required for GC/MS of fatty acids. The effects and dynamics of various degradation processes (such as ozonation or exposure to heat, UV rays and hydrogen peroxide) on the metabolic profile were also examined.

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THP-13: USE OF ENDOPHYTIC FUNGUS IN MITIGATING OF CADMIUM TOXICITY

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Cadmium is a widespread toxic pollutant, a nonessential element without any known benefits for organisms. It has been estimated that human activity releases approximately 13,000 tonnes of cadmium per year, and this pollutant may remain in the environment for up to 18 years. Unlike humans and animals, plants are sessile organisms that cannot escape the stress and actively compete above and below ground for the habitat. Cadmium can be rapidly taken up by the roots, transported, and accumulated in the stems. For these reasons, the extensive presence of cadmium in the soil causes deterioration symptoms in the aboveground part of plants, including chlorosis, necrotic lesions, wilting, and leaf deformation. Endophytic microorganisms are lowcost and environmentally friendly technology to improve food production and reduce abiotic stress, including cadmium toxicity. These microbes show a mutualistic relationship with their host plants and colonize both the intercellular and intracellular plant compartments without significant morphological change. The endophytes can significantly promote plant growth and induce tolerance to both biotic and abiotic stressors. Furthermore, they have beneficial effects on the host plant by improving nutrient uptake and modulating the level of phytohormones, siderophores, and enzymes. Here, we employed flax and analyzed its response to the presence of endophytic fungus under different cadmium ion concentrations. We found that the presence of fungi mitigated cadmium toxicity and improved plant growth. Our proteomics and metabolomics profiling provided the first molecular insights into this interaction and highlighted pathways underlying higher resilience in flax plants cultivated in the presence of fungi.

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FRP-14: *PHYTOPHTHORA* RESISTANCE MECHANISMS IN DIVERSE PLANT SPECIES

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Poplars are among the fastest-growing trees and significant resources in agriculture and forestry. However, rapid growth requires a large water consumption, and irrigation water provides a natural means for pathogen spread. That includes members of *Phytophthora spp.* that have proven to be a global enemy to forests. Here, the effects of artificial inoculation with two different representatives of aggressive species (*P. cactorum* and *P. plurivora*) were analyzed in the proteome of the *Phytophthora*-tolerant hybrid poplar clone T-14. Wood microcore samples were collected at the active necrosis borders to provide insight into the molecular processes underlying the observed tolerance to *Phytophthora*. The analysis revealed the pathogen impact on poplar primary and secondary metabolism, including carbohydrate-active enzymes, amino acid biosynthesis, phenolic metabolism, and lipid metabolism, all of which were confirmed by consecutive metabolome and lipidome profiling. The identified *Phytophthora* response proteins were compared to those previously found in trees with compromised defense against *Phytophthora*, namely, *Quercus spp.* and *Castanea sativa*. That provided a subset of candidate markers of *Phytophthora* tolerance, including certain ribosomal proteins, auxin metabolism enzymes, dioxygenases, polyphenol oxidases, trehalose-phosphate synthase, mannose-1-phosphate guanylyltransferase, and rhamnose biosynthetic enzymes. In summary, this analysis provided the first insight into the molecular mechanisms of hybrid poplar defense against *Phytophthora* and identified prospective targets for improving *Phytophthora* tolerance in trees.

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THP-15: ANALYSIS OF IMMUNOGENIC PEPTIDE IN VACCINE FORMULATION BY A CE-MS APPROACH

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With the growing interest in novel biologicals as peptide therapeutics and therapeutic peptide conjugates, the development of analytical methods for the quantitative analysis of peptides in pharmaceutical and/or biological matrices has become very important. Compared to small molecule drugs, peptide and protein biopharmaceuticals are large and heterogeneous (as a result of the biosynthetic process and subsequent manufacturing and storage), making their analysis very challenging. Analysis of large peptides and peptide conjugates is associated with the necessity to digest such large molecules. This procedure is most frequently performed by controlled enzymatic processes. Acid hydrolysis is one example of a complementary digestion technique, which was successfully tested and applied for “bottom-up” proteomic experiments.

Here, a robust analytical method based on on-line combination of capillary electrophoresis with tandem mass spectrometry (CE-MS/MS) was developed for determination of potentially immunogenic synthetic peptide in a conjugate with bovine serum albumin as carrier protein, and in a peptide conjugate formulated with an vaccine adjuvant – Alhydrogel® 2%. An effective non-enzymatic release step of the peptide from the final peptide conjugate based on acid hydrolysis with the use of 2% formic acid was successfully tested and implemented. Separation was performed in background electrolyte composed of 1M formic acid (pH 1.88). Moreover, in-capillary preconcentration strategy based on dynamic pH junction was investigated. Such approach was able to improve the signal intensity. The presented approach represent an effective tool for simple, rapid and robust quantification of immunogens in modern immunotherapeutics.

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FRP-16: HIGH-COVERAGE PROTEOME ANALYSIS OF RABBIT HEART SAMPLES USING 2D CONVENTIONAL-FLOW LC-MS

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Introduction

Anthracyclines, such as daunorubicin (DAU), are effective class of chemotherapeutic agents, however they are responsible for cumulative dose-dependent cardiotoxicity. Global proteomic approaches can help provide insight into its development. Due to high dynamic range of tissue proteomes, 2D liquid chromatography (LC) coupled to mass spectrometry (MS) can significantly enhance proteome coverage. However, long-term analysis can be negatively affected by instability of electrospray during nano LC-MS. Here, we present an optimized 2D conventional-flow LC-MS (68 µL/min) with aim to show its efficacy in proteomic analysis of rabbit hearts compared to nano LC-MS.

Methods

Samples of rabbit myocardium with or without DAU treatment (3mg/kg, weekly, 10 doses) were homogenized and lysed. Proteins were digested to peptides and derivatized by TMT isobaric labels. 2D peptide separation was realized using high-pH C18 sample fractionation followed by separation of collected fractions using conventional-flow (68 µL/min) and nano LC-MS (250 nL/min).

Results

Optimized 2D conventional-flow LC-MS was able to quantify more than 4000 proteins in TMT-labeled myocardial samples. The results showed that severe anthracycline cardiotoxicity phenotype was associated with marked global protein dysregulation, that involved profound remodeling of cardiomyocyte sarcomere, structural proteins and extracellular matrix. Pro-inflammatory proteins, proteasome subunits and multiple antioxidant proteins were markedly up-regulated, whereas the levels of mitochondrial proteins were declined compared to control myocardium.

Conclusion

2D conventional-flow LC-MS analysis was able to get high proteome coverage and comprehensive mapping of proteome changes in rabbit heart samples.

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THP-17: PROTEOMIC ANALYSIS OF EXTRACELLULAR VESICLES FROM PORCINE SEMINAL PLASMA - SEARCH FOR FERTILITY MARKERS

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Domestic pig (Sus scrofa) is considerable animal model for biomedical and translational research of various diseases and cancer. Moreover, pig has an irreplaceable role as a livestock, covering more than 30% of global meat consumption. Since the majority of sows on farms is inseminated artificially, assuring of stable fertility of boars and healthy litters is essential.

Seminal plasma is rich in proteins and is necessary for development and fertilizing capacity of sperms, thus male fertility. Seminal plasma is also enriched in extracellular vesicles, which participate in spermatozoa maturation, affect their motility, capacitation and survival.

We aimed to develop technique for isolation and proteomic characterization of small extracellular vesicles (average size 30-150 nm) from boar seminal plasma. We isolated extracellular vesicles by differential centrifugation and ultracentrifugation and verified their morphology and size by electron microscopy, flow cytometry and nanoparticle tracking analysis, as well as their purity by immunoblotting for selected markers (e.g. Alix, Lamin, UQCRC1, etc.). Using various detergents with FASP protocol we optimized protein identification and quantification with MS.

We identified almost 1,800 proteins in small extracellular vesicles (including 82% of the most often identified exosomal proteins), compared to 460 proteins in seminal plasma. Using SWATH-MS approach, we quantified almost 1,500 proteins and analyzed their abundances in small extracellular vesicles, sperms and seminal plasma, including known fertility markers.

Our workflow for isolation and MS-based proteomic characterization of extracellular vesicles is reproducible, robust and applicable to extracellular vesicle analyses from various body fluids or cell culture media.

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FRP-18: ION MOBILITY MASS SPECTROMETRY ANALYSIS OF AB42 OLIGOMERS

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Amyloid beta is widely accepted as one of the main causes of Alzheimer's disease (AD). Amyloid beta (1-42) and (1-40) are major components of senile plaques typically present in the grey matter of AD patients. Aβ(1-42) in β-sheet conformation forms a nucleation seed and promotes further aggregation of Aβ(1-40). Neurotoxic early stage oligomers aggregate into protofibrils and further prolongate to form mature fibrils and plaque deposits. Thorough characterization of soluble Aβ(1-42) oligomers is vital to comprehensive understanding of the oligomerization process. Native mass spectrometry can be utilized for such analysis as it combines using non-denaturing conditions with soft ionization techniques, therefore maintains non-covalent interactions. Optimization of experimental and instrumental conditions was preformed enabling detection of Aβ(1-42) oligomers formed in vitro. Moreover, ion mobility was used and optimized for separation of commonly occurring isobaric oligomer ions. Lower molecular weight oligomeric species up to hexamer (9 kDa – 27 kDa) were detected alongside the monomer.

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THP-19: MULTI-OMICS APPROACH TO A COMPREHENSIVE ANALYSIS OF BLOOD PLASMA FOR CLINICAL DIAGNOSTICS OF HEPATOCELLULAR CARCINOMA

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In 2020, hepatocellular carcinoma (HCC) takes fifth place worldwide in cancer mortality (more than 8% of the deaths). The early diagnosis of HCC would dramatically increase the chance for survival. Nowadays, most patients are diagnosed in advanced stages of the disease when no effective clinical treatment is possible and five-year survival rate across all stages reaches only 20%. HCC is often associated with at-risk group of patients with chronic liver disease. However, the current screening methods, based mainly on ultrasound examination, are not effective enough for early-stage detection (sensitivity being only around 50%).

Pathological processes associated with the presence of the disease in a human body may cause changes in the content and structure of various types of biomolecules. These changes may be observed in biofluids, e.g. blood plasma, which is collected using routine and almost non-invasive procedure and the collection is cost-effective. Blood plasma is in direct contact with tissues and contains a wide spectrum of signalling molecules, which may be affected by the current health condition.

This project aims to combine information from several analytical methods (FTIR, Raman spectroscopy, NMR, MALDI-TOF-MS, LC-ESI-Q-TOF) to create a panel of specific blood plasma-based biomarkers with the ability to differentiate HCC from controls (cirrhotic patients and healthy persons). To obtain different fractions of the sample for mass spectrometric techniques, we employed a combination of physical (plasma filtration with different cut-offs) and chemical separation methods (lipid separation using butanol/diisopropil ether (40/60)). MALDI-TOF-MS was used for analyses of the low- and high-molecular-weight blood plasma fractions as well as extracted plasma proteins.

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FRP-20: RESIDUAL HYPERLIPIDEMIC STRESS UNDER LIPID LOWERING TREATMENT MAY LEAD TOWARD IRREVERSIBLE NAFLD

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Background: Nonalcoholic fatty liver disease (NAFLD) includes a range of progressive disorders caused by excess lipids accumulation in the liver leading to hepatic steatosis and eventually fibrosis. Herein, we aim to identify the main signaling pathways and liver proteome alterations induced by hypercholesterolemia in an animal atherosclerotic model.

Methods: Using high-performance mass spectrometry, the effect of combined lipid lowering drugs (statins and anti-PCSK9 monoclonal antibody) were used after the interruption of the hypercholesterolemic diet in an experimental rabbit model to identify potential mediators, such as alarmins, responsible for the irreversible NAFLD build up under the hyperlipidemic sustained stress.

Results: The proteomic analysis revealed a number of differentially abundant proteins associated with Fatty acids degradation, Glycolysis / Gluconeogenesis and Non-alcoholic fatty liver disease. The mitochondrial dysfunction indicated alteration at the mitochondrial respiratory chain level and downregulation of NADH ubiquinone oxidoreductase, while the majority cytochrome expressions (cytochrome P4502E1, cytochrome b5, cytochrome c) are upregulated under lipid lowering treatment. The long term hyperlipidemic stress even under low fat diet and lipid lowering treatment is accompanied by alarmins release (annexins, galactins, HSPs, HMGB1, S100 proteins, calreticulin, fibronectin) that generate local inflammation and induced liver steatosis and aggressive fibrosis (by high abundance of galectin 3, fibronectin and calreticulin).

Conclusion: The novel knowledge uncovered by the present study are related to the residual effects of hyperlipidemic stress under the consistent combined lipid lowering treatment (statin and inhibitor of PCSK9).

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THP-21: THE OMICS HUNT FOR NOVEL MOLECULAR MARKERS OF RESISTANCE TO PHYTOPHTHORA INFESTANS

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Wild *Solanum* accessions are a treasured source of resistance against pathogens, including oomycete *Phytophthora infestans*, causing late blight disease. We analyzed *Solanum pinnatisectum*, *Solanum tuberosum*, and the somatic hybrid between these two lines, representing resistant, susceptible, and moderately resistant genotypes, respectively. Proteome and metabolome analyses showed that the infection had the highest impact on leaves of the resistant plant and indicated, among others, an extensive remodeling of the leaf lipidome. The lipidome profiling confirmed an accumulation of glycerolipids, a depletion in the total pool of glycerophospholipids, and showed considerable differences between the lipidome composition of resistant and susceptible genotypes. The analysis of putative resistance markers pinpointed more than 100 molecules that positively correlated with resistance. Putative resistance protein markers were targeted in an additional 12 genotypes with contrasting resistance to *P. infestans*. At least 27 proteins showed a negative correlation with the susceptibility including HSP70-2, endochitinase B, WPP domain-containing protein, and cyclase 3. In summary, these findings provide insights into molecular mechanisms of resistance against *P. infestans* and present novel targets for selective breeding.

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FRP-22: COMPARISON OF SWATH-MS DATA ANALYSIS PIPELINES ON PROTEOMIC DATA FROM HUMAN HUNTINGTON'S DISEASE IPSC AND NSC LINES

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Data-independent acquisition (DIA) is a robust, reproducible, high throughput quantitative MS approach that enables relative quantification of thousands of proteins in one sample. Several software tools and data analysis pipelines were developed over time to process DIA data. We used a specific variant of DIA known as SWATH-MS for characterization of induced pluripotent stem cells (iPSC) and neural stem cells (NSC) derived from Huntington's disease (HD) patients and healthy controls (WT).

Using TTOF 5600, we analysed at quantifiable level over 3000 proteins. We then processed the MS data using two different data pipelines. Pipeline #1 consisted of protein identification for spectral library creation using Mascot, followed by quantification using sample specific spectral library in Skyline/MSstats. Pipeline #2 consisted of identification and quantification in MaxDIA, using in silico generated human spectral library. Both approaches identified more changes between WT and HD lines at NSC stage, compared to iPSC stage. Using Pipeline #1, we quantified 3142 proteins and identified 129 differently abundant proteins, while using Pipeline #2, we quantified 3397 proteins, of which 91 were identified as differently abundant. 2125 proteins were common in both approaches, but only 11 proteins were identified as significantly changed by both pipelines.

This shows that different decisions at various stages of data analysis, from peptide identification to quantification of protein families or using only proteotypic peptides for quantification, can to large extent influence overall results and care should be taken in data verification and interpretation.

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THP-23: PHOSPHO-PROTEOMIC ANALYSIS TO EXPLORE THE MICROBE ASSOCIATED MOLECULAR PATTERNS (MAMPS) IN PLANT IMMUNITY

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Due to the climate change and agrochemicals, many important food crops have been suffering with serious challenges from various pests and diseases. The first layer in plant immunity to detect highly conserved components of microbes, such as flagellin and chitin, are called Microbe-Associated Molecular Patterns (MAMPs). These MAMP signalling pathways have been widely studied in the model plant Arabidopsis thaliana, but the overlap and differences among these mechanisms remain unclear in crop plants such as Brassica, Maize and Tomato. Phosphorylation is an excellent post-translational modification to focus on and could be identified by high-throughput Mass spectrometry (Orbitrap and timsToF pro). This project has applied MS-based phosphoproteomics to quantify the phosphorylation events in the plant immunity signalling, and it helped systematically map the conserved functional pathways and accurately identify orthologs among different plants. The mega-data will make great contributions to 'Precision Agriculture' which is an innovative technology and allows breeders to target specific genes and accelerate the breeding speed.

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FRP-24: PUTATIVE ROLE OF *PLASMODIOPHORA BRASSICAE* HSP70 IN *ARABIDOPSIS THALIANA* AND *BRASSICA NAPUS* INFECTION

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Plants contain several mechanisms that help them cope with specific conditions during their life, such as extreme temperatures or pathogen attacks. One of these mechanisms is the activity of heat shock proteins (HSP) that are not only involved in protein folding mechanisms but can also participate in signaling cascades and protein interactions. Our analysis revealed the putative role of *Plasmodiophora brassicae* HSP in the interaction with its plant host. Here, we employed proteome analysis and showed HSP70 dynamics in the course of *Plasmodiophora* infection. *Plasmodiophora brassicae* is a global pathogen of the Brassica family with significantly limited options for control strategies. Our results indicate that the HSP70 family could be a valid target for promoting resistance against *Plasmodiophora*.

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THP-25: USING MALDI-TOF MS FOR THE DETECTION OF PUTATIVE NEW *ACINETOBACTER* SPECIES FROM CATTLE FECES

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Question: *Acinetobacter* is a taxonomically diverse bacterial genus, with 72 validly named species and many provisional taxa [1]. The genus is ubiquitous, inhabiting plethora of natural ecosystems. *Acinetobacter spp.*, with *A. baumannii* in particular, have emerged as opportunistic human pathogens, associated with multidrug resistance and epidemic spread [2]. As the knowledge on *Acinetobacters* in non-human ecosystems is limited, we conducted a study on the impact of antibiotic use in livestock on the occurrence and spread of resistant and potentially pathogenic *Acinetobacter spp.* in cattle feces.

Methods: Samples of cattle feces were homogenized and cultured aerobically in a mineral medium supplemented with sodium acetate. The grown-up cultures were streaked onto both acetate agar and chrome agar plates. Up to 24 agar-grown colonies per sample were directly identified by MALDI-TOF MS using the current Bruker database supplemented with homemade entries of provisional *Acinetobacter* taxa. The spectra were further compared using cluster analysis to dereplicate isolates of the same strains.

Results: A total of 19 samples from 16 different cattle farms were analyzed. As many as 186 *Acinetobacter* isolates were obtained: *A. baumannii* (6), *A. courvalinii*/*A. vivianii* (2), *A. gandensis* (17), *A. haemolyticus* (1), *A. indicus* (26), *A. pseudolwoffii* (32), *A. variabilis* (13), Taxon 36 (9), genomic sp. 15/16 (1) and 79 unidentified isolates. Sixteen unidentified isolates formed a distinct and cohesive MALDI-TOF MS cluster, possibly representing a novel species.

Conclusion: We have applied an effective approach based on MALDI-TOF MS, which enables quick screening of multiple *Acinetobacter* isolates to assess their taxonomic diversity and to identify putative novel species.

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FRP-26: ASSESSMENT OF THE EFFECT OF PRETREATMENT OF BIRCH BARK BY SUPERCRITICAL EXTRACTION (SCCO_2) WITH THE FOLLOWING SOXHLET EXTRACTION: SEMIQUANTITATIVE ANALYSIS OF COMPOUNDS BY GC/MS

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The bark of trees is an important part of biomass. Many researchers have highlighted the potential of different species of tree bark in terms of their valorization to obtain value-added substances. Considerable attention has also been paid to the processing of bark from various species of the *Betulaceae* family. Extraction techniques are focused on isolating triterpenoids. The aim of this study was to assess the impact of pretreatment of birch bark by supercritical fluid extraction (scCO_2) with the following Soxhlet extraction, and semiquantitative analysis of extractive substances by GC/MS.

The method to obtain desired product was supercritical fluid extraction using carbon dioxide under various conditions, followed by Soxhlet extraction, where a mixture of acetone and petroleum ether was used as a solvent. Obtained extracts were analyzed by GC/MS. The separation of substances was carried out on a capillary column HP-5MS. The stationary phase consisted of (5%-phenyl)-methylpolysiloxane, and the carrier gas helium with a flow rate of 2 ml/min. The end of the capillary column was connected to MS with an electron ionizer and a quadrupole analyzer. The range of measured masses (m/z) was 50-600 amu. The substances found in the extracts were identified according to the National Institute of Standards and Technology mass spectra library.

The analysis of extracts confirmed the presence of triterpenoids lupeol, betulin, bergamotene, and friedelan-3-one and their derivatives. The semiquantitative analysis showed the most promising results for lupeol with yields up to 61%.

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THP-27: PROTEOMIC AND IMMUNOCHEMICAL CHARACTERIZATION OF *NAJA MOSSAMBICA* VENOMS FROM DIFFERENT GEOGRAPHICAL REGIONS OF AFRICA

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Naja mossambica is a representative of African spitting cobras which inhabits the southern parts of Africa. Its venom exhibits mainly cytotoxic effects and can lead to detrimental effects in the bitten victims. Currently, there is only one available polyspecific antivenom manufactured with the use of *N. mossambica* venom (SAIMR antivenom), and several others are produced with venoms obtained from closely related snakes species (e.g., Antivipmyn Africa, EchiTab-ICP-Plus, Inoserp Pan Africa). Unfortunately, some reports indicate that antivenin treatments are not effective in all countries of southern Africa. It turns out that in Mozambique and The Kingdom of Eswatini, common antivenoms do not exhibit therapeutic properties, which leads to terrible consequences for people from this region. Therefore, it seems that geographically induced changes in the venom proteomes might reduce the effectiveness of antivenoms in some parts of Africa.

This study focuses on the proteomic and immunochemical characterization of *N. mossambica* venom samples collected from three distinct regions of Africa: The Kingdom of Eswatini and two provinces of the Republic of Southern Africa (Limpopo and KwaZulu-Natal). Therefore the main objective of the research was to find a reason why antivenoms commonly used in treatments of *N. mossambica* bites are not effective in The Kingdom of Eswatini and Mozambique, whereas they exhibit regular activity in other countries of southern Africa. Moreover, the research expects to give thorough information about proteomic differences in analyzed venoms obtained from various parts of Africa. The comparison of these venoms will show to what extent geographical area could influence changes in *N. mossambica* venom proteomes.

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FRP-28: ARTERIAL VESSEL WALL PROTEOME ALTERATION INVOLVED IN THE REGULATION OF CELL DEATH MECHANISMS IN ATHEROSCLEROSIS

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Background. Atherosclerosis is an inflammatory lipid disease of the arterial vessel wall in which the balance between the predominant cell death mechanisms plays a critical role in dictating the clinical outcome. Unlike the immunologically silent apoptosis, non-apoptotic regulated cell death (necroptosis and ferroptosis) is a process in which affected cells release damage-associated molecular patterns (DAMPs) molecules, which can initiate and perpetuate a non-infectious inflammatory response in arterial wall.

Hypothesis. We hypothesize that DAMPs and non-apoptotic regulated cell death processes are critical players of artery plaque progression with inadequate response to lipid-lowering treatment.

Aim. We aimed to uncover the silent mechanisms that govern the existing residual risk of cardiovascular-related mortality in an experimental hyperlipidemic and hyperglycemic animal model.

Methodology. The study was conducted using proteomic and genomic approaches on the ascending aorta of control and hyperlipidemic and hyperglycemic rabbits with/without lipid-lowering treatment.

Results. All animals, except controls, presented hyperglycemia, numerous heterogeneous atherosclerotic arterial lesions, exhibited a high concentration of serum lipid parameters and increased lipid peroxidation oxidative stress markers. The shotgun proteomic analyses revealed that the aortic tissue level of DAMPs and proteins implicated in necroptosis and ferroptosis were significantly upregulated by the hyperlipidemic stress and some of them did not respond to lipid-lowering treatment.

Conclusion. These proteins could play a key role in the vascular disease silent evolution and may possess an unexplored therapeutic potential.

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THP-29: ADIPOCYTE AND ADIPOSE TISSUE PROTEOME SIGNATURES IN OBESITY

Pavel Hruška [1,2 *], Jan Kučera [3], Matěj Pekař [4,5], Pavol Holeczy [4,6], Miloslav Mazur [4], Marek Bužga [7,8], Daniela Kuruczová [9], Peter Lenárt [3], Jana Fialová Kučerová [1], David Potěšil [2], Zbyněk Zdráhal [2], Julie Bienertová-Vašků [1,3]

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The adipose tissue (AT) distribution is a significant factor in obesity pathophysiology and obesity-associated comorbidities. However, its role in obesity is still poorly understood. The tissue heterogeneity introduces intrinsic characteristics making the interpretation of the expression studies intricate. Therefore, we performed untargeted proteomics analysis of subcutaneous and omental primary adipocytes, the major AT cell type, followed by proteomics profiling of the respective AT. In total, we identified 3686 proteins in the adipocyte's proteome and 4506 proteins in the AT proteome. The differential expression analysis revealed 1140 and 1249 differentially expressed proteins in adipocytes and AT, respectively. Subsequent bioinformatics analyses highlight the most differentially expressed pathways and processes but also show tissue characteristics derived from adipocytes. Our analysis represents a valuable resource on protein expression in adipocytes and AT, highlighting the key features contributing to obesity pathophysiology.

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FRP-30: DEVELOPMENT OF A NOVEL APPROACH FOR APOCRINE SWEAT MICROSAMPLING AND ITS UTILIZATION IN BREAST CANCER SCREENING USING PSEUDOTARGETED LIPIDOMICS

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The intensive development of new non-invasive microsampling technologies is paving the way for a new era of patient friendly precision medicine. Since apocrine sweat (AS) has not yet been tested for this purpose so far, our aim was to look at the possibilities of its microsampling. We have designed a concept of 3D-printed attachment with porous glass filter disks called SLIDE (Sweat sampLIng DevicE) for easy sampling of AS. By applying pseudotargeted LC(C8)-MS(MRM) lipidomics the relevant lipids present in AS have been selected and semiquantified to evaluate the reproducibility and robustness of this novel approach. SLIDE was tested on 10 healthy individuals for three time points over a week, with mean variabilities for the lipid classes detected ranging from 26-64% and 51-119% for intraindividual and group variability (CVg), respectively. Probabilistic quotient normalization improved the CVg. The lipidomic content of AS was described in terms of identification and quantitation. A total of 240 lipids across 15 classes were identified. The lipid concentrations varied from 10-10 to 10-4 mol/L. The most numerous class of lipids was ceramides (Cer), while the free fatty acids (FFA) were the most abundant (10-5 mol/L on average). The applicability of SLIDE for breast cancer screening was tested on 20 clinically defined patients and 23 controls in parallel with the standard screening process in the mammology clinic. Patients were found to have increased concentrations of Cer and FFA and conversely decreased hexosylceramides. The main advantages of AS microsampling include the non-invasiveness of the procedure, speed of collection and patient comfort, while the application of SLIDE as an AS sampling technique brings new perspectives for use in modern clinical practice.

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THP-31: PROTEOMIC ANALYSIS OF HEART TISSUE – STUDY OF AMYLOIDOSIS

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Amyloidosis is a pathological deposition of misfolded protein with β-sheet conformation in various organ tissue. Amyloid is an insoluble degradation product which can lead to malfunction of an affected organ. The deposition can be caused by pathological conditions (e.g. chronic inflammatory disease) or it can be hereditary. For diagnostics, patients suspected of amyloidosis undergo a biopsy of an affected organ (1). Secure identification of protein causing amyloidosis is crucial for subsequent treatment. Antibody-based methods are commonly used for identification of amyloid protein. However, these methods have their limitations (2).

In this study, we focus on the analysis of transthyretin amyloidosis (ATTR) heart tissue from cryo-sections and formalin-fixed and paraffin-embedded (FFPE) sections using MS-based proteomic methods. After deparaffinization, samples are processed and digested using enhanced filter-aided sample preparation (eFASP). Digested samples are measured via LC-MS/MS system. For evaluation of relative abundance of proteins present in the sample we use MaxQuant software and the iBAQ intensities.

The described method was used for patient samples obtained from the Institute of clinical and experimental medicine (IKEM). Using this methodology, we are able to identify up to 1600 proteins in both cryo- and FFPE sections. Moreover, we are able to determine whether the amyloidosis is caused by transthyretin or other protein deposition.

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FRP-32: MACHINE LEARNING CLASSIFICATION UNVEILS SECRETS OF NATURAL INDIGO ANALYZED BY ION MOBILITY COUPLED TO MASS SPECTROMETRY

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Identification of pigments and dyes is essential for characterization and restoration of cultural heritage. Besides confirmation of colorants, which is already challenging due to minute amount of available samples, their provenance or biological origin is often required. Indigo, the most prominent historical blue colorant was produced from four sources: European woad, Indian indigo, Central and South American anil and Eastern Asian knotweed. Regardless of the used instrumental technique, traditional analytical approaches based on detection of its main components, indigotin and indirubin, were insufficient for such a demanding task. We present the first systematic comprehensive untargeted strategy for discrimination of indigo colorants from different biological sources. The analysis was based on FIA-ESI-IMS-MS, which captured all ionizable analytes including trace components. The massive of data consisting of natural pigments from various biological sources as well as reference paint samples embedded in common binders was statistically processed to select the important features and subjected to classification based on machine learning algorithms using a multi-method screening scheme. Combinations of eight feature selection methods and five classification algorithms were assessed with a set of independent samples using a nested (double) cross-validation process. The best-performing model based on a random forest classifier (average classification accuracy 94%) was used to classify historical samples. Important features were further identified based on their fragmentation patterns and confirmed by comparison with available standards. Flavonoids diosmetin and chrysoeriol were proposed as markers of woad and an indole alkaloid 2,2′ -biindolyl was confirmed in true indigo.

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THP-33: HDX-MS STUDY OF THE HDM2 PROTEIN INTERACTION WITH THE NUTLIN-3 COMPARED TO P53 PROTEIN

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Cancer is defined as uncontrolled cell division and tumour cell growth with the potential to spread or invade other body organs. The p53 protein, known as the guardian of the genome, plays important role in tumour suppression. Its interaction with human double minute (HDM2) inhibits p53 transcriptional activity and stimulates its ubiquitination followed by degradation. Overexpression of HDM2 in tumours inhibits p53 and therefore promotes uncontrolled cell proliferation. Inhibition of this interaction is important for cancer therapy. One of the promising inhibitors is Nutlin-3 (cis-imidazoline), which interacts with the HDM2 at the p53 binding site, thereby able to disrupt the p53 HDM2 complex. This leads to the release and activation of p53 and consequent cell cycle arrest or apoptosis.

In this work, hydrogen-deuterium exchange mass spectrometry (HDX MS) was employed for the study of HDM2 conformational dynamics after binding either with Nutlin-3 or p53 protein. The higher suppression of deuteration at the p53 binding domain in HDM2 protein was observed after the Nutlin-3 binding compared to the p53-HDM2 complex. Unlike p53-HDM2 interaction, the Nutlin-3 induced the allosteric changes in the C-terminal disordered region of the HDM2.

The results show that Nutlin-3 mimics the HDM2 binding site of p53. The different HDX suppression was observed in the case of Nutlin-3 interaction in comparison to p53 binding to HDM2. This pilot study will continue further with small molecule inhibitors which have the potential for cancer treatment.

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FRP-34: AUTHENTICITY OF ANIMAL FURS USING PROTEOMICS

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In order to reveal counterfeit fur, to protect protected mammalian species and for other forensic purposes, it is necessary to correctly determine the animal origin of the fur. One of the possibilities is to use of the proteomic approach that preferably targets the keratin protein, which is species-specific and, in addition, is present in large quantities in animal fur. In this work, we tried to overcome the most common obstacles in proteomic analysis of animal fur, which includes e.g. the presence of surface-bound lipids or the problem of obtaining the main protein of our interest – keratin. The reference materials were fur samples of fox, mink, muskrat and rabbit. The effects of disintegration of fur, delipidation and protein extraction on the results obtained by mass spectrometry techniques, namely matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and liquid chromatography coupled to electrospray ionization-quadrupole-time-of-flight mass spectrometry (LC-ESI-Q-TOF) are shown and evaluated. The potential species-specific peptide markers for both mass spectrometric methods were successfully found.

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THP-35: IDENTIFYING OF SINGLY AND DOUBLY PROTONATED DNA TRINUCLEOTIDE CODONS USING A GAS-PHASE DISSOCIATION

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Dissociations of DNA trinucleotide codons as gas-phase protonated ions were studied by tandem mass spectrometry. The cations showed different distributions of nucleobase loss from the 5', middle, and 3'-positions depending on the nucleobase nature as well as the charge state (+1 or +2).

In the case of structurally identical nucleobases in different positions (the dXXX and dXYX type), we achieved an unambiguous resolution of this identity by analyzing the dissociations of codons in which individual bases were distinguished by site-specific ^{15}N-labelling. Those derivatives were synthesized manually resulting in mass shifts by 5 Da for [$^{15}N_5$]-adenine and [$^{15}N_5$]-guanine, 3 Da for [$^{15}N_3$]-cytosine, and 2 Da for [$^{15}N_2$]-thymine. The combined results of our experimental and computational study will be discussed – especially the positional propensity of adenine, guanine, cytosine and thymine to dissociate as neutral molecules or protonated nucleobases.

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FRP-36: 5 YEARS OF DELVING INTO THE *NAJA ASHEI* VENOM PROTEOME

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Naja ashei is the biggest African spitting cobra found in Kenya, Ethiopia, Somalia, and Uganda. This cobra was considered to be a morphologically distinct variant of the species *N. nigricollis* however, molecular analyses showed that despite greater differences in morphology, this species is more closely related to *N. mossambica*, a spitting cobra found in Mozambique. Since 2007 it is considered a separate species.

For the past five years, our team has been the only one in the world to study the proteomic composition of *N. ashei* venom. In our study, we used various venom separation methods (2D electrophoresis [1], ultrafiltration [2], ion exchange chromatography [3], and SEC chromatography) and mass spectrometry techniques (MALDI ToF and LC-MS).

From the earliest analyses, our research has indicated that, as in other cobras, the major components of *N. ashei* venom are PLA_2 and 3FTx. All methods also identified SVMPs, CVF, CRISPs, and VNGF. With the increasing level of venom fractionation, new groups of proteins were discovered such as serine and cysteine proteases, L-amino acid oxidases, and antimicrobial peptides, among others. We have also found a mysterious group named by our team “Ig-like proteins” because it included proteins with an immunoglobulin domain, whose function is so far unknown. On the other hand, increasing the purity of the fractions for the most abundant protein groups resulted in the detection of many new peptides. Thus, it can be concluded that in the case of such a complex proteome as snake venom, in which individual groups of proteins clearly differ in abundance, the picture of the proteome is significantly affected by both the way proteins are fractionated and the way MS data are analyzed.

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THP-37: DEVELOPMENT AND VALIDATION OF A HIGH THROUGHPUT CZE-MS/MS METHOD FOR SENSITIVE QUANTIFICATION OF TRIPTORELIN IN PHARMACEUTICAL AND BIOLOGICAL MATRICES

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Triptorelin is a modulator of the gonadotropin-releasing hormone receptor and is clinically used in the treatment of hormone-responsive breast cancers, prostate cancer, endometriosis, female infertility, precocious puberty, and in the treatment of male hypersexuality. Triptorelin can also be abused in order to increase growth and improve athletic performance [1]. The aim of the present study is to develop a method based on capillary zone electrophoresis-tandem mass spectrometry (triple quadrupole) with multisegment injection and electrokinetic injection of the sample in order to achieve higher throughput and improved values of LOD and LOQ in both water and plasma matrices. The separation step was performed on a background electrolyte composed of 1000 mM formic acid at pH 1.88. We also compared the LODs of hydrodynamic and electrokinetic injections of triptorelin (0.25 and 0.005 µg/mL). The electrokinetic injection preconcentration technique reduces the LOD value 50 times compared to hydrodynamic injection. The multisegment injection approach, on the other hand, increases sample throughput 3 times. A highly selective and reliable determination of triptorelin was carried out in the MRM mode, using two ion transitions: $656.5 \rightarrow 328.3$ and $656.5 \rightarrow 249.0$. The developed and optimized method was then characterized by favorable performance parameters, such as limit of detection (5 ng/mL in water matrix, 25 ng/mL in plasma matrix), precision (relative standard deviation, 1.5-9.4% for intraday and 2.3-11.9% for interday reproducibility), and accuracy (relative errors in the range of 80-109%). Satisfactory performance parameters predetermine the proposed method for routine use in drug quality control laboratories and even in therapeutic drug monitoring.

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FRP-38: TARGETED MASS SPECTROMETRY TO IDENTIFY OPTIMAL CULTURE CONDITIONS FOR NEURAL DIFFERENTIATION

Rita Sucha [1], Jakub Cervenka [1,2], Martina Kubickova [1,2], Marian Hruska-Plochan [3], Dasa Bohaciakova [4], Katerina Vodickova Kepkova [1], Tereza Novakova [1], Katerina Budkova [1], Hana Kovarova [1], Petr Vodicka [1 *]

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In vitro-propagated neural stem cells require specific culture conditions to retain their multipotentiality or differentiate into adult neural cells. However, a defining signature of cells that differentiate upon defined conditions into desirable neuronal/glial phenotype remains insufficiently characterized. We applied SRM to measure levels of protein markers routinely used to probe neural differentiation in a panel of well-described conditions. Our method helped to identify the presence of pluripotent, multipotent as well as lineage-committed cells [1]. Since the capacity of the measurement allows to quantify differentiation markers together with other relevant proteins simultaneously, we aimed to develop assays for additional markers that would provide a system-wide view. We browsed manually curated databases and selected 360 proteins that report on specific biological processes on the basis of literature evidence. The proteins are mechanistically employed in biological processes of neural differentiation, including cell proliferation, autophagy, apoptosis, etc. Such an expanded assay could allow the optimization of novel specific propagation and differentiation protocols, further increasing the accuracy in detecting targeted cell populations.

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THP-39: POTENTIAL OF ASAP-MS TECHNIQUE AND ITS APPLICATION FOR METABOLITE PROFILING IN PLANT MICROSAMPLES

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In this communication we introduce a new atmospheric solids analysis probe mass spectrometric (ASAP-MS) technique for direct analysis of solid plant microsamples. Lab modified glass capillaries were used as probe in connection with a high-resolution tandem mass spectrometer Cyclic IMS (Waters). The sampling was performed using a defined micromanipulation under microscopic control.

Long chain fatty acids (LFA), their hydroxy-derivates (HLFA) and alkyl esters of caffeic acid (AECA) were detected and identified by this technique in intact microsamples of pea seed coat parts. Effect of external degradation factors (UV light, ozonation or heat) on metabolite profile was studied. Significant degradation of (H)LFA and AECA was observed. On the other hand, sterols (e.g. β-sitosterol, stigmasterol and campesterol) and phenolic compounds (sinapic acid, catechin etc.) were identified in all microsamples without significant changes. Moreover, new degradation products (e.g. a signal at m/z 413.3811) appeared in exposed microsamples and their identification is an objective of present research.

Combination of micromanipulation with microscopic control for sampling and ASAP-MS analysis using modified capillaries appeared to be appropriate approach for metabolite profiling in plant microsamples.

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FRP-40: LC-MS/MS DETERMINATION OF DIRECT ORAL ANTICOAGULANTS IN PLASMA OF LUPUS POSITIVE PATIENTS

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Direct oral anticoagulants (DOAC) are targeted inhibitors of coagulation factors that are commonly used for anticoagulation therapy. Functional assays used for their determination, are suspect of possible interference with antibodies present, especially in patients who have developed lupus anticoagulans (LA). An alternative option for the determination of DOAC is LC-MS, which should give correct results unencumbered by interferences. To evaluate the efficacy of DOAC treatment in lupus-positive patients, 31 plasma samples were investigated. All patient samples were spiked with three types of DOAC (dabigatran, rivaroxaban, and apixaban) at concentrations that significantly affected the screening test for LA and may mask the presence of LA. Determination of DOAC levels was performed both by routinely used functional assays and by our developed LC-MS/MS method. An UltiMate 3000 RS liquid chromatography system (Dionex, Sunnyvale, CA) coupled with a Triple Quad 6500 tandem quadrupole mass spectrometer (Sciex, Foster City, CA) was used for LC-MS/MS analysis. Separation was performed using a Luna Omega C18 polar column (Phenomenex, Torrance, CA) within 3 min. The results showed significant differences between these two approaches. When comparing them before and after DOAC binding, the fold change for the functional assays ranged from 1.4-1.7, whereas for LC-MS 62-183. This work suggests that the presence of LA-type antibodies substantially affects the determination of DOAC by functional assays and in this case the LC-MS/MS method should be used for their determination.

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THP-41: INSIGHTS INTO THE TRANSPORT DYNAMICS OF PROKARYOTIC CHLORIDE/PROTON ANTIPORTER

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Chloride channel family includes transmembrane channels and transporters, which are involved in many cellular processes and their mutation can cause serious illnesses. To treat these diseases, a description of their transport mechanism is needed. Chloride transporter CLC-*ec1* from *E. coli* is often used as a model protein to study the protein family of chloride channels. The protein functions as an antiporter of a single proton for two chloride anions. During transport of ions, the protein converts between inward and outward-facing conformations. Outward-facing state is induced by protons, when Glu residues which are involved in ion transport are protonated. This state can be mimicked by a QQQ mutant, in which three key Glu residues are mutated to Gln residues [1]. So far, CLC-*ec1* transport has been studied by X-ray crystallography that provided detailed but static images. Here we used hydrogen/deuterium exchange mass spectrometry to extend the recent findings and provide more detailed insight into the transport dynamics of this protein. Full-length wild type and QQQ CLC-*ec1* were overexpressed in bacteria, isolated via detergent solubilization and purified by affinity chromatography and gel filtration. HDX-MS based digestion was carefully optimized to provide full sequence coverage with reasonably sized peptides. Next, HDX-MS experiment was conducted at different conditions. First, we compared WT and QQQ proteins at pH 7.4, which pointed to differences between the two forms underlying their different conformations. Next, to address the role of protonation, both proteins were followed at four pH values spanning pH range 4.4-7.4. These data highlight the stepwise protonation and the associated structural changes across the ion transport path.

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FRP-42: CYTOKINE EXPRESSION IN SPONTANEOUS REGRESSION OF MELANOMA IN MELANOMA-BEARING LIBECHOV MINIPIG MODEL

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Melanoma is highly malignant skin cancer originating from transformed melanocytes. Interestingly, an immune response to melanoma can be spontaneously triggered in the body, leading to partial disappearance of the tumor, i.e. spontaneous regression.

In our study, we monitored the expression of cytokines in Melanoma-bearing Libechov Minipig (MeLiM) model of hereditary melanoma. We aimed to characterize differences in cytokine expression between MeLiM animals undergoing melanoma progression and spontaneous regression to identify regulatory molecules in these processes and possible markers distinguishing both groups.

RT-PCR and Luminex xMAP assays were used to quantify cytokine expression on mRNA and protein levels in melanoma tissue, normal skin and blood plasma.

Spontaneous regression was characterized by elevated levels of interleukin 12 (IL-12) and cytokines activating IL-1 receptor. In addition, high IL-6 expression was found in melanoma compared to healthy skin, and the effect of recombinant IL-6 on proliferation and migration of MeLiM melanoma-derived cells was studied in vitro.

Our data suggest that the spontaneous regression in MeLiM is accompanied by pro-inflammatory environment supporting recruitment of immune cells. Identified immunoregulatory molecules might find future implications in the research of human disease and therapy.

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THP-43: DEVELOPMENT OF A METHOD FOR LIPOPEPTIDE SEPARATION USING LIPOSOMES

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Liposomes - vesicles formed by a phospholipid bilayer - are commonly used as a model of cell membranes in vitro experiments. The so-called Liposome-binding assay is a simple and inexpensive method how to prepare these vesicles and it has been used in many projects to investigate the interactions of whole proteins with the cell membrane (1,2). However, it is not known that liposomes have been used to test the interaction of membranes with lipopeptides, which are formed by cleavage of the entire lipoprotein and are directly responsible for binding to the phospholipid bilayer.

The aim of this project is to verify the functionality of the peptide-liposome system, as a possible way of separation of peptides with hydrophobic properties from a complex matrix, thus enable their qualitative and quantitative analysis, which is important for monitoring pathological processes, a differentiation or synaptic transmission in cells (3).

With modified Liposome-binding procedure, it was possible to isolate lipopeptides from mixture of peptides obtained by enzymatic digestion of Mouse mammary tumor virus matrix protein (MMTV). Almost 100% of the lipopeptides were separated and concentrated in the liposomal fraction.

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FRP-44: MASS SPECTROMETRY – BASED IDENTIFICATION OF 5'-RNA CAPS

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It is well known that most of the RNAs are post-transcriptionally modified on the nucleobases or ribose. Increased sensitivity of next-generation sequencing and especially mass-spectrometry based analysis revealed new class of modifications on 5' end of RNA, which are called RNA caps [1]. They are mostly derived from metabolites, cofactors and dinucleotide polyphosphates [2,3]. It is believed that their presence can affect the RNA stability, cellular metabolism and even mRNA translation, however their exact role is still not well described. The physicochemical properties of these molecules (high hydrophilicity, acidic phosphate functional groups, nucleobases) makes their analysis very challenging. This work will present workflow for qualitative and quantitative analysis of (non)canonical 5'-RNA caps in bacteria and mammalian tissue cell cultures.

RNA was isolated from various *in vitro* and *in vivo* samples and analyzed by UHPLC (Acquity H-class, Waters) equipped with dual mode reversed phase-anion exchange column (Atlantis Premier BEH C18 AX, Waters) coupled to high resolution mass spectrometer (Xevo G2-XS qTOF, Waters).

We have developed a robust and reproductible workflow for RNA caps analysis. In all digested RNAs we have observed signals of different canonical and non-canonical caps such as NAD, CoA, dinucleotide polyphosphates and their methylated analogues. Their structure was validated based on the precise retention time, *m/z* ratio and compared to the commercial standards. In other cases, fragmentation spectra were acquired to confirm the identity of the cap.

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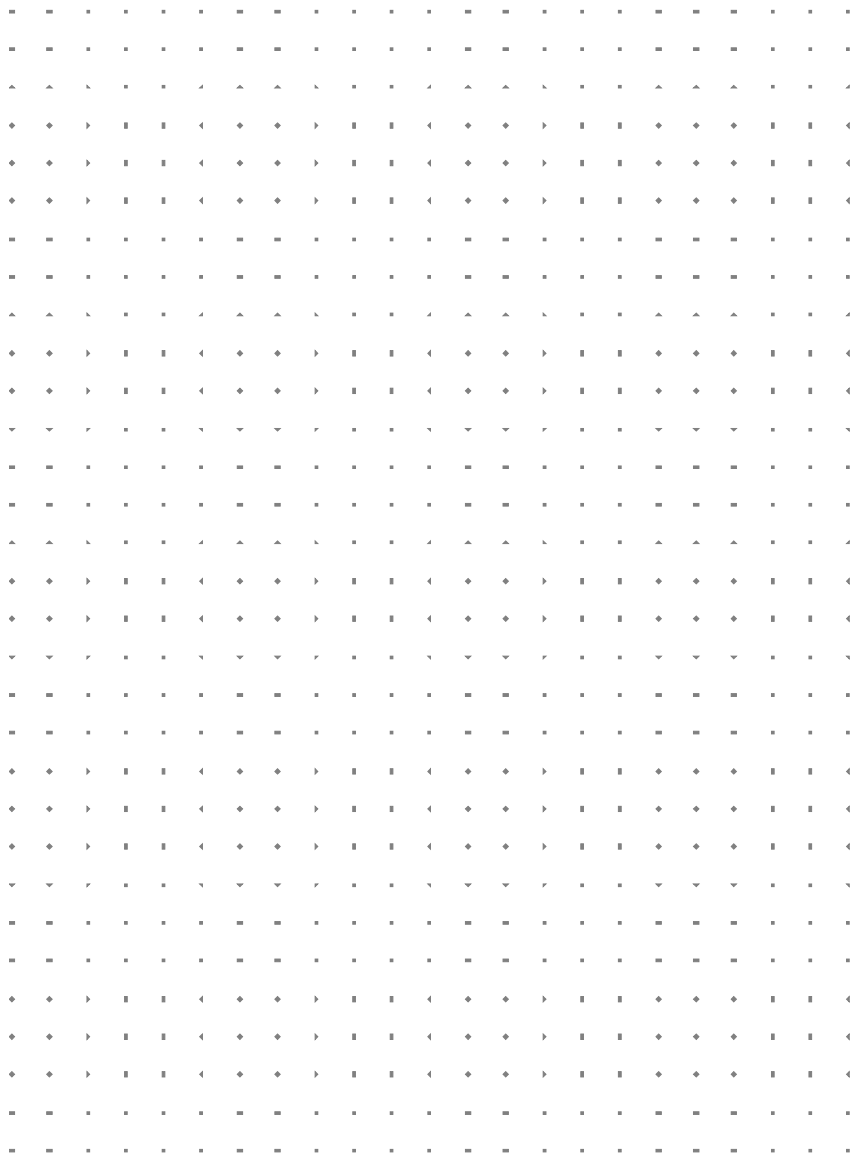

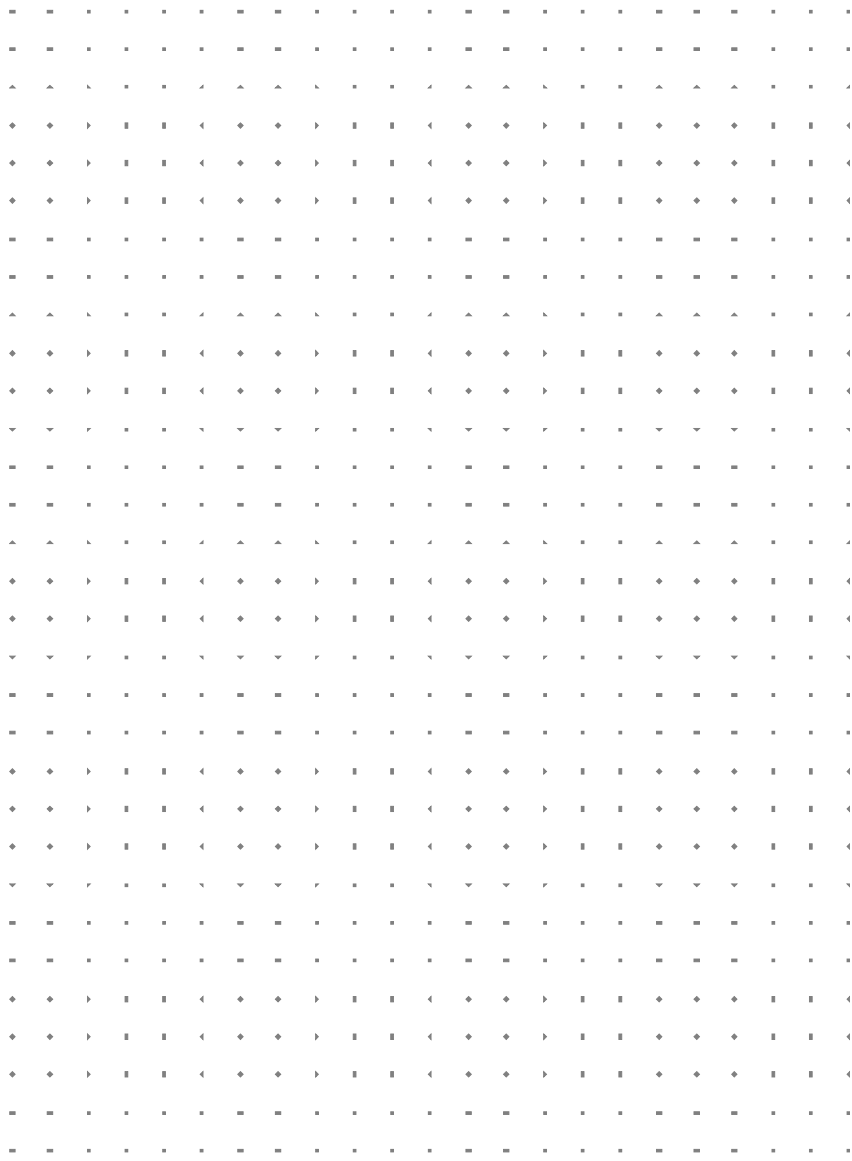

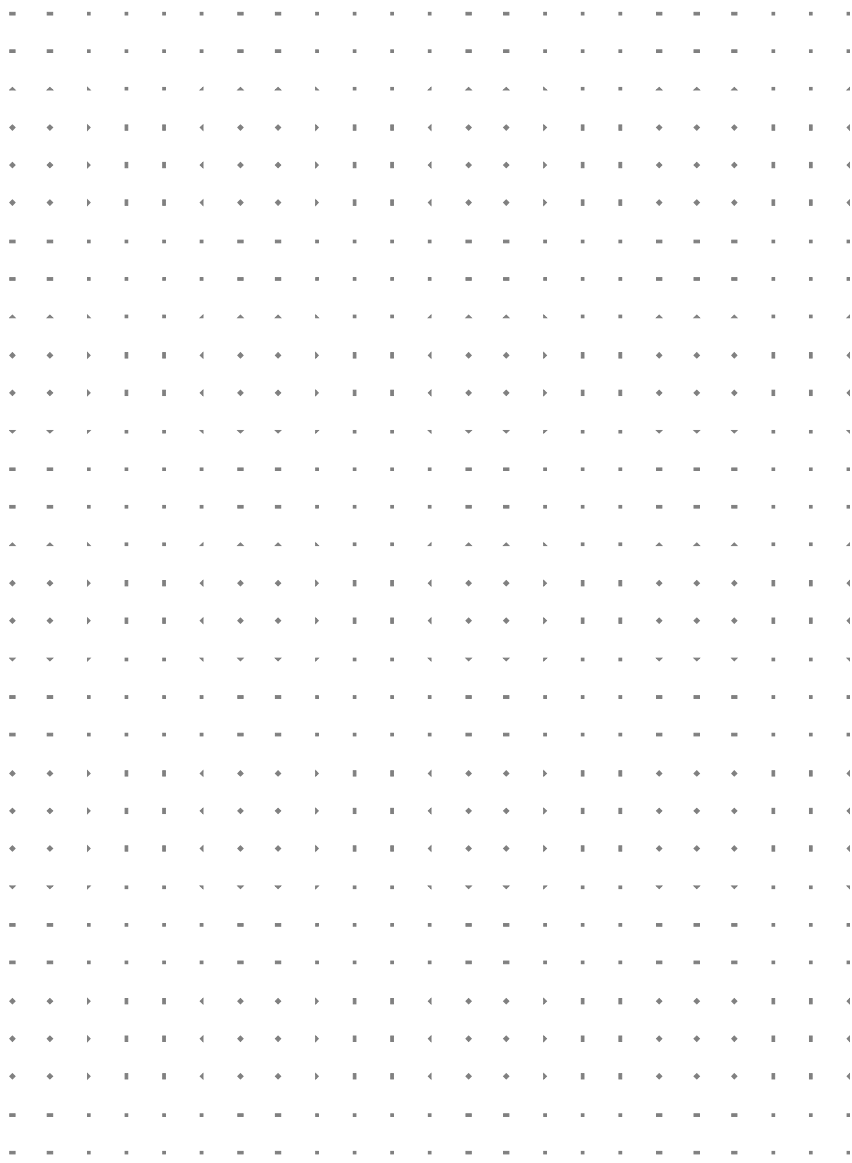

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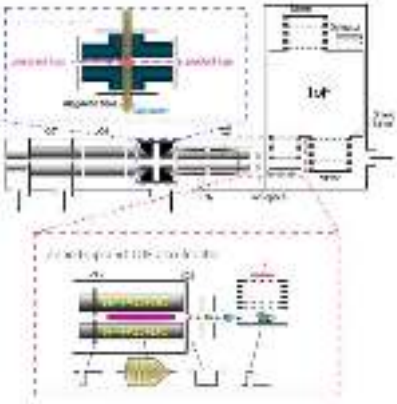

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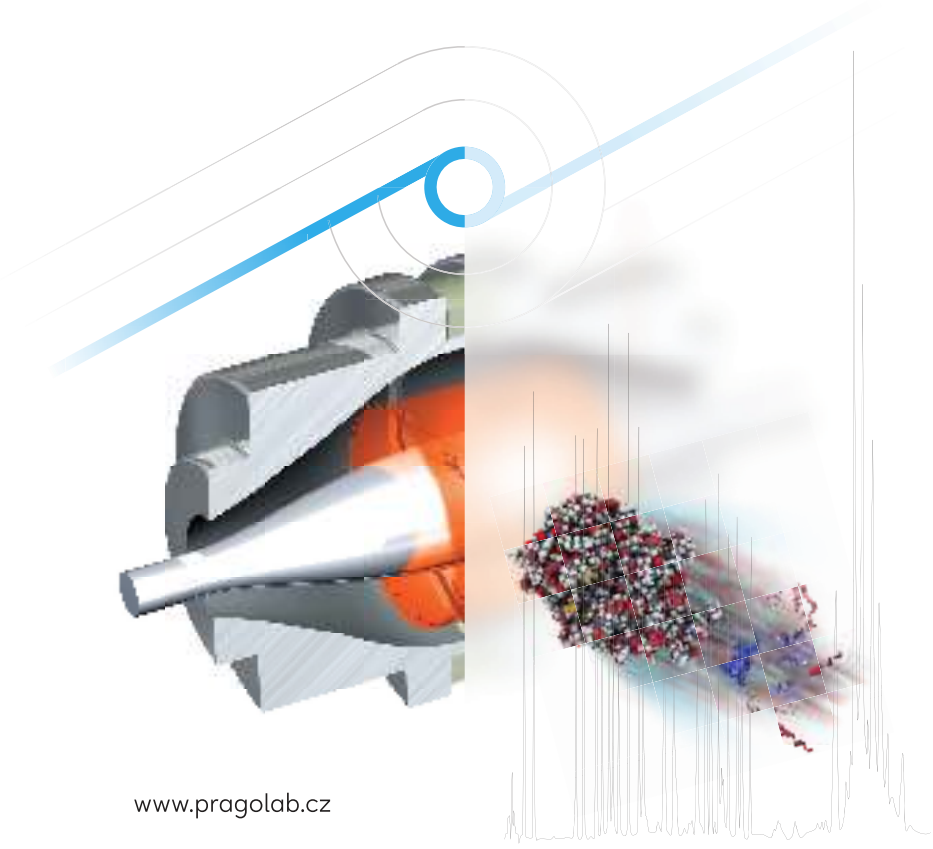

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