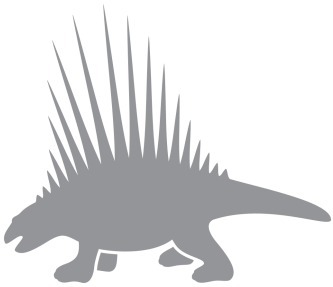

ELEVENTH ANNUAL CONFERENCE OF THE CZECH SOCIETY FOR MASS SPECTROMETRY

Brno, June 19 – June 21, 2023

BOOK OF ABSTRACTS

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

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Czech Society for Mass Spectrometry

Brno 2023

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Eleventh Annual Conference of the Czech Society for Mass Spectrometry

Date

19th June – 21st June 2023

Venue

Moravian Museum (Moravské zemské muzeum)

Dietrichstein Palace

Zelný trh 8

659 37 Brno

Czech Republic

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Czech Society for Mass Spectrometry, Olomouc

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Faculty of Science, Charles University, Prague

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Monday 19th June 2023

12:00 – 19:30	Registration
10:00 – 11:30	Excursion at Thermo Fisher Scientific
12:30 – 12:40	Conference opening
12:40 – 13:30	**Plenary lecture I: Jan Kratzer** (Chairperson: Jan Preisler)
PL-1	*ICP-MS: the other mass spectrometry*
13:30 – 14:30	Company Workshop – Pragolab s. r. o.
14:30 – 14:50	Coffee break
14:50 – 16:10	**Session I**
	(Chairperson: Lenka Hernychová)
14:50 – 15:10	Karel Harant
MoO-01	*Orbitrap Ascend – experience in proteomics core facility*
15:10 – 15:30	Tomáš Oždian
MoO-02	*The characterization of proteome and protein-tissue analysis in the samples of the cervical mucus*
15:30 – 15:50	Adam Paulin Urminský
MoO-03	*Paclitaxel Neuropathy: A Glycoproteomic Approach to Predictive Biomarkers in Breast Cancer*
15:50 – 16:10	Tomáš Ječmen
MoO-04	*Perturbed N-glycosylation of IgG expressed in HEK293T cells in the presence of fluorinated monosaccharide analogs determined by MS*
16:10 – 16:30	Coffee break

16:30 – 17:50	**Session II**
	(Chairperson: Jan Fiala)
16:30 – 16:50	Tomáš Korba
MoO-05	*Identification and Characterization of Compounds Using Electron Activated Dissociation on SCIEX ZenoTOF 7600 System*
16:50 – 17:10	Karel Chalupský
MoO-06	*Lipids and bone dismorphology in insig1 gene-deficient mice*
17:10 – 17:30	Josef Dvořák
MoO-07	*Novel desorption-ionization ms affinity enzyme-linked assay for Clostridium bacteria family toxin activity detection*
17:30 – 17:50	Aleksandr Melikov
MoO-08	*Investigation of human Hsc70 oligomerization*
17:50 – 18:20	Poster talks (Chairperson: Tomáš Ječmen)
MoS-1	*Jana Schwarzerová: A Comprehensive Tool for State-of-the-Art Pre-processing Analysis in Metabolomics included in updated App: COVAIN v2.0.0*
MoS-2	*Jasmína Portašiková: Insights into the transport dynamics of prokaryotic chloride/proton antiporter*
MoS-3	*Valérie Procházková: Quantitative Cross-linking Mass Spectrometry Using Data-Independent Acquisition*
MoS-4	*Michael Karpíšek: Fast FluoroAlkylation of Proteins (FFAP): A Novel Cross-linking Strategy for Aromatic Residues*
18:20 – 22:00	Drinks and poster session

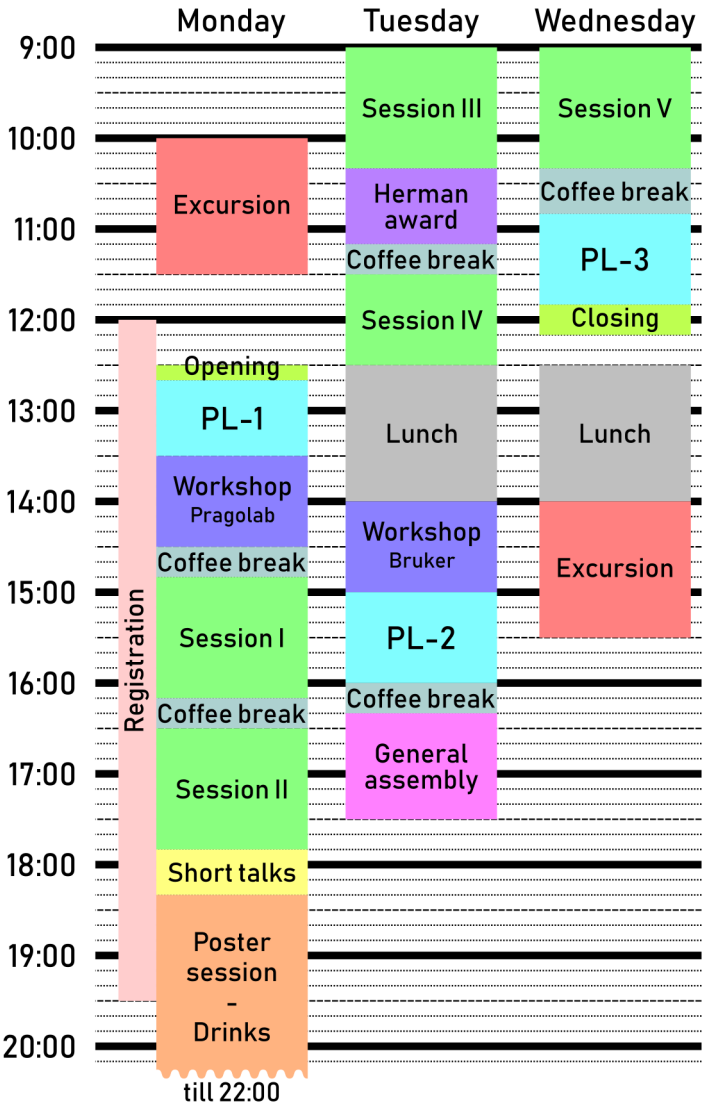
Tuesday 20th June, 2023

9:00 – 10:20	**Session III**
	(Chairperson: Olga Součková)
9:00 – 9:20	Isabel Riba
TuO-09	*Unravelling Biological Sample Complexity With Advanced Mass Spectrometry*
9:20 – 9:40	Marianna Nytka
TuO-10	*Multiple linear regression of mobilograms of isomeric new synthetic drugs analyzed by cyclic ion mobility*
9:40 – 10:00	Anton Škríba
TuO-11	*RNA-modifications analysis by hydrophilic interaction liquid chromatography-mass spectrometry*
10:00 – 10:20	Rutuja Patil
TuO-12	*Aspergillus fumigatus and Pseudomonas aeruginosa interplay at a host interface*
10:20 – 11:10	**Zdeněk Herman Award presented by Resonance Foundation and presentation of the winning thesis**
11:10 – 11:30	Coffee break
11:30 – 12:30	**Session IV**
	(Chairperson: Rutuja Patil)
11:30 – 11:50	Stanislav Kukla
TuO-13	*Drawing a better map: Optimizing LC-MS analysis of biologicals*
11:50 – 12:10	Ondřej Bucek
TuO-14	*Application of the mass spectrometry to identify protein markers of Alzheimer's disease*
12:10 – 12:30	Roman Tuzhilkin
TuO-15	*Photo-Methionine, Azidohomoalanine and Homopropargylglycine Have Distinct Effect on the Growth of Auxotrophic and Prototrophic E. coli in Minimal Medium, Alter Protein Expression Levels and Incorporate into Newly Synthesized Proteins at Different Rates*
12:30 – 14:00	Lunch
14:00 – 15:00	Company Workshop – Bruker s. r. o.

15:00 – 16:00	**Plenary lecture II: Graham Cooks** (Chairperson: Michael Volný)
PL-2	*Mass Spectrometry for Chemical Synthesis and Analysis: including an appreciation of the work of Zdenek Herman (on-line)*
16:00 – 16:20	Coffee break
16:20 – 17:30	General assembly of the Society

Wednesday 21st June, 2023

9:00 – 10:20	**Session V**
	(Chairperson: Adam Pruška)
9:00 – 9:20	Gary Kruppa
WeO-16	*The benefits of trapped ion mobility for single-cell proteomics*
9:20 – 9:40	Alan Kádek
WeO-17	*Zapping ions – using powerful lasers for more informative mass spectrometry*
9:40 – 10:00	Marek Polák
WeO-18	*Fast photochemical oxidation of nucleic acids coupled to high-resolution MS analysis*
10:00 – 10:20	Hynek Mácha
WeO-19	*Internal standard normalization in MALDI mass spectrometry imaging*
10:20 – 10:50	Coffee break
10:50 – 11:50	**Plenary lecture III: Adam Schröfel** (Chairperson: Alan Kádek)
PL-3	*Introduction to three dimensional cryo-electron microscopy*
11:50 – 12:10	Poster prize, Final remarks
12:30 – 14:00	Lunch
14:00 – 15:30	Excursion at Thermo Fisher Scientific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Registration
Excursion
Opening
PL-1
Workshop
Pragolab
Coffee break
Session I
Coffee break
Session II
Short talks
Poster
session
-
Drinks
till 22:00
Session III
Herman
award
Coffee break
Session IV
Lunch
Workshop
Bruker
PL-2
Coffee break
General
assembly
Session V
Coffee break
PL-3
Closing
Lunch
Excursion

PL-1: ICP-MS: the other mass spectrometry

Jan Kratzer [1 *]

1. Institute of Analytical Chemistry ASCR, v.v.i., Brno

The aim of this lecture is to bridge the gap between the users and specialists in the field of organic mass spectrometry and the community working in inorganic (i.e. element) mass spectrometry employing the inductively coupled plasma mass spectrometry (ICP-MS) technique. ICP-MS is nowadays the most sensitive, multi-element and universal detector that allows detection of elements, especially metals, down to the $pg \cdot ml^{-1}$ level. ICP-MS instrumentation has many similarities with organic mass spectrometers but it also shares many common features with other atomic spectrometric detectors used in trace element analysis. Analogously to other MS techniques, the ICP-MS consists of: 1) ion source, 2) mass analyzer and 3) the detector. ICP torch is the ion source realized by rf-driven (27 or 40 MHz) plasma sustained in argon at atmospheric pressure. This highly energetic plasma (8000 K, 1600 W) is responsible for atomization, excitation and finally ionization of analyte. The ions produced enter the low pressure environment of MS. They are separated according their mass to charge ratio (m/z) in a single quadrupole mass analyzer. Due to its low resolution (1 amu) strategies have been developed to avoid isobaric (on mass) interferences to distinguish between atomic ion of analyte and polyatomic ions as interferents having the same m/z, e.g. $^{75}As^{+}$ (analyte) versus $^{40}Ar^{35}Cl^{+}$ (interferent). For that purpose, the ICP-MS spectrometers are equipped with collision/reaction cells while their newest generation relies on triple quadrupole (ICP-MS/MS) configuration. The ions are subsequently detected by electron multiplier. Sample introduction is an inherent part of ICP-MS analysis affecting also the detector sensitivity. Like in other spectrometric detectors employed in element analysis liquid sample nebulization is the most common approach. Sample introduction efficiency is limited by the efficiency of the nebulizer (5-10%). Alternative approaches to sample introduction include solid sampling by laser ablation (LA) or gas phase sampling by vapor generation (VG). LA-ICP-MS is suitable for hard-to-degrade samples and enables also element imaging. VG-ICP-MS relies on selective analyte conversion from liquid to gas phase improving analyte introduction efficiency and decresing the risk of interferences due to matrix separation. Analysis of (metal) nanoparticles is also feasible by single particle (sp)-ICP-MS bringing information about their concentration and size distribution. ICP-MS is not only used to determine the total element content but it can be also applied to the speciation analysis, i.e. determination of different chemical species of the same element. Since ICP-MS is a ″destructive″ detector not preserving structure information about the species due to the atomization/ionization processes in the ICP torch, separation of the species by HPLC or GC have to precede their detection with speciation information derived from retention times. Applications of ICP-MS to trace element and speciation analysis in environmental and clinical samples will be discussed. Examples from the fields of metallomics will be demonstrated where both organic and inorganic MS can be combined as HPLC detectors to receieve simultaneously information on structure (ESI-MS) and ultratrace metal content (ICP-MS) in metalloproteins.

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PL-2: Mass Spectrometry for Chemical Synthesis and Analysis: *including an appreciation of the work of Zdeněk Herman*

Graham Cooks [1 *]

1. Department of Chemistry, Purdue University, West Lafayette, IN, USA

Over the course of a long career, entirely spent doing mass spectrometry, a major privilege has been my interactions with outstanding scientists, especially those who were also distinguished in other spheres. The late Zdeněk Herman is a prime example and this talk includes coverage of topics where our science overlapped as well as recollections of Zdeněk himself.

One such shared topic concerns **collisions of ions with surfaces** and the associated kinematics. This topic includes inelastic and reactive ion scattering from surfaces, energy partitioning and ion thermochemistry. This presentation reviews fundamentals of energy partitioning, angular scattering and target and projectile effects. It also deals with applications, including those of surface induced dissociation (SID) and with ion soft landing on surfaces, both topics of considerable current interest.

I shared with Zdeněk Herman, and many others of an earlier era of mass spectrometry, a strong interest in the **instrumentation**. Novel work is greatly facilitated by newly designed and built instruments and the scattering instruments at Prague and Purdue share fascinating and noteworthy differences and similarities.

In some topics, my style was not embraced by Prof. Herman. His strength in mathematics and fundamental physical chemistry led to his polite disinterest in my **kinetic method of making thermochemical determinations**. Zdenek is well-known for his cartoon representations of scientists he knew, and I rather think that the red steed in his semi-satirical cartoon me driving an out-of-control team of horses represents the kinetic method.

Zdenek Herman's city, **Prague**, is one of the epicenters of world science. I illustrate its effect during a recent visit. This concerns **accelerated reactions in microdroplets**, which I will briefly present, followed by a consideration of the **Hofmeister series** and the possible role of the water radical cation $\mathbf{(H_2O)^{+\cdot}}$ in explaining this effect.

In closing I reflect briefly on the boundless nature of creative work and how Prof. **Zdeněk Herman** demonstrated this and so serves as a model to which we should aspire.

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PL-3: Introduction to three dimensional cryo- electron microscopy

Adam Schröfel [1 *]

1. Faculty of Science, Charles University, Prague

This talk provides a concise introduction to three-dimensional cryo electron microscopy (3D cryo-EM) and electron tomography. We will cover the basic principles of sample preparation, imaging, and data collection using cryo-EM techniques, as well as the reconstruction methods involved in deriving high-resolution structures. Additionally, we will explore the application of electron tomography, a powerful extension of cryo-EM, for studying three-dimensional cellular architectures and larger macromolecular complexes. The talk aims to provide participants with a foundational understanding of 3D cryo-EM and electron tomography, showcasing their potential for advancing structural biology and uncovering intricate cellular details.

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MoO-01: Orbitrap Ascend - experience in proteomics core facility

Karel Harant [1 *]

1. Univerzita Karlova v Praze

Our core omics facility at Biocev has been using the first generation orbitrap tribrid mass spectrometer – Orbitrap Fusion Tribrid for the last eight years. This year, we have installed the latest member of the tribrid family Orbitrap Ascend Tribrid Mass spectrometer. We were able to compare the instruments' performance for label free and isobaric labeling analysis. In addition, we tested several new features of the Orbitrap Ascend.

Isobaric labeling is a popular alternative to the label-free approach. It allows to design experiments that are not easily possible with label free work flows. One of the major limitations is ratio compression. By providing additional specificity, MS3 fragmentation solves this problem, but the solution comes at the expense of slowing down the data acquisition. Slower acquisition then results in reduction of the protein identifications obtained. The novel real-time search functionality, which was introduced with the Orbitrap Eclipse Tribrid Mass spectrometer, removes this time penalty and makes the isobaric labeling fully competitive to the label free work-flows in quantitative proteomics.

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MoO-02: The characterization of proteome and protein-tissue analysis in the samples of the cervical mucus

Tomáš Ožd ian [1 *], Jan Vodička [2], Jiří Dostál [2], Dušan Holub [1], Michal Ješeta [3], Pavla Kouřilová [1], Radovan Pilka [2], Igor Crha [3], Petr Džubák [1], Marián Hajdúch [1]

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The cervical mucus is the viscous plug of the cervix uteri, which dramatically changes its properties during the menstrual cycle and pregnancy. At the same time, it is a body fluid originating from cervical and uterine glands, which is easily and non-invasively obtained. The main task of this talk is to characterize the proteomic composition of healthy women undergoing artificial insemination with natural or stimulated menstrual cycles. According to known peristaltic properties of the uterus, we have also tried to assess proteins to the possible tissue origin using the human protein atlas database. As a result, we have identified 4370 proteins and 621 of them were differentially expressed in the samples with natural or stimulated cycles and we have found proteins with expression specific to the testis, liver, placenta, and neural tissues. Those findings further support the hypothesis of cervical mucus as the potential source of biomarkers for future studies.

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MoO-03: Paclitaxel Neuropathy: A Glycoproteomic Approach to Predictive Biomarkers in Breast Cancer

Adam Paulin Urminský [1,2], Noortje de Haan [3], Yassene Mohammed [3], Tomas Henek [4], Manfred Wuhrer [3], Jana Halamkova [5], Lenka Hernychova [4 *]

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4. Research Centre for Applied Molecular Oncology, Masaryk Memorial Cancer Institute, Brno, Czech Republic
5. Department of Comprehensive Cancer Care, Masaryk Memorial Cancer Institute, Faculty of Medicine, Masaryk University, Brno, Czech Republic

Paclitaxel, a potent chemotherapeutic agent, is extensively used in the treatment of breast cancer, one of the most prevalent forms of cancer worldwide. Despite its efficacy, a significant challenge in the clinical use of paclitaxel is its association with peripheral neuropathy, a severe and often debilitating side effect that can dramatically affect patients' quality of life. This neuropathy is characterized by numbness, tingling, or pain in the patient's hands and feet, limiting their daily activities and often necessitating dose reduction or treatment discontinuation.
Currently, the capacity to predict which patients are more likely to develop Paclitaxel-induced PN is lacking. This inability to forecast this adverse effect hampers the clinicians' ability to personalize treatment plans, potentially compromising treatment efficacy and patient quality of life.
To address this critical gap, our project aims to explore the blood serum glycoproteome of patients prior to paclitaxel treatment who had acquired paclitaxel neuropathy or whose treatment had not caused these issues. Glycoproteomics, the study of changes in the glycosylation status of proteins, holds promise in uncovering potential biomarkers for disease states and responses to treatment. We hypothesize that distinct patterns of protein glycosylation could signal an elevated risk of developing peripheral neuropathy in response to paclitaxel treatment.
Following our in-depth glycoproteomic investigation, our plan is to use machine learning methods to determine whether there is a particular combination of these proteoforms that could serve as a predictive model for paclitaxel-induced peripheral neuropathy.

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MoO-04: Perturbed N-glycosylation of IgG expressed in HEK293T cells in the presence of fluorinated monosaccharide analogs determined by MS

Tomáš Ječmen [1 *], Celeste Abreu [1], Zuzana Urbanova [1], Jindřich Karban [2], Ondřej Vaněk [1]

1. Department of Biochemistry, Faculty of Science, Charles University, Hlavova 2030, 12840 Prague, Czech Republic

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Different fluorinated monosaccharide analogs were shown to inhibit the biosynthesis of characteristic glycan structures without being incorporated and have the potential to become a part of the treatment of diseases such as multiple sclerosis or certain types of cancer. Here, we investigate the possibility of determining whether the cellular glycosylation perturbed by different monosaccharide fluoro derivatives can be detected on a single glycoprotein level, and the inhibitor-specific N-glycan profiles can be distinguished on the affected glycoprotein. We overexpressed His-tagged Fc fragment of human IgG in HEK293T cells cultivated in the presence of one of the seven acetylated monofluoro analogs of hexosamines or fucose at 50μM concentration and in untreated cells serving as control. The proteins were digested in solution with trypsin and analyzed by RP-ESI-qTOF MS. Ion chromatograms for 19 glycoforms differing in monosaccharide composition, which were attached to a single peptide backbone, for the unglycosylated form of this peptide and two other peptides lacking glycosylation site were extracted, the areas under the curves were calculated and compared among the samples. For five of seven fluoro saccharides tested as potential glycosylation inhibitors, we observed an unchanged overall level of glycosylation at the concentration used. This was manifested as only a minimal amount of unglycosylated form of the peptide with a glycosylation site and was also observed in the controls. Also, the relative proportions of individual glycoforms did not differ significantly when compared to the proportions of the glycoforms in the respective controls. In contrast, a 1-2 orders of magnitude more abundant non-glycosylated form of the peptide was observed for the other two analogs, whose glycoprofiles differed markedly from the controls and also between them. For the acetylated 4-fluoro analog of GalNAc, the enriched glycoforms ($Hex_{3-5}HexNAc_2$) suggest that the later steps of the glycosylation pathways localized in the Golgi are predominantly affected by this inhibitor. In comparison, the rate of fucosylation was dramatically decreased in the presence of acetylated 2-fluoro fucose. A slight difference in total fucosylation level was also noticed for one of the controls, but the effect was much less pronounced than in the presence of 2-fluoro fucose.

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MoO-05: Identification and Characterization of Compounds Using Electron Activated Dissociation on SCIEX ZenoTOF 7600 System

Tomáš Korba [1 *]

1. AMEDIS

Electron Activated Dissociation (EAD) offers additional fragmentation technique to traditional collision induced dissociation (CID). In EAD, 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 (1). 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. This was mainly due to the mating of the continuous beam coming from the quadrupole ion path with time-of-flight (TOF) analysis, a pulsed, discontinuous measurement technique. High capacity ion trap just after the CID collision cell (Q2) and before the pusher region of the TOF, with optimized release of ions from this trap, allows the duty cycle losses to be mitigated leading to MS/MS sensitivity gains of 4-20 fold (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). Examples of EAD utilization presented in the talk include distinguishing of isomer and isobar small molecule compounds and middle down characterization of proteins in single analysis.

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REFERENCES:

1. *Baba T. et al.: Dissociation of Biomolecules by an Intense Low-Energy Electron Beam in a High Sensitivity Time-of-Flight Mass Spectrometer. J Am Soc Mass Spectrom.32, 1964-1975 (2021).*
2. *Loboda A.V., Chernushevich I.V.: A novel ion trap that enables high duty cycle and wide m/z range on an orthogonal injection TOF mass spectrometer. J Am Soc Mass Spectrom.; 20: 1342-1348 (2009).*
3. *Baba T. et al.: Distinguishing cis and trans isomers in intact complex lipids using electron impact excitation of ions from organics (EIEIO) mass spectrometry. Anal. Chem. 2017, 89: 7307–7315 (2017).*

MoO-06: Lipids and bone dismorphology in INSIG1 gene-deficient mice

Karel Chalupský [1 *], Klára Dohnalová [1,2], Kryštof Klíma [1], František Špoutil [1], Jan Procházka [1], Radislav Sedláček [1]

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Rare diseases encompass a diverse range of medical conditions that affect a small number of individuals within a population. One intriguing aspect of rare diseases is their association with lipids, which are essential molecules involved in various biological processes. Lipids play a crucial role in cellular structure, energy storage, and signal transmission. In some cases, rare diseases result from abnormalities or dysfunction in lipid metabolism, and these conditions lead to the accumulation or depletion of lipids in various tissues, causing a wide array of symptoms.
One specific protein involved in the regulation of lipid metabolism and intracellular cholesterol synthesis is INSIG1 (Insulin-induced gene 1). To investigate the effects of reduced INSIG1 function, we utilized a strain of mice with a deletion of the Insig1 gene (Insig1 KO) and looked primarily on their liver lipidome, together with other phenotyping tests.
Our analysis of hepatic lipidome revealed an accumulation of cholesterolesters and triacylglycerols in the livers of Insig1 KO mice. Additionally, we observed alterations in metabolites of vitamin D in their plasma. Furthermore, Insig1 KO mice exhibited craniofacial bone development and finger malformation.
The Insig1 KO phenotype is an example of far-reaching consequence of alterations of lipid spectra. Through our study, we demonstrated the biological significance of cholesterol metabolism and its connection to bone abnormalities in a model of Insig1 deficiency. These findings shed light on the intricate relationship between lipid metabolism, cholesterol homeostasis, and rare diseases, contributing to a better understanding of the underlying mechanisms and future diagnostics.

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MoO-07: Novel desorption-ionization ms affinity enzyme-linked assay for *Clostridium* bacteria family toxin activity detection

Josef Dvořák [1,2 *], Lukáš Fojtík [1,2], Michael Volný [1,2], Jaroslav Hrabák [3], Petr Novák [2,1], Petr Pompach [1,4]

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Clostridium difficile is a dangerous human pathogen causing inflammation, diarrhea, or life-threatening pseudomembranous colitis. Toxin B produced by the bacterium catalyzes the transfer of glucose from UDP-glucose to a threonine 37 of Ras homolog family member A protein (RhoA). The modification by glucose inactivates RhoA GTPase activity and leads to the disruption of the intestine cytoskeleton. The novel approach for C. difficile detection is based on in-situ enrichment of glucosylated intact RhoA using functionalized chips prepared by ambient ion soft-landing and MALDI-ToF measurement. Affinity protein-modified chips were used prior to the enzymatic reaction for binding the affinity-tagged recombinant RhoA protein. The reaction buffer containing different concentration levels of recombinant Toxin B was applied on the affinity MALDI plate and let incubate. The effectivity of the enzymatic reaction was monitored after matrix application by Autoflex speed MALDI-ToF mass spectrometer operated in linear positive mode. Data were searched for a mass shift of 162 Da corresponding to glucose modification of the RhoA. The intact RhoA was observed by a linear MALDI-ToF as a singly charged ion at m/z 25 000 and as a doubly charged ion at m/z 12 500. The glucosylated form of the RhoA was observed at m/z 25 162 and at m/z 12 581, resp. The glucose-modified RhoA was observed after in-situ enrichment using affinity protein chips at the lowest concentration of 2.4 ng/mL of toxin B. Changes in the workflow and several modifications of the reaction buffer allowed to keep RhoA protein intact and possible to detect by MALDI-ToF after transferring this method to a real-life complex human stool isolate sample.

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REFERENCES:

1. *Di Bella, S.; Ascenzi, P.; Siarakas, S.; Petrosillo, N.; Di Masi, A. Clostridium difficile Toxins A and B: Insights into Pathogenic Properties and Extraintestinal Effects. Toxins 2016, 8, 134*
2. *Ashleigh S. Paparella, Sean M. Cahill, Briana L. Aboulache, and Vern L. Schramm ACS Chemical Biology 2022 17 (9), 2507-2518*
3. *Anal. Chem. 2016, 88, 17, 8526–8534 Publication Date:August 1, 2016*

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MoO-08: Investigation of human Hsc70 oligomerization

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Heat shock cognate protein 70 (Hsc70) is a 71 kDa chaperone protein belonging to the ubiquitous family of heat shock proteins 70 (Hsp70). The representatives of this protein family are considered as molecular machines with ATPase activity facilitating correct folding of spatial protein structure, both in normal and stressful conditions (hypoxia, heat shock, pH fluctuations etc.). In addition, Hsc70 was identified as an uncoating enzyme for triskelion meshwork on the surface of clathrin-coated vesicles. Among other roles, Hsc70 prevents protein aggregation and assists polypeptide maturation, it facilitates the protein transport into organelles, such as endoplasmic reticulum and mitochondria. It is involved in targeting proteins for lysosomal degradation and in many others important cellular processes related to protein homeostasis 1. Therefore, the regulation of Hsc70 and other Hsp70 proteins is believed to be highly important, especially in a context of cellular stress.

Based on the experimental observation, the mechanism of inactivation through oligomerization was hypothesized. The dimer and trimer species of Hsp70 proteins were identified both in case of prokaryotic and eukaryotic homologs 2,3. It was also speculated that Hsp40 cofactors promote oligomerization to even higher-order oligomers2. This and other possible oligomerization models of wild type HSC70 and the subset of HSC70 mutants were investigated by cross-linking mass spectrometry. The distance constraints imposed by different cross-linker lengths allowed to build structural models of Hsc70 oligomeric species. To decipher between intra and intermolecular cross-links, the studied protein and its mutants were produced in 15N-labeled form as well.

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REFERENCES:

1. *Liu, T., Daniels, C. K. & Cao, S. Comprehensive review on the HSC70 functions, interactions with related molecules and involvement in clinical diseases and therapeutic potential. Pharmacol. Ther. 136, 354–374 (2012).*
2. *King, C., Eisenberg, E. & Greene, L. Polymerization of 70-kDa Heat Shock Protein by Yeast DnaJ in ATP. J. Biol. Chem. 270, 22535–22540 (1995)*
3. *Sarbeng, E. B. et al. A Functional DnaK Dimer Is Essential for the Efficient Interaction with Hsp40 Heat Shock Protein. J. Biol. Chem. 290, 8849–8862 (2015).*

TuO-09: Unravelling Biological Sample Complexity With Advanced Mass Spectrometry

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Mass spectrometry has seen huge innovations over the last 30 years. Significant advancements in computing coupled with high-speed electronics has allowed huge strides in improvements in MS performance that have expanded the research capabilities of MS. Using hyphenated Mass Spectrometry techniques, we can determine what is in our samples and how much there is and even where analytes are.

Due to increasing sample complexity and the need for better quality data, high specificity and selectivity in the analysis method is required. Depending on the question being asked, different mass spectrometer platforms can be utilized to gain the required answer. In this presentation we will provide an overview of Waters Discovery Mass Spectrometry Platforms and explore how the latest innovations are being used to help scientist better characterize their samples.

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TuO-10: Multiple linear regression of mobilograms of isomeric new synthetic drugs analyzed by cyclic ion mobility

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Cyclic ion mobility uses traveling wave technology with a closed-loop separator, increasing the resolving power with a higher number of passes[1]. The higher-resolution ion mobility can provide the separation of protomers[2] or conformers. In this study, three isomeric pairs were analyzed by a SELECT SERIES Cyclic IMS (Waters Corporation, UK) in the form of $[M+H]^+$ and $[M+Cat]^+$ (Cat^+ ~ Na^+ or Li^+). For protonated molecules, a higher number of passes provided a wider isomer's peak due to the separation of protomers/conformers. Although the peaks overlapped, the individual isomer's contribution was successfully determined by applying Composite Spectrum Regression (CSR) (OriginPro 2019b, OriginLab). The coefficient of determination (COD) for $[M+H]^+$ was: 0.9718 and 0.9856 for 3-MMC/Buphedrone (7 passes) and 3-FMC/Flephedrone (10 passes), respectively. BDB and Methedrone significantly differed in their fragmentation which did not enable acquiring the appropriate ion signal intensity of $[M+H]^+$ for both isomers. For comparison, the ion mobility separation of $[M+Cat]^+$ was carried out too. Already in the 1 pass experiment, the pair BDB/Methedrone was baseline separated for both adducts (COD 0.9879 for ($[M+Li]^+$). Such separation needed 10 and 25 passes for 3-MMC/Buphedrone (0.9930, $[M+Na]^+$) and 3-FMC/4-FMC (0.9620, $[M+Li]^+$), respectively. The multiple linear regression of mobilograms provided results comparable to adducts' separation for 3-FMC/4-FMC and 3-MMC/Buphedrone. This approach can be useful if isomeric adducts are not separated or not generated with sufficient signal intensities. It is useful in distinguishing overlapped peaks.

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REFERENCES:

1. *Giles, K. et al. A Cyclic Ion Mobility-Mass Spectrometry System. Anal Chem 91, 8564–8573 (2019).*
2. *Lalli, P. M. et al. Protomers: Formation, separation and characterization via travelling wave ion mobility mass spectrometry. Journal of Mass Spectrometry 47, 712–719 (2012).*

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TuO-11: RNA-modifications analysis by hydrophilic interaction liquid chromatography-mass spectrometry

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The post-transcriptional modification of RNA on nucleotides is a well-known phenomenon. Recent advancements in next-generation sequencing and mass-spectrometry based analysis have identified a new class of RNA modifications on 5' end of RNA, termed RNA caps. These caps are typically derived from metabolites, cofactors, and dinucleotide polyphosphates [1,2]. Although their presence is believed to influence RNA stability, cellular metabolism, and mRNA translation, their exact role remains poorly understood. Currently, these molecules are analyzed by reversed-phase chromatography with ion-pairing agents, such as trialkylammonium salts. It is well established technique, however the presence of high salt concentration is suppressing the ionization and lowering the sensitivity of mass spectrometry detection. We have developed an alternative method, relying on HILIC (hydrophilic interaction chromatography), which does not need such strong ion-pairing agents and can be used even on longer oligonucleotides [3].
This work presents a qualitative and quantitative analysis of canonical and non-canonical 5'-RNA caps in bacteria and mammalian tissue cell cultures. The protocol was applied on investigation of NAD cap in RNA from HIV-infected MT4 cells, coenzyme A in bacteria, and hypermethylation of canonical cap in small nuclear RNA. The structural identification of these caps was validated based on retention time, m/z ratio and compared to commercial standards. In some cases, fragmentation spectra were acquired to confirm the identity of the caps. The results are currently used to elucidate the different biological roles of these caps.

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REFERENCES:

1. *Cahova H. et al.: Nature 519, 374-377 (2015)*
2. *Hudeček O. et al.: Nature Comm. 11, 1052 (2020)*
3. *Lobue P. et al.: J. Chromatogr. A. 1595, 39-48 (2019)*

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TuO-12: *Aspergillus fumigatus* and *Pseudomonas aeruginosa* interplay at a host interface

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Introduction: Invasive aspergillosis caused by the opportunistic pathogen *Aspergillus fumigatus* (Af) is responsible for 600,000 deaths in 2019 [1]. After entering the host, Af is confronted with stage-specific innate immune responses. Moreover, in immunocompromised individuals with respiratory disorders, Af co-exist with the bacterial pathogen *Pseudomonas aeruginosa* (Pa) leading to a worsened prognosis. Af secretes siderophores and mycotoxins to survive and defend inside host body. It is therefore, clinically significant to understand molecular interactions of activated secondary metabolism between pathogens and host cells to develop appropriate therapeutic strategies. Methods: For dual culture, Af and Pa were grown in an iron-limited medium and interaction was visualized using scanning electron microscopy. For Af-immune cell interaction, neutrophils were incubated with Af mycelia in RPMI medium. Siderophores were extracted using liquid-liquid whereas mycotoxins and human neutrophil peptides were extracted by solid phase extraction. All analytes were quantified by liquid chromatography and mass spectrometry. CycloBranch [2] and MassLynx 4.1 softwares (Waters) were used for data analysis.

Results: Co-existence of both microbes was observed in a co-culture. Af siderophore production suppressed from stationary phase of Pa growth. Conversely, Af siderophore secretion increased by 48h during interaction with neutrophils. Exposure of Af by neutrophils resulted in mycotoxins; gliotoxin and bis-methyl gliotoxin production. All three forms of human neutrophil peptide secretion increased in the presence of Af compared to control samples. Our results showed that Af siderophore and mycotoxin production is crucial during combat with Pa and neutrophils.

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REFERENCES:

1. *Dobiáš, R. and Havlíček, V., Microbial and natural macromolecules, pp. 57-72. Academic Press (2021).*
2. *Novak, J., et al.:Analytical Chemistry. 92(10), pp.6844-6849 (2020).*

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TuO-13: Drawing a better map: Optimizing LC-MS analysis of biologicals

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Newer, faster, and more efficacious strategies for peptide mapping and analysis of biologicals are needed to help bring vital and potentially life-saving therapeutics to market as quickly as possible. In this talk we will discuss new strategies for bottom-up analysis of therapeutic proteins using faster enzymes, new buffer systems, and optimal column chemistries that enable analysts to perform peptide mapping experiments faster, in as little as an afternoon, and with fewer artifacts.

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TuO-14: Application of the mass spectrometry to identify protein markers of Alzheimer's disease

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Alzheimer's disease is the most common dementia, affecting approximately 55 million people worldwide. It is estimated that around 2050 this number will likely exceed 150 million. Today, mutations in genes are known that predispose to Alzheimer's disease in 5 % of cases (familial Alzheimer's disease), but other cases are idiopathic. The most important genetic risk factor is the isoform of the lipid carrier apolipoprotein E, APOE4. There are various hypotheses for the origin of this disease. We focused on hypotheses related to neuroinflammation and dysregulation of lipid metabolism. Today's diagnostic methods are not suitable for early diagnosis of Alzheimer's disease and population screening. Positron emission tomography and markers in the cerebrospinal fluid are mainly used for diagnosis, but these are invasive methods. Therefore, markers detectable in the blood serum are needed. Based on the literature, we selected a panel of protein markers playing a role mainly in lipid metabolism or immune reactions relevant to Alzheimer's disease. We used blood serum and cerebrospinal fluid from patients and healthy individuals. We used ultra-high-performance liquid chromatography with mass spectrometry to quantify the panel of selected proteins.

Additionally, we measured proteins in cerebral organoids, cellular 3D models derived from human induced pluripotent stem cells, to determine the effect of apolipoprotein E genotype on the abundance of proteins characteristic of individual cell types and proteins related to Alzheimer's disease. The results can contribute to the investigation of the molecular mechanisms of the disease and help gradually reveal its principles by focusing on proteins with altered expression in certain stages of the disease. New protein markers could be used for early diagnosis of Alzheimer's disease.

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TuO-15: Photo-Methionine, Azidohomoalanine and Homopropargylglycine Have Distinct Effect on the Growth of Auxotrophic and Prototrophic *E. coli* in Minimal Medium, Alter Protein Expression Levels and Incorporate into Newly Synthesized Proteins at Different Rates

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Residue-specific incorporation of non-canonical amino acids (ncAAs) is a technique introducing bio-orthogonal functionalities into proteins that find applications in protein characterization and quantification. In this work, protein expression with three methionine analogues – photo-methionine (pMet), azidohomoalanine (Aha) and homopropargylglycine (Hpg) – in proto-trophic *E. coli* BL-21 and auxotrophic *E. coli* B834 was investigated, and the effect of the ncAAs on bacterial growth and expression of two recombinant proteins – cytochrome b_5 (b_5M46) and green fluorescence protein construct (MBP-GFP) – were assessed. ncAA incorporation achieved in the auxotrophic *E. coli* strain after 26-hour expression was 50-70% for pMet, and approximately 50% for Aha; with medium and low expression levels of MBP-GFP and b_5M46, respectively. In contrast to the auxotrophic strain, higher protein expression was achieved in the prototrophic host but with a sharp drop in the ncAA content after the first hours of expression. Similar protein expression levels and incorporation rates of 70-80% were achieved for both bacterial strains with Hpg. Our findings can provide guidance for expressing proteins with high content of the ncAAs, highlight the pitfalls when determining levels of methionine replacement by ncAAs using MALDI-TOF mass spectrometry, and point to a possible systematic bias in metabolic labelling techniques using Aha or Hpg.

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WeO-16: The benefits of trapped ion mobility for single-cell proteomics

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Thought to be an unachievable dream just a few years ago, it is now possible to achieve coverage of the proteome in a single cell from a human cancer cell line to depths of >4000 protein groups, sufficient to begin unraveling biological differences between cells. This talk will present details of a new mass spectrometer with the highest sensitivity to date for single cell proteomics and other proteomics applications requiring ultimate sensitivity such as immunopeptidomics and PTM analysis. The mass spectrometer includes a trapped ion mobility separation (TIMS) device in the front end and the advantages of this configuration for single cell proteomics will be discussed. The best currently achievable results will be presented as well as perspectives for future developments.

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WeO-17: Zapping ions – using powerful lasers for more informative mass spectrometry

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Ever since the deciphering of human genome, both structural biologists and mass spectrometrists working in the field of proteomics have realized, that there exists yet another level of complexity involved in the regulation and controlling of protein functions. Through splicing, mutations as well as post-translational modifications and involvement in larger non-covalent assemblies, individual proteins generate a complex landscape of so-called proteoforms with huge implications on their functioning in organisms. [1] Therefore, over the last decade or so proteomics has been aiming increasingly at the analysis of specific proteoforms through the means of top-down mass spectrometry, especially in its native implementation working with whole protein assemblies. However, this approach relies heavily on efficient and informative means of fragmentation for which diverse MS dissociation techniques and possibly their combinations are often sorely needed.

This contribution will report on the successful implementation of both 10.6 μm CO_2 and 193 nm ArF lasers for infrared multi-photon dissociation (IRMPD) and ultraviolet photodissociation (UVPD), respectively, for in-cell dissociation inside the 15T SolariX Fourier-transform ion cyclotron resonance (FT-ICR) mass spectrometer at BIOCEV in Prague. Their benefits as well as potential for collaborations will be showcased on examples of both proteins and smaller molecules. Also, future directions of research aimed at native mass spectrometry of large non-covalent protein assemblies will be discussed.

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REFERENCES:

1. *Smith, L.M. & Kelleher, N.L. Nat Methods 10, 186–187 (2013).*

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WeO-18: Fast photochemical oxidation of nucleic acids coupled to high-resolution MS analysis

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Recent years have seen significant growth in the methods of structural proteomics, which have had had a significant impact in the field of structural and molecular biology. These methods may address questions related to structure and dynamics of protein and protein complexes, making them highly favorable for studying protein-DNA interactions. One specific method, radical covalent labelling, has emerged as an effective analytical tool for characterization of biomolecules. Fast Photochemical Oxidation of Proteins (FPOP), the most common radical labelling method, is now exclusively employed for mapping the structure and interaction of proteins. However, in the early stages of radical chemistry, it was primarily utilized for studying structure and interaction of nucleic acid with transcription factor.

In this study, we applied FPOP oxidation to investigate the interaction between a double-stranded nucleic acid known as a Insulin Response Element (IRE) and DNA binding domain of FOXO4 transcription factor. The IRE in the absence and the presence of FOXO4 was subjected to fragmentation/oxidation in the FPOP platform. Residual protein was digested using Proteinase K, and the resulting DNA fragments were analyzed using high-resolution 15T-FT-ICR mass spectrometry. The study also emphasizes the analytical aspects and highlights the benefits and drawbacks associated with analyzing DNA fragments throughout the experimental process, including FPOP oxidation, LC-MS analysis, and data analysis.

Analysis of separated IRE fragments revealed that hydroxyl radicals cleave the DNA nonspecifically, creating a set of all possible 3'OH, 3'P, 5'OH and 5'P terminal fragment ions. Complementary fragment ions were found in the LC-MS trace and subsequently quantified. Comparison of IRE fragment ions revealed a significant protective effect around the bingind sequence in the major groove of DNA, also lower protection in the minor groove.

Obtaining detailed information about solvent accessibility of IRE might enable *ab initio* design of FOXO4/IRE structural model. This is potentially valuable because the corresponding crystal structure is currently unclear.

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WeO-19: Internal standard normalization in MALDI mass spectrometry imaging

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Mass spectrometry generally suffers from the strong influence of an analyzed matrix and its impact on the ionization process, affecting a targeted compound's yield. This matrix effect is highlighted in the matrix-assisted laser desorption/ionization (MALDI) mass spectrometry imaging, where two key factors must be considered. The first comes from the principle of ionization, in which the matrix has to be co-crystalized with analytes and ensure efficient ionization. The second is the heterogeneity and the integrity of a sample, usually a tissue section, causing enhancement or suppression of the ionization. Thus, MALDI MSI is recognized as a qualitative method where an internal standard, which would compensate for the matrix effects, is absent. We developed an internal standardization approach to extend a possible use of MALDI MSI to quantitative analysis. With the use of ^{13}C labeled glucose, ^{13}C labeled glutamic acid, ^{15}N labeled adenosine monophosphate, and ^{13}C labeled and species-specific peptides, a broad range of analytes, including saccharides, amino acids, nucleosides, and peptides can be precisely and spatially mapped to decipher region-specific tissue metabolomic processes. The presented approach was used in the *Escherichia coli*-induced neuroinfection model and opened new opportunities for relative and absolute quantitation.

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MoS-1: A Comprehensive Tool for State-of-the-Art Pre-processing Analysis in Metabolomics included in updated App: COVAIN v2.0.0

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In mass spectrometry (MS)-based metabolomics data, effective data preprocessing plays a crucial role in enabling advanced analysis and facilitating meaningful insights for comparative studies. With this objective in mind, our primary focus was on enhancing and implementing a cutting-edge preprocessing method in our newly developed application, COVAIN v2.0.0, for advanced metabolomics data processing.

Our goal was to provide users with a streamlined and user-friendly environment that allows for intuitive data processing, offering various options to generate comprehensive results for final assessment. The preprocessing section alone encompasses a wide range of functionalities, including different imputation methods for handling missing values such as zero, half minimum (HM), mean, median, random forest (RF), singular value decomposition (SVD), k-nearest neighbors (kNN), and quantile regression imputation of left-censored data (QRILC).

Furthermore, subsequent preprocessing steps include outlier adjustment, where default options are provided to reduce outliers based on the winsorization transformation technique. Lastly, the transformation step offers multiple normalization options, including log(), ln(), and Z-score transformations. Additionally, this section incorporates the calculation of the ANOVA test for group selection, along with the ability to adjust the p-value.

Overall, our application, COVAIN v2.0.0, strives to deliver an advanced and user-friendly solution for metabolomics data processing, ensuring robust preprocessing techniques and a comprehensive suite of analysis tools.

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MoS-2: Insights into the transport dynamics of prokaryotic chloride/proton antiporter

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Chloride channel family includes transmembrane channels and transporters, which are present in both eukaryotic and prokaryotic organisms. They are involved in many cellular processes and their mutation can cause serious illnesses. To treat these diseases, description of their transport mechanism is needed. Bacterial chloride transporter CLC-ec1 from E. coli is often used as model protein to study protein family of chloride channels. It functions as an antiporter of a single proton for two chloride anions. During transport of ions, the protein undergoes conformational changes that convert inward and outward-facing conformation. 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 to 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 over-expressed in bacteria, isolated via detergent solubilization and purified by affinity chromatography and gel filtration. Next, HDX-MS experiment was conducted at different conditions. First, we compared WT and QQQ proteins to address the role of protonation. Both proteins were followed at four pH values spanning pH range 4.4-7.4. The deuteration rate of WT protein at pH 4.4 did not completely match the deuteration of the QQQ protein, we decided to run an experiment at even lower pH 3. These data are highlighting the step-wise protonation and the associated structural changes across the ion transport path.

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REFERENCES:

1. *T. S. Chavan et al.," Elife, vol. 9, pp. 1–30, 2020, doi: 10.7554/eLife.53479.*

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MoS-3: Quantitative Cross-linking Mass Spectrometry Using Data-Independent Acquisition

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Chemical cross-linking in combination with mass spectrometry (CXMS) has been developed into a powerful tool for mapping interaction networks and three-dimensional structures of proteins and their complexes. However, proteins are intrinsically dynamic, and they can form different conformations. Adding quantitative information to CXMS offers a unique opportunity to study flexibility and structural rearrangement of proteins. In this study, we report the benefits of utilizing data-independent acquisition and novel urea-based isotopically labeled cross-linkers.

First, we used different mixtures (9:1, 1:1 and 1:9) of MS-cleavable DSPU and its isotopically labeled analogue to modify model proteins (BSA, BCA) testing quantitative potential of our strategy. Subsequently holo and apo forms of calmodulin and myoglobin were modified by isotopically labeled and non-labeled reagents to quantify the structural rearrangement upon calcium or heme binding, respectively. Both, the cross-link formation and quantification were performed in single data-independent experiment where tryptic peptides were measured in the broad-band mode with mass accuracy below 1 ppm and subsequently fragmented without isolating precursor ions at fixed collision energy resulting in sub-ppm accuracy for fragment ions as well. The raw data were converted into hybrid mgf file which was interpreted by MEROX search engine.

Acquired data on model proteins clearly demonstrate the potential of our quantitative cross-linking strategy. The high mass accuracy in MS1 and MS2 modes enables unambiguous identification of cross-linked peptides. Moreover, the quantitative information is not derived from the MS1 experiment only. Due to the presence of isotopically labelled reporter ions in MS/MS spectra, it is possible to improve the qualitative and quantitative aspects of quantitative cross-linking experiments. Observed changes nicely overlap with high resolution structural models and previously published data. Our results lead to an assumption that presented data-independent acquisition method can be utilized for quantitative cross-linking experiments studying structure and dynamics of proteins and protein assemblies in solution.

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MoS-4: Fast FluoroAlkylation of Proteins (FFAP): A Novel Cross-linking Strategy for Aromatic Residues

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Chemical cross-linking in combination with mass spectrometry (CXMS) has been developed into a powerful method for mapping protein structures, dynamics and interaction networks including molecular interfaces in protein-protein and protein-nucleic acid complexes. Although many cross-linkers have been developed in last two decades, majority of CXMS analyses still utilizes lysine-specific cross-linking reagents based on N-hydroxysuccinimide esters. Other cross-linking reagents have only limited use for various reasons such as low reactivity, low occurrence of targeted residues, unwanted side products etc. In this study we show new generation of cross-linkers based on recently published Fast FluoroAlkylation of Proteins (FFAP) technology that enables targeting of aromatic amino acids residues.

Cross-linking reaction is based on two step FFAP mechanism which includes activation of hypervalent iodine using metal ions or lewis acid and subsequent attack of aromatic residues by the resulting radical. Since the induction of fluoroalkyl radicals is triggered by ascorbic acid and the labeling pulse is stopped by tryptophan, it enables to perform protein labeling experiments in quench flow system in short time range (3s). The studied proteins were analyzed by bottom up approach using high resolution mass spectrometry (solariX XR 15T, Bruker Daltonics) where samples were digested by trypsin protease, separated on reverse phase column online coupled to mass spectrometer.

The results on several protein models such as horse heart myoglobin complex clearly demonstrate the potential of our novel cross-linking strategy. The cross-links of aromatic residues generated by fluoroalkyl radicals nicely correspond with protein crystal structures of studied protein and provide information not attainable by lysine-specific cross-links. Our data lead to assumption the cross-linker based on fluoroalkyl radicals could be use as fast and powerful method to study protein structure and dynamics in solution.

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MoP-01: Sulfated phenolic acids are common plant metabolites

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Sulfated metabolites are mainly typical for marine organisms, including seagrasses (e.g. zosteric acid), algae (e.g. sulfated phenolic acids and sulfated polysaccharides), invertebrates (e.g. sulfated steroids) and sponges (e.g. psammaplin A sulfate). On the contrary, plant sulfated metabolites, represented by e.g. glucosinolates, sulfated flavonoids and phytosulfokines, are much less common and explored. Here, we report a novel group of plant sulfated compounds, sulfated phenolic acids, that were originally detected only in algae and seagresses.
The untargeted UHPLC-QTOF-MS metabolomic analysis of more than 50 methanolic extracts from plants, fungi and algae was performed. Based on the detection of the neutral loss of sulfur trioxide (monoisotopic mass 79.9568 Da) in MS/MS spectra, we identified ferulic acid 4-sulfate, vanillic acid 4-sulfate, 4-(sulfooxy)benzoic acid and 4-(sulfooxy)phenylacetic acid in plants for the first time. In addition, we detected zosteric acid in land plants. These compounds were quantified in several edible species, including oat, wheat, barley, tomato, carrot, broccoli, celery, cabbage, banana, pineapple, radish and olive oil. While concentrations of sulfated phenolic acids ranged from 0.34 - 22.18 μg/g DW, the corresponding non-sulfated acids were mostly undetected or present at lower concentrations.

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MoP-02: Development of sample pretreatment methods for the analysis of selected growth factors in various biological fluids by CZE-MS

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Growth factors are a heterogeneous group of proteins that are secreted by various tissues in the human body. Their presence in biological matrices may indicate the development of multiple diseases, thus there is a growing need to develop new high-performance analytical methods and procedures for their reliable identification and quantification. However, these substances are present in biological fluids at very low concentration levels compared to other high-abundant proteins that can mask the presence of growth factors and make their analysis impossible. This work aims to develop a sample pretreatment method for the purification of complex biological samples before their analysis by online hyphenation of capillary zone electrophoresis and mass spectrometry (CZE-MS). Optimization and comparison of different conditions for solid phase extraction and protein precipitation to reach the compatibility with the CZE-MS method and online sample preconcentration was performed on biological fluids (urine, plasma and serum) fortified with a mixture 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).

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MoP-03: Optimisation of infliximab middle-up analysis by capillary electrophoresis - mass spectrometry

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Infliximab (IFX) is a chimeric monoclonal antibody (mAb) widely used for treatment of multiple types of diseases. IFX is a 150 kDa protein consisting of heavy and light chain subunits of molecular weight approximately 50 kDa and 25 kDa, respectively. Middle-up mass spectrometry (MS) mAb analysis has gained popularity over the last years as an alternative to a full protein enzymatic digestion [1]. Capillary electrophoresis (CE) is a high-resolution separation electromigration technique that was shown to be advantageous for separation of proteins [2]. In this work, two reducing conditions were investigated and compared while the parameters for CE-MS analysis of IFX reference standard were optimised. A range of concentrations of dithiothreitol (DTT) and tris(2-carboxyethyl)phosphine hydrochloride (TCEP) being 50 - 500 mM and 50 μM – 1 mM, respectively, were tested for the IFX reduction. The analysis of reduced IFX was performed on a 6520 qTOF mass spectrometer (Agilent, Santa Clara, US). CE experiments, focused on optimisation of IFX middle-up analysis, were carried out on a CE 7100 instrument coupled to a 6410 triple-quadrupole mass spectrometer (both Agilent, Santa Clara, US). Background electrolyte (BGE) systems (including formic and acetic acids) were tested and the optimal results were achieved by using 1 M acetic acid. Both reducing agents (with appropriate concentration) were shown as suitable and heavy and light chain subunits were observed in the mass spectra. Generally, a difference in charge state distribution of both subunits was observed for the two of the investigated reducing agents. Middle-up CE-MS analysis was capable of detecting mAb light chain in single ion monitoring and scan modes, showing a promise for the future mAb CE-MS analyses.

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REFERENCES:

1. *Lermyte F. et al. J. Am. Soc. Mass Spectrom., 30(7), 1149–1157 (2019)*
2. *Chen D. et al. Mass Spectrom. Rev., 42(2), 617–642 (2023)*

ACKNOWLEDGEMENT:

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MoP-04: Development of modern analytical methods for the characterization of keyhole limpet hemocyanin (KLH) as immunogenic carrier protein in biopharmaceuticals

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Keyhole limpet hemocyanin (KLH) is a large glycoprotein composed of KLH1 and KLH2 subunits, each of which has a molecular weight in the range of 400 kDa. The aforementioned subunits then assemble into decameric (4 million Da), di-decameric, and multi-decameric structures of enormous sizes [1].

Its antigenic properties cause strong antibody responses. Therefore, KLH is widely used in the field of immunobiology. The conjugation of nonimmunogenic peptides or other haptens with KLH results in highly immunogenic preparations. KLH has high potential in the treatment of various types of cancer. It can be used alone in the treatment of bladder cancer or as a carrier in vaccines against non-Hodgkin lymphoma, skin melanoma, or breast cancer. Similarly, KLH can be conjugated to HIV or HPV-4 peptides or proteins for potential AIDS therapy and prevention of HPV-related diseases [2].

The aim of the present study was to develop sufficient and reliable analytical methods for the assessment of KLH structure. Comprehensive characterization was achieved using three approaches: size exclusion chromatography, peptide mapping (LC-MS), and intact protein analysis (MS – Q-TOF). Moreover, specific KLH peptides (characteristic for each KLH subunit) were selected for perspective quantitative approach based on targeted LC-MS analysis.

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REFERENCES:

1. *Swerdlow R. et al.: Keyhole limpet hemocyanin: Structural and functional characterization of two different subunits and multimers, Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology. 113(3), 537–548 (1996).*
2. *Pichichero M.: Protein carriers of conjugate vaccines, Human Vaccines & Immunotherapeutics. 9(12), 2505–2523 (2013).*

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MoP-05: An application of UPLC-QQQ MS to characterize changes in free cholesterol and cholesterol esters in lung cells and lung tissue after mice exposure to metal oxide nanoparticles

Markéta Laštovičková [1 *], Daniela Kristeková [2], Kamil Křůmal [1], Pavel Coufalík [1], Lukáš Alexa [1], Marcela Buchtová [2], Pavel Mikuška [1]

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Cholesterol is essential compound for normal cell function. It is a component of cell membranes and a precursor of steroid hormones and other key metabolites. In animal tissues, cholesterol occurs in free form (free cholesterol, FC), esterified to long-chain fatty acids (cholesterol esters, CEs) or bound in lipoproteins. CEs are the preferred form for transport of cholesterol in plasma and as a biologically inert storage form to compensate for the excess. Abnormal cholesterol levels have been observed in a variety of diseases. Thus, quantitative or qualitative changes in various forms of cholesterol can be used to monitor the progress of treatment or as useful biomarkers revealing the molecular mechanism of disease.

The lead oxide nanoparticles (PbO NPs) are emitted into the environment during high-temperature and other technological processes. The inhalation of PbO NPs severely damaged target organs of exposed mice. These nanoparticles crossed the lung barrier and were distributed through the blood to secondary target organs where they caused numerous pathological changes. Our optimized LC-MS method, including reverse-phase UPLC combined with triple quadrupole mass spectrometry (UHPLC-QQQ-MS), was used to compare the profile of lipids extracted from a control lung cell line (and lung tissue from control mice) compared to lung cells and tissue from mice exposed to elevated concentrations of airborne PbO NPs. Preliminary results showed that the levels of selected CEs (e.g. CE 18:1; CE 18:2, CE 22:6, CE 24:5; CE 24:6) in lung cells were increased after exposure to PbO NPs. Our study revealed a association of alteration of cholesterol metabolism after exposure to metal NPs, which is the subject of our further detailed study.

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MoP-06: Monitoring of conformational changes in transmembrane proteins using HDX and FFAP radical labeling

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The chloride channel family is divided into two groups: channels and antiporters. Channels are known for their large conformational changes of subunits during ion transport. In contrast, antiporters have only tiny movements near the transport pathway. Transporters are involved in many cellular processes, and their mutation can cause serious diseases [1]. The CLC-ec1 is a Cl^-/H^+ antiporter of a single proton for two chloride ions from *Escherichia coli*. Ion transport is supposed to be accompanied by a structural change between inward and outward-facing conformation, but this transition has not been captured so far. During the transport cycle, the protonation of key Glu residues should induce an outward-facing state. This was mimicked through a mutation of three Glu residues for Gln[3]. We used this mutation to reveal the role of protonation in the ion transport mechanism of CLC-ec1. CLC-ec1 and CLC-QQQ mutant were expressed in the *E. coli* system and purified by IMAC chromatography in ŋ-decyl maltoside as a solubilization reagent[1]. Solubilized protein was transferred to the saposin nanodisc by size exclusion chromatography. We then used hydrogen/deuterium exchange and radical labeling with Togni reagent (FFAP) coupled to high-resolution mass spectrometry to capture the structural changes alongside the backbone as well as on the side chains of aromatic residues The optimization of the digest workflow for both methods provided full sequence coverage with reasonable lengths of the peptides. As the best conditions for the HDX we determined online digest on the column of co-immobilized pepsin-nepentesin 2 whereas for FFAP we got better sequence coverage by offline digest via cyanogen bromide and trypsin. HDX and FFAP were subsequently used for the study of the structural differences between CLC-ec1 and the CLC-QQQ mutant at pH 7.4. To prove the role of protonation, a comparison experiment at four different pH levels, from 4.4 to pH 7.4, was performed for both proteins. Results showed a critical connection between protonation and structural changes in the ion transport path and conformational change in the transmembrane helixes.

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REFERENCES:

1. *S. M. Elvington et al., EMBO J., vol. 28, no. 20, pp. 3090–3102, 2009*
2. *R. Dutzler et al., Nature, vol. 415, no. 6869, pp. 287–294, 2002*
3. *T. S. Chavan et al., Elife, vol. 9, pp. 1–30, 2020*

MoP-07: The ion mobility/mass spectrometry method for analysis of phosphorylated tau peptides in cerebrospinal fluid

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A variety of neurodegenerative disorders, including Alzheimer´s disease (AD), are associated with neurofibrillary tangles composed of tau protein in the form of toxic tau oligomers. Aberrant tau phosphorylation is a hallmark in many tauopathies, and hyperphosphorylation promotes the formation of paired helical filaments which are the main constituents of neurofibrillary tangles in the brain. While cerebrospinal fluid levels of total tau and tau phosphorylated at threonine residue 181 are established core biomarkers for AD, the values of alternative phosphorylation sites, which may have more direct relevance to pathology, for early diagnosis are not yet known. To characterize the specific phosphorylation of tau peptides in CSF, we have applied an innovative mass spectrometry workflow. Using ion mobility separation, we were enabled to provide valuable structural information and separate different isobaric tau peptides. This is the most critical step for the development of ultra-sensitive immunoassays allowing the quantification of novel biomarkers at sub-picogram levels.

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MoP-08: LC-MS analysis of internal DNA modification-method development

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Methylation of nucleic acids can play a crucial role in biological processes including regulation of gene expression. The most common DNA modifications are methylations of adenosine and cytosine such as 5-methyldeoxycytosine, 5-hydroxymethyl-deoxycytosine, N4-methyl-deoxycytosine and N6-methyldeoxyadenine (m6dA). [1] These modifications are mostly detected by LC-MS in the form of nucleosides with reverse phase chromatography as a separation method. This protocol first requires an enzymatic digestion by Nuclease P1 (NUP1) followed by dephosphorylation using alkaline phosphatase (CIP). [2]

Our aim was to simplify the sample preparation and to increase the sensitivity of the method. The optimized protocol includes only one enzymatic digestion of DNA by NUP1 followed by hydrophilic interaction chromatography (HILIC). The modifications are analysed in the form of nucleoside monophosphates by UHPLC (Acquity H-class, Waters) equipped with BEH Amide HILIC column coupled to high resolution mass spectrometer (Xevo G2-XS qTOF, Waters). The developed method was used to detect and quantify the most widespread internal modification on DNA, m6dA. Moreover, we also studied the changes of dAMP methylation in *Bacillus subitilis* under different growth conditions.

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REFERENCES:

1. *Bilyard M.K. et al.:Curr. Opin. Chem. Biol. 57, 1-7 (2020)*
2. *Schiffers S. et al.:Angew. Chem. Int. Ed. 56(37), 11268-11271 (2017)*

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MoP-09: Analysis of Insulin-like Growth Factor 1 in Pharmaceutical Preparations by On-line Capillary Electrophoresis-Mass Spectrometry

Radovan Tomašovský [1,2 *], Martina Opetová [1,2], Jana Havlikova [1,2], Peter Mikus [1,2], Katarína Maráková [1,2]

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Insulin-like growth factor-1 (IGF-1) is a 70-amino acid single-chain polypeptide which has found application in diagnostics as a biomarker of growth hormone disorders and as a therapy for growth failure in children and adolescents. Due to its strong anabolic effects, it is often abused by athletes for doping purposes in various pharmaceutical forms, including tablets, capsules, and injectable solutions. Here, we developed on-line hyphenated method based on capillary zone electrophoresis and triple quadrupole mass spectrometry detection with electrospray ionization for the determination of IGF-1 in pharmaceutical matrices. We achieved an efficient separation and well-shaped peaks with favorable migration times (<15 min) by optimizing the background electrolyte (500 mM formic acid + 5% acetonitrile, pH 1.96) and a separation voltage (+30 kV). Highly efficient, accurate, repeatable, sensitive (sub µg/mL levels), and selective analysis of IGF-1 was achieved with further optimization of ESI and MS parameters. Optimized and validated CZE-ESI-MS method was successfully applied for the determination of IGF-1 in injectable solutions (Increlex®) and its presence was also confirmed in various nutritional preparations.
This is the first work that uses validated CZE-ESI-MS method for the determination of IGF-1 in pharmaceutical matrices and reveals the potential of capillary electrophoresis for its use in drug quality control laboratories with benefits such as high separation efficiency, high-speed analysis, low sample consumption, as well as environmental and cost aspects compared to the more established liquid chromatography methods.

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MoP-10: The effect of domain order on modulation of fusion two-domain proteins structure and its dynamics studied by hydrogen-deuterium exchange

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INTRODUCTION

Is the function of the multidomain proteins only determined by the existence of domains that are embedded in the sequence or the position and order of the domains matters? In such protein, hypothetically, each domain structure or function could be influenced by close neighbours. To address this hypothesis, we used hydrogen deuterium exchange structural method (HDX-MS) to investigate possible perturbations in structure of PDZ3 domain of Zonula occludens 1 protein (PDZ3). This domain has a natural peptide ligand with known binding site. We took PDZ3 domain and fused with TrpCage, in silico designed artificial domain, in both forward (PDZ3-TrpCage: PsLT) and reverse (TrpCage-PDZ3: TlLP) version of the sequence. Our aim was to determine peptide regions directly influenced by TrpCage position and find the differences between PsLT and TlLP proteins.

METHODS

We applied HDX analysis in a traditional HDX set-up and also in set-up with low temperature and short labelling times to characterize the deuterium accessibility of PDZ3 peptides and regions. Subsequently, we performed the experiments with peptide ligand (JAMAP6),

RESULTS

The data shows that, in general, TrpCage destabilizes the PDZ3 structure. The peptide localiation also shows that some of the PDZ3 peptides with different behaviour in PsLT compared to TlLP were localized in ligand binding site suggesting that TrpCage affects also function of PDZ3. The results of the ligand binding experiments, surprisingly, counteract the effect of TrpCage more effectively in TlLP compared to PsLT. According to our results, we suggest the methodology to study the domain structural differences in the context of other domains using HDX-MS.

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MoP-11: Simple UHPLC-MS amino acid analysis for determination of immunogenic proteins in biopharmaceuticals

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We developed a modern and progressive analytical approach for the determination of innovative protein therapeutics present in biopharmaceuticals, based on the highly sensitive and selective amino acid analysis performed by reversed phase ultra high-performance liquid chromatography (UHPLC) and single quadrupole mass spectrometry. Such analytical approach is accompanied with the use of fast and effective targeted amino acids derivatization procedure which enables to enhance the sensitivity and selectivity of the analysis and also to ensure appropriate retention properties of the analytes in reversed phase chromatographic separation mode. Moreover, the on-line connection of ultra high-performance liquid chromatography with single quadrupole mass spectrometer enabled an unambiguous identification of amino acids derivatives and their exact quantification. The proposed method was successfully validated and applied in the field of quality control of modern therapeutic proteins present in biopharmaceuticals. The UHPLC-MS can be advantageously used as routine method in quality control laboratories, and with promising potentialities also for other and/or more complex matrices.

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MoP-12: Single particle inductively coupled plasma mass spectrometry imaging of immunochemically labeled spheroid sections

Vilém Svojanovský [1], Jan Preisler [1 *], Marek Stiborek [1], Viktor Kanický [1], Jiří Kroupa [2]

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Spheroids are one of the 3D biological models in which the arrangement of proliferating and necrotic cells resembles the tumor microenvironment. One of the markers of proliferation is Ki-67 protein, which is expressed by cells in the active phase of the cell cycle. Immunohistochemical labeling with a primary antibody and a fluorophore-labeled secondary antibody followed by fluorescence microscopy analysis is commonly employed for two- and three-dimensional imaging of the target protein in spheroid sections. We have successfully switched from fluorophore labeling of Ki-67 to nanoparticle labeling combined with LA ICP MS. Recently, gold nanoparticles were used as very sensitive immunochemical tags in conventional UV laser ablation ICP MSI.[1,2] In our approach, intact 20 nm gold nanoparticles tags were desorbed from the spheroid section with 2940 nm laser ablation system.[3]

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REFERENCES:

1. *Cruz-Alonso, María, et al., Microchimica Acta 185 (2018): 1-9*
2. *Tvrdonova, Michaela, et al., Analytical and bioanalytical chemistry 411 (2019): 559-564*
3. *Stiborek, Marek, et al., Analytical Chemistry (2022)*

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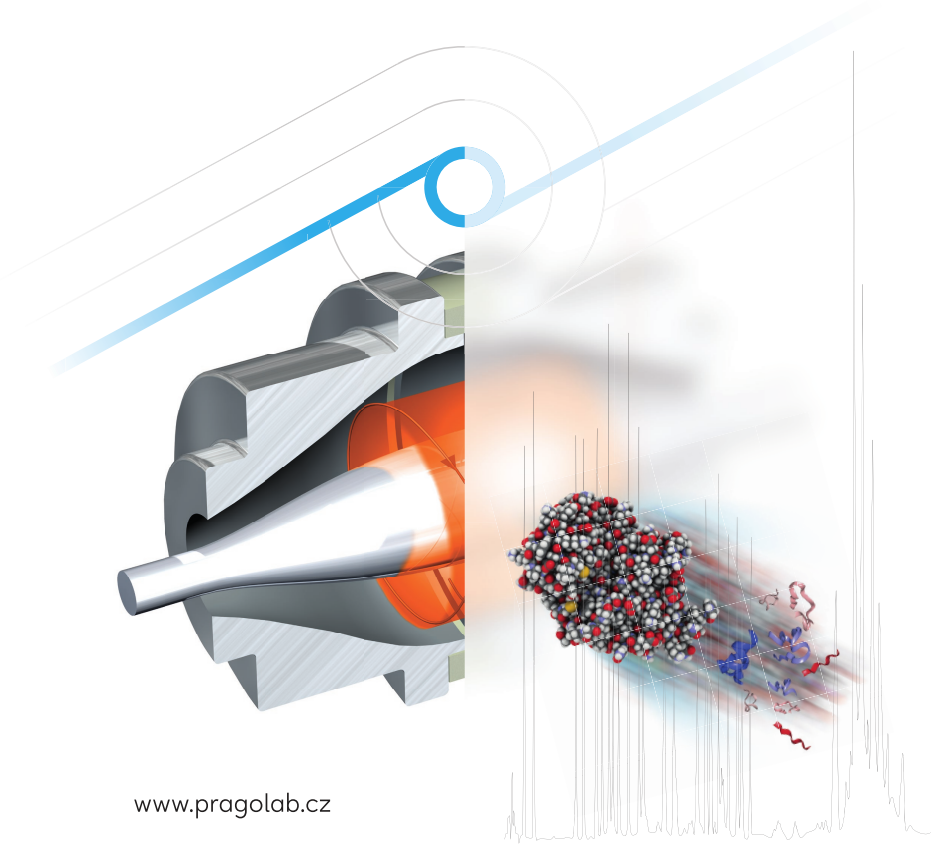

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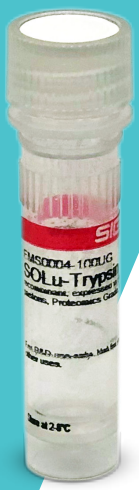

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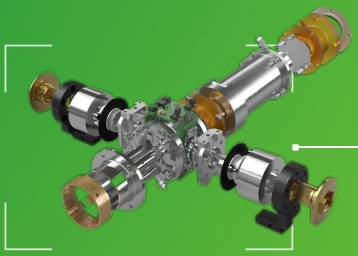

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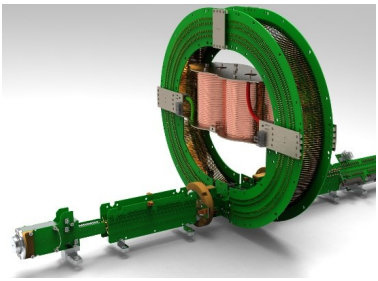

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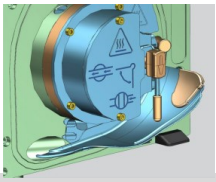

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Altiur

Agilent Bravo robot pro metabolomiku a lipidomiku. Obsluhu zvládne opravdu každý člen týmu!

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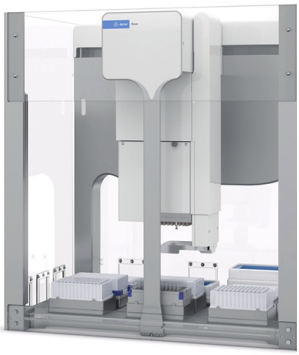

- **Jednoduchý a kompaktní robot** pro automatizaci metabolické a lipidomické přípravy vzorku.
- Práce s **jamkovými destičkami** (formát 96, 384, 1536, deep wellpates) a s **1 ml zkumavkami na vzorky.**
- Optimalizovaný **workflow pro analýzu metabolitů v plazmě** pomocí precipitace proteinů a deplece lipidů na EMR lipid SPE.
- **Snadné ovládání** pomocí software VWorks s hotovými nebo uživatelsky nastavenými postupy.
- Možnost využití také pro **proteomickou a genomickou** přípravu vzorku.

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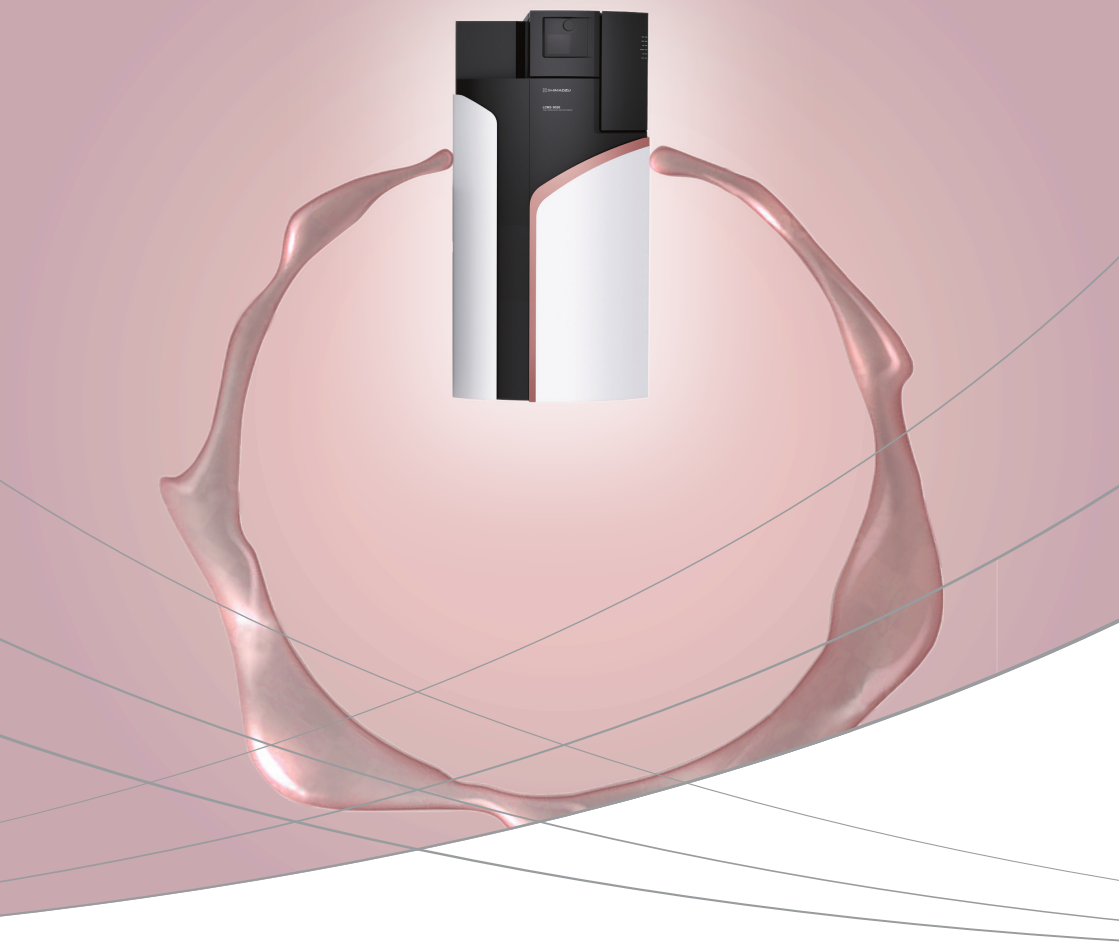

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