NINTH ANNUAL CONFERENCE OF THE CZECH SOCIETY FOR MASS SPECTROMETRY

Prague, October 11 – October 12, 2021

BOOK OF ABSTRACTS

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

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Ninth Annual Conference of the Czech Society for Mass Spectrometry

Date

11th October – 12th October 2021

Venue

CTU in Prague, SÚZ- congress department

Thákurova 1

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Czech Republic

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Monday 11th October 2021

8:00 – 9:30	Registration
9:30 – 9:40	Conference opening
9:40 – 10:30	**Plenary lecture I.: Zdeněk Lánský** (Chairperson: Petr Novák)
PL-1	*Tau regulates access to microtubules*
10:30 – 10:50	Coffee break
10:50 – 11:35	Company Workshop – Pragolab s. r. o. *Lukáš Plaček, Michaela Ščigelová, Lars Kristensen a Zuzana Snížková* *Lars Kristensen, Thermo Fisher Scientific: The Vanquish Neo UHPLC system sets new performance standards and provides ultimate flexibility for proteomics applications* *Michaela Ščigelová, Thermo Fisher Scientific: Global Proteome Quantification Using Internal Standard Triggered Targeted Analyses*
11:35 – 12:55	**Session I.** *(Chairperson: Martin Hubálek)*
11:35 – 11:55	Zdeněk Spáčil
MoO-01	*Advances in mass spectrometry-based metabolomics and quantitative proteomics*
11:55 – 12:15	Katarína Molnárová
MoO-02	*Analysis of glycopeptides using liquid chromatography-mass spectrometry*
12:15 – 12:35	Siddharth Jadeja
MoO-03	*The use of mobile phase with a low formic acid concentration in LC-MS proteomic analysis leads to enhanced electrospray ionization and improved sensitivity*
12:35 – 12:55	Markéta Nezvedová
MoO-04	*Profiling cell-specific, developmental and neurodegenerative protein markers in cerebral organoids*
12:55 – 14:00	Lunch
14:00 – 15:20	**Session II.** *(Chairperson: Petr Fryčák)*

14:00 – 14:20	Anton Škríba
MoO-05	*Bacterial virulence factors as an infection biomarkers*
14:20 – 14:40	Aleš Kvasnička
MoO-06	*Alternative biofluids and novel microsampling techniques for applications in clinical analysis*
14:40 – 15:00	Rutuja Patil
MoO-07	*Extracellular secretome in polarized Aspergillus fumigatus growth triggers the invasive aspergillosis in a host*
15:00 – 15:20	Jiří Houšť
MoO-08	*Aspergillus fumigatus: from the iron acquisition strategies to the siderophore-based monitoring of invasive pulmonary aspergillosis*
15:20 – 15:40	Coffee break
15:40 – 16:25	Company Workshop – Merck s. r. o.
	Ing. Jan Havlíček, RNDr. Martin Šušla, PhD: Milli-Q® – Ultrapure Water for Demanding Instrumental Analyzes
16:25 – 17:20	Poster talks I (Chairperson: Petr Man)
MoS-01	*Jana Jaklová Dytrtová: Copper(II) Interactions with Triazoles under ESI Conditions*
MoS-02	*Adéla Pravdová: Analysis of lipoprotein samples: preparation, LC-MS optimization, data processing*
MoS-03	*Jakub Červenka: Human neural stem cell proteomics – from basic towards translational research*
MoS-04	*Hynek Mácha: Increased level of norepinephrine, epinephrine, and α-tocopherol following neonatal brain hypoxic-ischemic injury in rats analyzed by mass spectrometry imaging*
MoS-05	*Roman Tuzhilkin: Azurin: a model metalloprotein to study an electron transfer process*
17:20 – 22:00	Poster party

Tuesday 12th October, 2021

9:00 – 10:40	**Session III.**
	(Chairperson: David Friedecký)
9:00 – 9:20	Darshak Gadara
TuO-01	*Systematic Feature Filtering in Untargeted LC-MS Metabolomics: Application towards Biomarker Discovery*
9:20 – 9:40	Durga Jha
TuO-02	*Characterization of membrane lipids in cerebral organoids*
9:40 – 10:00	Petra Švecová
TuO-03	*Ultra-trace determination of oxaliplatin and its compendial impurities by sweeping-MEKC-ICP-MS*
10:00 – 10:20	Barbora Piskláková
TuO-04	*Diagnosis of inherited metabolic disorders by LC-MS/MS*
10:20 – 10:40	Dominika Luptáková
TuO-05	*Neuropharmacokinetic Visualization of Regional and Subregional Unbound Drug Transport Across Blood-Brain Barrier Enabled by Quantitative Mass Spectrometry Imaging*
10:40 – 11:00	Coffee break
11:00 – 11:45	Company workshop – HPST s. r. o.
	Jitka Zrostlíková, HPST: Agilent workflow for lipidomics using LC/MS QTOF
	Karel Chalupsky et. al. , Czech Centre for Phenogenomics, IMG, AV ČR: Hyperbilirubinemia Is Associated with Changes in Cholesterol Metabolism and Fat Breakdown
11:45 – 13:00	Votes
13:00 – 14:00	Lunch
14:00 – 15:20	**Session III.**
	(Chairperson: Petr Pompach)
14:00 – 14:20	Dmitry Loginov
TuO-06	*Hydroxyl radical footprinting analysis of a human haptoglobin-hemoglobin complex*

14:20 – 14:40	Lukáš Fojtík
TuO-07	*Fast Fluoroalkylation of Proteins Uncovers Structure and Dynamics of Biological Macromolecules*
14:40 – 15:00	Tereza Kadavá
TuO-08	*Variable-Temperature nESI analysis of protein-DNA complexes*
15:00 – 15:20	Adam Pruška
TuO-09	*Temperature-controlled Mass Spectrometry: Through the Mass to Thermodynamics of Non-canonical Nucleic Acid Complexes*
15:20 – 15:40	Coffee break
15:40 – 16:25	Company workshop – Bruker s. r. o.
	Gary Kruppa: New developments in ultra-high sensitivity and single cell proteomics.
	Daniel Vláčil: Next level of targeted and nontargeted screening with the Ion Mobility hrMS
16:25 – 17:25	**Plenary lecture II. + surprise: Vladimír Havlíček** (Chairperson: Zdeněk Kukačka)
PL-02	*Infection metallomics*
17:25 – 17:30	Closing remarks

PL-01: Tau regulates access to microtubules

Zdeněk Lánský [1 *]

1. Institute of Biotechnology of the CAS, Prague, Czech Republic

Tau is an intrinsically disordered microtubule-binding protein involved in a number of neurodegenerative diseases. Malfunction of tau and its detachment from axonal microtubules are correlated with axonal degeneration. However, mechanistic understanding of this process is still missing. We recently showed that tau molecules cooperatively form cohesive, selectively permeable, envelopes on the microtubule surface. Importantly, these tau envelopes can protect microtubules from microtubule-degrading enzymes. I will discuss the mechanism of the tau envelope formation and the roles of canonical neurodegeneration-related factors on the tau envelope dynamics.

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PL-02: Infection metallomics

Vladimír Havlíček [1 *]

1. Mikrobiologický ústav AV ČR, v.v.i.

Infection metallomics is a mass spectrometry (MS) platform established based on the central concept that microbial metallophores are specific and sensitive biomarkers of invasive infectious diseases. During infection in critically ill patients, the pathogen activates secondary bacterial, mycobacterial, and fungal metabolism, leading to the production of metallophores increasing its chance of survival in the host. Here, clinical applications of metallophores in medical microbiology from historical and functional perspectives will be reviewed and under-studied and emerging applications with high diagnostic potential for the post-covid era will be discussed [1,2].

MS with isotope data filtering is central to infection metallomics; it has been used to study the interplay between "frenemies" in hosts and to monitor the dynamic response of the microbiome to antibiotic and antimycotic therapies. Infection metallomics was used for the noninvasive diagnosis of invasive aspergillosis. An array of triacetylfusarinine C (TafC)/ferricrocin (FC)/gliotoxin (Gtx) was detected in a cohort of 35 patients (13 positive and 22 controls). In the clinical study of critically ill, mostly non-neutropenic humans, the TafC/FC/Gtx sensitivity in urine was 100% compared to standard GM in serum (36%). Further, invasive coinfection of A. fumigatus with carbapenem-resistant P. aeruginosa was noninvasively detected in humans by monitoring levels of the respective siderophore, TafC and bacterial pyoverdin E, pyochelin, and 2-heptyl-4-quinolone [3]. MS may also discriminate invasion from benign colonization based on well-defined thresholds distinguishing proliferation from the colonization steady state.

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ACKNOWLEDGEMENT:

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MoO-01: Advances in mass spectrometry-based metabolomics and quantitative proteomics

Darshak Gadara [1], Markéta Nezvedová [1], Durga Jha [1], Eliška Benešová [1], Zdeněk Spáčil [1 *]

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INTRODUCTION: Mass spectrometry-based metabolomics and proteomics allow for the exploration of biomarkers with high selectivity and sensitivity. However, developing robust protocols to profile metabolites and proteins is challenging and thus not fully exploited in biology and medicine. We present our latest contributions to exploratory metabolomics [1] and targeted proteomics [2,3].

METHODS: UHPLC systems (1290 Infinity II; Agilent Technologies and Nexera X2, LC-30AD, Shimadzu Corp.) equipped with reversed-phase (RP) C18 pre-column and analytical column (CSHTM, 1.7 µm, Waters Corp.), coupled to HRMS (Orbitrap Fusion, Thermo Scientific Corp.) or a triple quadrupole mass spectrometer (Agilent 6495B, Agilent Corp.).

RESULTS: Exploratory metabolomics produces up to 85% false positives due to the inefficient elimination of chimeric signals and chemical noise. Our novel workflow addresses the limitation. Features were detected in UHPLC-MS1 data by XCMS Online, filtered using blank subtraction and reproducibility assessment, and annotated to generate tentative identifications. Next, we compared predicted and experimental RP-UHPLC retention times using a model based on a linear regression of 42 retention indices (cLogP from −6 to 11). The workflow reduced tentatively identified metabolites by 88%, from initially detected 6940 features in XCMS to 839 tentative identifications [1].

A proper internal standard choice is critical for accurate, precise, and reproducible mass spectrometry-based proteomics assays. However, a consensus on the design of a winged peptide for absolute quantification is missing. We first evaluated the sequence extension's length and position influence on synthetic extended "winged" peptides' quantitative performance [2,3].

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MoO-02: Analysis of glycopeptides using liquid chromatography-mass spectrometry

Katarína Molnárová [1 *], Petr Kozlík [1]

1. Katedra analytické chemie PřF UK

Protein glycosylation plays a key role in many biological processes including protein stabilization and folding, cellular localization and receptor activation [1]. The analysis of glycoproteins is challenging due to their microheterogeneity (variations of glycan structure at a specific site) and macroheterogeneity (site occupancy or completeness of glycosylation) [2]. The standard procedure of glycoprotein analysis includes their proteolytic digestion followed by the analysis of resulting intact glycopeptides by liquid chromatography coupled to electrospray tandem mass spectrometry (LC-ESI-MS/MS). In our study, we separated the glycopeptides of hemopexin in both reversed phase chromatography (RP-LC) and hydrophilic interaction liquid chromatography (HILIC). After the separation, the glycopeptides were analyzed in information-dependent acquisition mode by maXis™ Q-TOF mass spectrometer (Bruker Daltonics, Bremen, Germany). The glycopeptide fragmentation was achieved via collision−induced dissociation (CID) of the three most intense precursors. The collision energy was set at 70 eV and the mass spectra were acquired simultaneously at lower and higher collision energies. In the fragmentation spectra characteristic oxonium ions were observed at m/z at 138, 168, 204, 274, 366. Moreover, in HILIC mode, based on the fragmentation spectra we were able to assign the isobaric glycoforms to core and outer arm fucosylation.

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MoO-03: The use of mobile phase with a low formic acid concentration in LC-MS proteomic analysis leads to enhanced electrospray ionization and improved sensitivity

Siddharth Jadeja [1 *], Hana Sklenářová [1], Juraj Lenčo [1]

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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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MoO-04: Profiling cell-specific, developmental and neurodegenerative protein markers in cerebral organoids

Markéta Nezvedová [1 *], Tereza Váňová [2], Jan Raška [2], Dáša Bohačiaková [2], Zdeněk Spáčil [1]

1. RECETOHX, Faculty of Science, Masaryk University
2. Dept. of istology and Embryology, Faculty of Medicine, Masaryk University

Immune-based assays are frequently used to quantify proteins.[1] However, they suffer from selectivity issues and limited throughput and multiplexing capacity. The development of a new immunoaffinity assay is time-consuming and costly. Recent advances in analytical mass spectrometry[2] offer a unique opportunity to investigate diseases with elusive causes, such as neurodegenerative disorders. Three-dimensional cell cultures, cerebral organoids (COs), represent an emerging model system to study biological processes leading to brain diseases. The COs recapitulate the brain tissue's cytoarchitecture, mimicking the brain development *in vivo*.[3] However, this technology has limitations, i.e., batch-to-batch reproducibility and heterogeneity of produced COs, and requires suitable tools to characterize the cellular composition and neuronal maturation adequately. Our study aims to profile a panel of protein markers in a single CO using selected reaction monitoring (SRM) quantitative proteomics. We developed an SRM assay to quantify 50 protein targets per analysis in a single CO. The total protein content in single COs ranged between 7 and 141 µg. We used a minimum of 1 µg of the total protein equivalent per analysis. We quantified 27 out of 37 targeted cell markers to characterize organoids' cytoarchitecture (neural stem cells, radial glial cells, neurons, astrocytes) and neurodevelopmental stages (markers of cell maturity) in all analyzed COs. We found COs a suitable *in vitro* model system that recapitulates *in vivo* developmental features. The remaining 13 targeted neurodevelopmental and neurodegenerative proteins were quantified in all COs. We discovered an age-dependent variance in cytoarchitecture and protein marker levels in COs.

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MoO-05: Bacterial virulence factors as an infection biomarkers

Anton Škríba [1], Tomáš Pluháček [1,2], Radim Dobiáš [3,4], Miloš Petřík [5], Petr Hubáček [6], Jan Hrbáček [7], Vladimír Havlíček [1 *]

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Antimicrobial-resistant pathogens mainly members of ESKAPEEc family, (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter spp.* and *Escherichia coli*) [1] are the main cause of threats to our healthcare system. They are responsible for increased morbidity and mortality among patients at-risk thereby, rapid diagnostic as well as new therapeutic approaches has to be discovered.

Motivated by the importance of fast, non-invasive and sensitive detection of microbial infections, we developed a method detecting bacterial secondary metabolites such as siderophores [2] and quorum sensing molecules responsible for cell-to-cell communication [3]. We used liquid chromatography-mass spectrometry based monitoring of these virulence factors in biological specimens such as urine, aspirate and even breath condensate.

This work will present two types of infections, one caused by *Pseudomonas aeruginosa* which can cause pneumonia in patients with bronchiectasis and chronic obstructive pulmonary disease. Initially we started with *in vitro* models, followed by *in vivo* rat models, and finally, we tested the method on five patient, where pyoverdines and 2-heptyl-4-quinolone quorum sensing molecule were quantified at ng/mL levels. Second type of infection will be related to the uropathogenic bacteria, causing severe urinal tract infections. From 39 patients samples obtained from Department of Urology of the Thomayer hospital in Prague, we quantified siderophores relative to the creatinine levels. These data were then correlated to microbial cultivations.

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MoO-06: Alternative biofluids and novel microsampling techniques for applications in clinical analysis

Aleš Kvasnička [1,2 *], David Friedecký [1,2], Zdeněk Zadák [3]

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2. Laboratory for Inherited Metabolic Disorders, Department of Clinical Chemistry, University Hospital
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Microsampling techniques are gaining increasing scientific interest due to the largely non-invasive and rapid sampling procedure that can also be done at home, the possibility to send samples by mail and the potential of high throughput analysis and automation of the entire process. As a model example from practice, newborn screening is performed from a sample of dry blood spot, which is collected from newborns by semi-invasive surface puncture of the heel. Other alternative biological materials that could be routinely analysed in the future include saliva, sweat or tears. We have also contributed in this area, namely by designing a concept of 3D-printed attachment with porous glass filter disks—SLIDE (Sweat sampLIng DevicE) for easy sampling of apocrine sweat. By applying advanced mass spectrometry coupled with the liquid chromatography technique, the complex lipid profiles were measured to evaluate the reproducibility and robustness of this novel approach. Data transformation using probabilistic quotient normalization (PQN) significantly improved the analytical characteristics and overcame the 'sample dilution issue' of the sampling itself. The lipidomic content of apocrine sweat from healthy subjects 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 main advantages of apocrine sweat microsampling include: (a) the non-invasiveness of the procedure and (b) the unique feature of apocrine sweat, reflecting metabolome and lipidome of the intracellular space and plasmatic membranes. The SLIDE application as a sampling technique of apocrine sweat brings a promising alternative, including various possibilities in modern clinical practice.

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MoO-07: Extracellular secretome in polarized *Aspergillus fumigatus* growth triggers the invasive aspergillosis in a host

Rutuja Patil [1,2], Dominika Luptáková [1], Radim Dobiáš [3,4], Tomáš Pluháček [1,2], Andrea Palyzová [1], Vladimír Havlíček [1,2 *]

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Aspergillus colonization in immunocompromised patients can develop into invasive pulmonary aspergillosis causing 200,000 deaths in 2019 [1].Distinction between *A. fumigatus* (Af) caused colonization and infection in a host is challenging. Conidial germination is the crucial process for initiating fungal proliferation[2]. Iron-containing fungal virulence factors called siderophores secreted during polarized growth can provide a new borderline between asymptomatic colonization and invasive infection caused by Af.

Methods:-Af human isolate was grown in an iron limited mineral medium at 37°C for 72h. Germination of conidia was observed using bright field microscopy.Intracellular ferricrocin (FC), hydroxy-ferricrocin(HFC) and extracellular triacetylfusarinine C(TafC) and fusarinine C(FusC) siderophores were extracted using liquid-liquid extraction and quantified by LC-FTICR mass spectrometry(MS).

Result:-Different morphotypes can be distinguished during conidial germination: dormancy, isotropic growth and polarized growth. HFC essential for conidial iron storage is synthesized during non-pathogenic dormancy. From isotropic to polarized phases, conidia increase expression of both intracellular and extracellular siderophores to sustain conidial and hyphal iron storage using extracellular iron uptake. FC gradually increased from isotropic growth while TafC secretion was triggered from 8 h incubation of polarized growth of conidia.

Conclusion-Qualitative and quantitatively different profiles of siderophores are characteristic for each Af morphotypes.The triggering of TafC,FusC and FC synthesis in fungal cells defines a borderline characterizing enhanced fungal proliferation. This switching pathogenicity expression systems on can be monitored in animals and humans by MS.

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MoO-08: *Aspergillus fumigatus*: from the iron acquisition strategies to the siderophore-based monitoring of invasive pulmonary aspergillosis

Jiří Houšť [1,2 *], Anton Škríba [1], Tomáš Pluháček [1,2], Miloš Petřík [3], Hubertus Haas [4], Vladimír Havlíček [1,2]

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Invasive pulmonary aspergillosis (IPA) is the most severe fungal infection predominantly found in immunocompromised patients [1]. To ensure sufficient iron supply, Aspergillus fumigatus (Af) applies two high-affinity iron uptake systems, including enzymatic reduction of iron (RIA) or its chelation into siderophores (SIA) [2]. Siderophores, in particular, can contribute to an early, non-invasive, and specific IPA diagnosis [3].

We determined the viability of Af in the presence of no iron, insoluble iron oxide, and soluble ferrous sulphate in vitro. We cultivated both wild type and mutant strains affecting both the SIA and RIA. We monitored the biomass and siderophore production. Next, we simulated the IPA development in neutropenic rats (not) undergoing antifungal therapy in vivo. We monitored the infection progress by simultaneous detection of siderophores, posaconazole, and their metabolites in urine.

In in vitro experiment, all strains showed the slowest growth rate in iron-restricted media which, conversely, contained the most concentrated siderophore content compared to other iron conditions. To utilize ferric cation from iron oxide, Af preferentially biosynthesized the extracellular siderophore fusarinine C rather than its triacetyl derivative. The SIA-lacking mutant produced less biomass compared to the mutant incapable to use RIA. In in vivo experiment, we detected the siderophores two days post-inoculation at the latest in all rats. Whereas non-treated rats died within five days after inoculation, rats receiving posaconazole survived as reflected by decreased siderophore content in urine.

We confirmed and highlighted the importance of siderophores as iron chelators and virulence factors. We demonstrated early, non-invasive, and specific IPA monitoring.

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TuO-01: Systematic Feature Filtering in Untargeted LC-MS Metabolomics: Application towards Biomarker Discovery

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Exploratory mass spectrometry-based metabolomics generates a plethora of features in a single analysis. However, >85% of detected features are typically false positives due to inefficient elimination of chimeric signals and chemical noise not relevant for biological and clinical data interpretation. The data processing is considered a bottleneck to unravel the translational potential in metabolomics. Here, we describe a systematic workflow to refine exploratory metabolomics data and reduce reported false positives. We applied the feature filtering workflow in a case/control study exploring common variable immunodeficiency (CVID). In the first stage, features were detected from raw LC-MS data by XCMS Online processing, blank subtraction, and reproducibility assessment. Detected features were annotated in metabolomics databases to produce a list of tentative identifications. We scrutinized tentative identifications' physicochemical properties, comparing predicted and experimental reversed-phase liquid chromatography retention time. A prediction model used a linear regression of 42 retention indices with the cLogP ranging from -6 to 11. The LC retention time probes the physicochemical properties and effectively reduces the number of tentatively identified metabolites, which are further submitted to statistical analysis. We applied the retention time-based analytical feature filtering workflow to datasets from the Metabolomics Workbench, demonstrating the broad applicability. A subset of tentatively identified metabolites significantly different in CVID patients was validated by MS/MS acquisition to confirm potential CVID biomarkers' structures.

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TuO-02: Characterization of membrane lipids in cerebral organoids

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Current development in three-dimensional cell culture systems like cerebral organoids (COs) is being utilized as a developing human brain model. The brain is a lipid-rich organ with membrane lipids playing a crucial role in maintaining the structure of the neural cells. Analyzing membrane lipids in COs can provide a new avenue to study aging and Alzheimer's disease (AD)-related cerebral changes. Our study focused on gangliosides (GSs), a class of sialic acid-containing glycosphingolipids abundant in the plasma membrane implicated in AD pathophysiology. We profiled GSs using selected reaction monitoring (SRM) and ultrahigh performance liquid chromatography (UHPLC-ESI-MS/MS) in a single CO. The current study profiled longitudinal changes in GSs levels in wild-type (WT) COs harvested at five time-points – 50, 85, 110, 130, and 160 days. We also profiled the GSs in WT and AD COs with different Apolipoprotein E (ApoE) isoforms, namely ApoE3/3 and ApoE3/4, since ApoE is a significant risk factor for AD. Simple GSs were the most abundant GSs sub-class in COs. GSs from the same sub-class with different ceramide chains portrayed similar trends, and ceramides with shorter fatty acyl chains were the most abundant in COs. Neuron-specific GSs – GM1, GD1a, GD1b, and GT1b, were observed to increase gradually till day 110, followed by a decline at later time points. In the ApoE3/3 line, there was a higher level of GSs in AD COs than WT COs. This result contrasted with the ApoE3/4 line, where the AD COs had lower levels of GSs compared to WT. In summary, our study using induced pluripotent stem cell-derived (iPSC) COs recapitulates GSs changes with time as reflected in brain tissues and suggests ApoE related changes in membrane lipids in COs.

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TuO-03: Ultra-trace determination of oxaliplatin and its compendial impurities by sweeping-MEKC-ICP-MS

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Oxaliplatin is a platinum-based cancerostatic drug widely used for the treatment of various types of cancer. Oxaliplatin production can be theoretically accompanied by four platinum-based impurities: impurity B, C, D, and E. The development of the sensitive method for the simultaneous determination of impurities in concentrated oxaliplatin drugs is needed to study their potential effects on living entities. Capillary electrophoresis (CE) employing online preconcentration has already been used for the determination of a plethora of cancerostatic drugs and their impurities.

In our work, we used online sweeping preconcentration micellar electrokinetic chromatography connected with inductively coupled plasma mass spectrometry (sweeping-MEKC-ICP-MS) for the ultra-trace determination of oxaliplatin, impurity B and C [1]. All experiments were performed on the CE7100 instrument connected with the ICP-MS 7700x instrument via a lab-made interface. Under the optimal conditions, 25 mM sodium phosphate pH 2.15 with 175mM sodium dodecyl sulphate (SDS), injection time of 90 s at 50 mbar, the baseline separation of all compounds was achieved. The sweeping-MEKC-ICP-MS method was fully validated in terms of linearity, the limits of detection, quantification, accuracy, precision, and migration time reproducibility. The limits of detection at ng mL-1 levels and wide dynamic ranges up to six orders of magnitude were achieved. This method is able to determine really low levels of oxaliplatin impurities (0.0003%); therefore, it can be used in routine pharmaceutical laboratories for quality control purposes.

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TuO-04: Diagnosis of inherited metabolic disorders by LC-MS/MS

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Urinary organic acid (OA) analysis is an important part of the diagnosis of inherited metabolic disorders (IMD), monitoring of their treatment and the possible development of the metabolic crisis. Clinical manifestations of IMD are usually non-specific, thus laboratory analysis of specific biomarkers is crucial for the differential diagnosis. Routinely, OA analysis is performed by GC-MS, which although is a method with a high separation efficiency and selectivity, it also has certain shortcomings. These include mainly the time-consuming and laborious extraction and derivatisation of analytes from samples and a low sensitivity for acylglycines. There has been developed an LC-MS/MS method covering a total of 147 metabolites from a range of organic acids, acylglycines and acylcarnitines for the diagnosis of more than 90 IMD. Sample preparation is simple and includes diluting a urine sample to the creatinine concentration of 1 mmol/l and adding a mixture of internal standards. Analytes are separated in 26 minutes on the Acquity HSS T3 column (Waters) and analysed by a triple quadrupole analyser (QTRAP 6500, AB Sciex). Diagnosis is based on changes in levels of specific biomarkers, which are assessed using a modified z-score whose values are linked to the Cytoscape software. A metabolic map of IMD has been created in this software, where the change in biomarker levels of each patient and the affected metabolism can be visually monitored. This method is partially validated and more than 500 urine samples have been already analysed by this platform together with internal and external quality control samples. The method serves not only for the diagnosis of IMD, but also for the detection of false positives from the newborn screening and for the monitoring of patients with IMD.

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TuO-05: Neuropharmacokinetic Visualization of Regional and Subregional Unbound Drug Transport Across Blood-Brain Barrier Enabled by Quantitative Mass Spectrometry Imaging

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Introduction: Comprehensive determination of the extent of drug transport across the region-specific blood-brain barrier (BBB) is a major challenge in preclinical studies. To understand the biological mechanisms of low-molecular-weight drug transport, it is essential to recognize that only an unbound (free) molecule can cross the membrane to interact with targets in the brain and initiate a pharmacological response [1].

Methods: To study, spatially visualize and quantify unbound BBB drug transport, we combined in vivo brain neuropharmacokinetics (neuroPK) and in vitro brain slice drug distribution studies with MALDI Fourier transform ion cyclotron resonance quantitative mass spectrometry imaging (qMSI) and established a novel approach the qMSI for unbound drug determination (qMSI-uD). Using this method, we studied antipsychotic drugs risperidone, clozapine, and olanzapine, displaying different neuroPK properties, and we determined the partition coefficient (Kp) of unbound drug (uu) in the brain (Kp,uu,brain) [2].

Results: qMSI-uD enabled visualization of the extent of unbound drug regional and subregional BBB transport characteristics at 20 µm lateral resolution and the post-BBB cerebral drug distribution. We showed significant differences in all three drugs between total and regional Kp,uu,brain, higher unbound drug BBB transport in gray matter compared to white matter, and differences in between brain cortex subregions [2].

Conclusions: qMSI-uD method allows investigation of heterogeneity in BBB transport and presents new possibilities for molecular psychiatrists and neuroscientists by facilitating the interpretation of regional target-site exposure results and decision making.

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TuO-06: Hydroxyl radical footprinting analysis of a human haptoglobin-hemoglobin complex

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Methods of structural mass spectrometry have become more popular to study protein structure and dynamics. Among them, fast photochemical oxidation of proteins (FPOP) has several advantages such as irreversibility of modifications and more facile determination of the site of modification with single residue resolution. In the present study, FPOP analysis was applied to study the hemoglobin (Hb) – haptoglobin (Hp) complex allowing identification of respective regions altered upon the complex formation. FPOP footprinting using a timsTOF Pro mass spectrometer revealed structural information for 84 and 76 residues in Hp and Hb, respectively, including statistically significant differences in the modification extent below 0.3 %. The most affected residues upon complex formation were Met76 and Tyr140 in Hbα, and Tyr280 and Trp284 in Hpβ. The data allowed determination of amino acids directly involved in Hb – Hp interactions and those located outside of the interaction interface yet affected by the complex formation. Also, previously modeled interaction between Hb βTrp37 and Hp βPhe292 was not confirmed by our data

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TuO-07: Fast Fluoroalkylation of Proteins Uncovers Structure and Dynamics of Biological Macromolecules

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Covalent labeling of proteins in combination with mass spectrometry is a powerful tool for structural and dynamic studies of biomolecules in solution. This method can be used as a complementary technique to classical structural methods like X-Ray, NMR, or Cryo-EM. The current labeling methods such as FPOP or HDX enable monitoring protein solvent-accessible areas with sufficient spatial resolution, however, they impose strict requirements on the sample analysis and therefore it is still high demand for alternative, less complicated and inexpensive approaches. In this study, we introduce Fast Fluoroalkylation of Proteins (FFAP) a new stable covalent labeling method based on producing radicals from Togni reagent. Fluoroalkyl radicals were incubated with studied proteins in a quench flow system when the timescale of the reaction pulse is in seconds. The modified proteins were subsequently reduced, digested, separated on reverse phase column and analyzed using high-resolution mass spectrometry. The extent of modification was calculated from the results of triplicate measurements that were statistically analyzed.

As the model proteins for the first structure characterization study, we choose apo/holomyoglobin and the protein complex hemoglobin-haptoglobin (Hb-Hp). The obtained results for both forms of myoglobin show that the protein is significantly modified by radicals in absence of heme. In the case of Hb-Hp complex, our data uncover the interaction interface of the complex. All results were in perfect agreement with previously published HDX and FPOP results[1]. In summary, we introduce the FFAP a new powerful radical labeling method using fluoroalkyl radicals that represent an inexpensive alternative for structural analysis of proteins in an aqueous buffer.

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TuO-08: Variable-Temperature nESI analysis of protein-DNA complexes

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Variable temperature nanoelectrospray ionization (vT-nESI) is an evolving technique that enables the analysis of samples having various temperatures (25-95°C) prior the ionization. In comparison with other biophysical techniques such as circular dichroism (CD) or differential scanning fluorimetry (DFS) employing mass spectrometry (MS) could be highly advantageous. This approach to studying the melting of biomolecules gives us a unique insight into sample composition even analyzing various protein complexes. Moreover, ion mobility mass spectrometry (IMS-MS) is an effective tool to monitor also structural changes and transitions upon heating.

In this study, we present an integrative approach to characterize the interaction of TEAD and FOXO transcription factors DNA binding domains with DNA. We use vT-nESI IMS-MS in combination with biophysical techniques such as CD and DFS to describe processes occurring upon melting of these protein-DNA complexes. Since transcription factors are playing an important role in various eukaryotic regulatory processes understanding their interaction with DNA is crucial. TEAD family proteins are partly responsible for cell differentiation and apoptosis. Proteins from the FOXO family are responsible for protein expression in response to environmental signals.

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TuO-09: Temperature-controlled Mass Spectrometry: Through the Mass to Thermodynamics of Non-canonical Nucleic Acid Complexes

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Repeated nucleic acid (NA) sequences account for more than 50% of our genome. These sequences can fold into non-canonical DNA structures that play essential roles in cellular function. Guanine-rich repeats can form G-quadruplexes (GQs) and partially complementary strands can form branch-like structures that converge toward a central branchpoint, such as three-way junctions (TWJ) or Holliday junctions (HJ). The persistence of these structures can lead to structural instability that can cause severe cellular damage. To develop specific ligands that target non-canonical structures, detailed information about the binding site, binding affinity, and thermodynamics is required. Challenges using conventional methods arise from the structural complexity and variability of NA complexes. We overcome this by laboratory-built temperature-controlled nanoelectrospray ionization (TCnESI) mass spectrometry, which simultaneously analyzes various forms of non-covalent complexes and characterizes their thermodynamics. The source design, which includes a nESI emitter placed between two copper blocks that guarantee uniformly distributed heat, is coupled with either a Synapt G2S or a cyclic IM-MS instrument (both from Waters Corp.). Using TCnESI, we investigated a set of multi-domain complexes that contain various GQ and duplex forming sequences. Mutual interaction of adjacent DNA domains and ligand binding were identified using melting temperatures followed by van't Hoff analysis. Changes in the thermodynamics of NA complexes were found to depend on the number of domains and their position. The TWJ and HJ unfolding pathways were identified and thermodynamically characterized. The stabilization effect of branchpoint-binding ligands, TrisPOB and 2,7-TrisNP, on TWJ and HJ was also studied.

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MoS-01: Copper(II) Interactions with Triazoles under ESI Conditions

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Under electrospray copper(II) is highly reactive. Its ability to be reduced to Cu(I) depends on the character of possible ligands present. Triazoles are synthetic compounds widely used in agriculture and medicine for antifungal treatment. Their triazole heterocycle represents one centre for the creation of dative bond with copper cations. Usually, copper is also bound by another bond coming from the side chain of triazoles (chlor-phenyl ring, hydroxyl group). This contribution presents information how the character of triazole moieties interaction with copper(II) influences Cu oxidation state [1], complex stability [2] and creation of metabolites of triazoles [3].

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MoS-02: Analysis of lipoprotein samples: preparation, LC-MS optimization, data processing

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Proteins are the key elements in all living organisms. They play a crucial role in all phases of a cell cycle from the replication of genetic information to the cell aging. Many of these processes would not be possible without specific proteins modifications like fosforylation or glycosylation.[1] Lipidation, less common but still very important posttranslational modification, enables protein interactions or association of proteins with a cell membrane. Lipoproteins defects are associated with diseases like Huntington's disease, schizophrenia or cancer, [2] which makes the analysis of lipoproteins a highly needed procedure. However, due to their specific properties and because of lack of a standard lipoprotein mixture, it is quite challenging.

Our study was focused on lipoproteins, which are modified on N-terminus by myristic acid - matrix protein of mouse mammary tumor virus and matrix protein of Mason-Pfizer monkey virus. The aim of this study was to optimize the process of analysis from sample preparation, through the LC separation, to MS detection and data processing. The enhanced filter-aided sample preparation combined with liquid-liquid or solid-phase extraction was tested. After finding the optimal conditions of the LC method, MS detection was optimized. A synthetic peptide modified by N-terminal myristoylation was fragmented using different fragmentation techniques (CID, HCD, UVPD).

The optimized LC-MS method was applied to a sample with different preparation method, containing human cell lysate, MMTV and MPMV lipoproteins. The final experiment showed differences in results depending on the sample preparation procedure and confirmed that the optimized LC-MS method is able to detect N-terminal lipomodification of proteins in complex samples.

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MoS-03: Human neural stem cell proteomics – from basic towards translational research

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Human neural stem cells (NSCs) represent a promising treatment for currently incurable neurodegenerative diseases or central nervous system injuries. Transplanted cells are expected to repair the damaged tissue by replacing dead cells or by modulation of microenvironment in the grafting site by protein secretion. However, the transplantation of multipotent NSCs may lead to the differentiation into undesirable cell type(s) or even to unlimited proliferation and tumour formation. Therefore, development of novel approaches for streamline differentiation into desired cell type(s) and a comprehensive characterization of such cells are required.

Firstly, we focused on the characterization of cellular proteome and secretome and their changes during *in vitro* differentiations of human NSCs using data-independent acquisition (DIA) approach and Luminex multiplex immunoassay. We identified activation of VEGF, HIF-1 and Wnt signaling pathways and increased secretion of IL-6 and VEGF during differentiation. Our follow-up study proved that VEGF121, but not VEGF165, supports survival and induces proliferation of spontaneously differentiating NSCs [1]. These results encourage the hypothesis that transient VEGF121 supplementation is advantageous during the cell therapy.

Secondly, we aimed to develop a multiplexed selected reaction monitoring (SRM) method for routinely used cell type-specific markers: OCT4, SOX2, NES, DCX, TUBB3, MAP2, S100B, GFAP, GALC, and OLIG1. This allowed us to assess the neural differentiation potential of NSCs between several *in vitro* differentiation protocols [2]. An expanded assay with additional markers may provide higher data reliability compared to antibody-based methods, representing a method of choice for quality control of NSCs.

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MoS-04: Increased level of norepinephrine, epinephrine, and α-tocopherol following neonatal brain hypoxic-ischemic injury in rats analyzed by mass spectrometry imaging

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Introduction: Neonatal brain hypoxic-ischemic (HI) injury represents a major cause of mortality and morbidity in newborns characterized by two types of brain energy metabolism failure [1]. Primary energy metabolism failure occurs due to lack of oxygen; delayed in time, secondary energy metabolism failure occurs due to hypoxia-induced membrane depolarization and intracellular calcium overload. Within 6-24 hours after the birth, the degree of the secondary failure underlies the severity of the HI insult that per se determines the neonate's outcome.

Methods: Using the Rice-Vannucci model in 7-day old rats, we studied time-dependent neuromodulation changes in the HI-insulted brains. Neurotransmitters and metabolites were spatially visualized and relatively quantified in brain regions and subregions by high-resolution MALDI mass spectrometry imaging at 12, 24, and 36 hours after the insult.

Results: Monoamine neurotransmitters changes were apparent from 24 hours, including norepinephrine and epinephrine levels significantly increased in the regions of caudate putamen, nucleus accumbens, and hypothalamus in the HI-insulted hemisphere. α-tocopherol, a neuroprotective agent [2], remained unchanged for up to 24 hours. Commencing 36 hours, a significant α-tocopherol increase in the HI regions of globus pallidus, hippocampus, and thalamus was observed with a similar trend in other regions of the brain.

Conclusion: Monoamine alterations after HI injury could induce apoptosis inseverely affected regions [3]. Contrary, α-tocopherol could reduce ischemic edema and protect the blood-brain barrier [2].

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MoS-05: Azurin: a model metalloprotein to study an electron transfer process

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Pseudomonas aeruginosa azurin is a small blue copper protein commonly used as a model in electron transfer experiments due to its characteristic UV-VIS spectrum. In this study we have introduced a structural photoinducible analogue of canonical amino acid Met – L-2-amino-5,5-azi-hexanoic acid (photo-Met) – into azurin structure to study both electron transfer reaction and oligomerization of the protein. Photo-Met was inserted into two types of azurin: wild-type (WT) azurin and Az2W mutant where two adjacent W residues with confirmed role in electron hopping across protein-protein interface are present. Our results revealed electron transfer reaction occurring in both proteins after photoactivation (UV-VIS spectroscopy) and higher oligomerization yield in Az2W mutant (SDS-PAGE & MS). Additionally, we have experimented with preparation and evaluation of differently metallized azurins due to Zn-form being a persistent contaminant in azurin recombinant expression and purification. Previously unreported application of basic native polyacrylamide gel electrophoresis (nPAGE) to assess apo/Cu/Zn-azurin ratio thanks to their differing pI was explored. Moreover, nPAGE paired with MS identification revealed further oligomerization processes occurring in the samples under native conditions. To conclude, in this study we have shown several intriguing properties of azurin mutants and their metal-forms and have also introduced promising new techniques which can be utilized in their analysis.

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MoP-01: Analysis of triazine and neonicotinoid pesticides in soil samples by UHPLC-Q-Orbitrap

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The aim of this study was to investigate the levels of persistent triazine herbicides and neonicotinoid insecticides in samples of soils from the Czech-Bavarian border. Ultrahigh-performance liquid chromatography connected to quadrupole Orbitrap high-resolution tandem mass spectrometer (UHPLC-Q-Orbitrap) was used for the determination of pesticide residues and their metabolites in soil samples. Soil samples and their upper and lower horizons from more than 50 localities were obtained from the border areas of western Bohemia and Bavaria. Pesticides were extracted from the soil samples using the QuEChERS (quick, easy, cheap, effective, rugged, and safe) procedure [1]. One general extraction procedure followed by one targeted UHPLC-Q-Orbitrap MS analytical method was used to monitor the compounds. The most frequently detected pesticides were triazine herbicides and their metabolites. The levels of individual pesticides (almost exclusively triazine metabolites) in some Czech soil samples exceeded the concentration of 0.01 µg/g. In contrast, neonicotinoid concentrations were relatively very low. At the same time, a significant decrease in triazine pesticides and their metabolites was recorded between the upper and lower soil horizon. Overall, our study demonstrated acceptable usage of UHPLC-Q-Orbitrap for quantification of multiple pesticide residues both in fresh and archive soils. This is also the first survey of multiple pesticide residues in various soil profiles, which is closely linked to their occurrence in underground water including an insight into their relationships to site and pesticide chemical properties.

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MoP-02: Changes in acidic glycosphingolipids in aging and neurodegeneration

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Glycosphingolipids are amphipathic lipids composed of sphingoid base and fatty acyl attached to mono-, or oligosaccharide moiety. Gangliosides and sulfatides belong to a group of acidic glycosphingolipids and numerous studies report their involvement in neurodevelopment, aging and neurodegeneration. By interacting with signalling pathways in lipid rafts they play role in processes such as apoptosis, myelin-axon interactions and pathogen binding. Neuroprotective properties of gangliosides have been reported in models of neurodegenerative diseases and neuronal injury which highlights their potential as future terapeutic agents. However, the mozaic of glycosphingolipid involvement in aging and neurodegeneration is still scarcely understood.

In this work, we have performed screening of acidic glycosphingolipids in transgenic rat model expressing human truncated tau protein. A total number of 117 ganglioside species and 36 sulfatide species were positively identified in rat brain tissue. Two ganglioside subclasses containing 11 species were newly discovered and characterized using LC-MS/MS approach. Semiquantitative analysis of characterized lipids in brain tissue was performed to determine the impact of aging and neurodegeneration on glycopshingolipid profiles. Our results show gradually declining levels of several sulfatide species during aging and elevated levels of GM3 gangliosides in rat brains affected by neurofibrillary degeneration.

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MoP-03: Separation of tau isobaric phosphopeptides from Alzheimer's disease brain by cyclic ion mobility mass spectrometry

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Tauopathies are sporadic or familial neurodegenerative disorders characterized by intracellular inclusions of abnormal hyperphosphorylated and truncated tau protein. Tauopathies involve around 20 different neurodegenerative diseases, including the most frequent tauopathy - Alzheimer´s disease (AD). To resolve the complexity of tau-phosphorylation sites, we have used the new cyclic ion mobility(cIMS) instrumental platform for the first time to analyze specific phosphorylated sites on a tau protein isolated from Alzheimer's disease brain. We have performed the single and multipass separation cycle experiments for closely positioned phosphorylation sites S214, T212, and T217 of a tryptic peptide from the proline-rich region of tau – TPSLPTPPTREPK. Such modifications were not resolved previously using classic MS or IMS designs. We confirm all three major phosphorylated sites at different ratios in tau isolated from the human brain. The results show that cIMS is the ultimate technique for characterizing isobaric tau peptides phosphorylated on different amino acid residues.

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MoP-04: Nontargeted metabolomic analysis of quinoa (*Chenopodium quinoa* Willd.) using UHPLC-Q-Orbitrap mass spectrometer

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Quinoa (*Chenopodium quinoa* Willd.) is a pseudo-cereal from the Chenopodiaceae family firstly domesticated in the mountain region of South America. This highly adaptable and stress-tolerant plant is recognizing increasing popularity in the human diet and because of its superior nutritional properties, quinoa gained the status of a superfood. Moreover, quinoa also contains numerous secondary metabolites that play a significant role in plant protection, many of which are also considered valuable to human health. Five main groups of biologically active metabolites found in quinoa are phenolic acids, flavonoids, terpenoids, steroids, and nitrogen-containing compounds. With the aim of analyzing the metabolomics profile of 5 quinoa varieties, nontargeted analysis on UHPLC-Q-Orbitrap high-resolution tandem mass spectrometer was used. Obtained data were processed in Compound discoverer 3.0. As a result, 110 different chemical compounds were identified, from which the largest chemical classes detected in this study were flavonoids (38 compounds), fatty acids (13 compounds), and carboxylic acids (8 compounds). According to available scientific literature [1,2], 95 compounds have not been identified in quinoa before. The most interesting representatives of the flavonoids group were, for example, morin, cirsimaritin, miquelianin, hosludin, cerarvensin, or flemiwallichin D. These findings showed that quinoa possesses a superior variability in secondary metabolites, which deserves further research in this area.

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MoP-05: Proteomic analysis of phospholipid-binding proteins in the regulation of plant cell polarity

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Specific protein-lipid interactions are fundamental for all organisms. They regulate reproduction, growth, morphology, and responses to pathogens and more. In plants, lipid-protein interactions have not been satisfyingly described yet. Our laboratory has already proved that distribution and relative amount of anionic phospholipids, such as phosphatidylinositol phosphates, phosphatidylserine, and phosphatidic acid, on plasma membrane are affecting plant cell polarity. Various proteins are specifically bound to anionic lipids in membranes and these interactions then start various signaling processes, also involving exocytosis and endocytosis. We hypothesize that distinct combinations of anionic lipids are responsible for regulation of vesicular transport processes like exocytosis and endocytosis. Aim of this project is to identify peripheral membrane proteins interacting with anionic phospholipids, to analyse comparatively the specificity of these interactions towards distinct anionic lipids and to analyze the effect of membrane curvature on proteinphospholipid interactions. We will reach this aim by binding the proteins to prepared lipid vesicles with various composition and size. After the binding, we will isolate and analyze the proteins on MALDITOF MS and LC-MS/MS. This research will improve our understatement of cell polarization mechanisms and signalling protein targeting in cell.
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MoP-06: Separation of human IgG glycopeptides using different mixed-mode hydrophilic interaction/ion-exchange liquid chromatography stationary phases

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Immunoglobulin G (IgG) is the most common type of human serum antibody, which protects the body from infection. The glycosylation of its Fc region (a part of the IgG heavy chain) influences physiological states and functionality of the antibody, and the composition of attached N-glycans has been linked to several autoimmune, infectious and metabolic diseases. Protein glycoprofiling is a challenge due to the micro- and macro-heterogeneity of glycosylation, and the low abundance of individual glycoforms attached, but is desirable for clinical diagnostics. The separation of intact glycopeptides by hydrophilic interaction liquid chromatography (HILIC) followed by mass spectrometry show to be a rewarding approach allowing protein glycoprofile characterization. Nowadays, a number of HILIC columns differing in used chemistries are commercially available, however, there is a lack of comparative studies assessing their performance and helping the selection of the appropriate one for a specific application.

Here, we compare three recently developed ACE HILIC columns – with unfunctionalized (HILIC-A), polyhydroxy functionalized (HILIC-N), and aminopropyl functionalized (HILIC-B) silica – in the separation of human IgG glycopeptides. All three columns showed higher separation power for different glycoforms than widely used reversed-phase chromatography, and we show that each column separates a different group of glycopeptides more effectively than the other two. Moreover, HILIC-A and HILIC-N columns separated the isobaric A2G1F1 glycopeptides, and thus showed the potential for the elucidation of the structure of isomeric glycoforms.

The presented results should provide a guidance for the selection of an adequate stationary phase for different glycoproteomics applications.

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MoP-07: Suborganelle proteomics, especially analysis of nucleus and chloroplast, as a tool for a deeper clarification of the plant response to drought

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Analysis of plant leaves is a challenge in the field of proteomics, mainly due to the high concentration of the essential enzyme Rubisco. One solution to get more accurate results of less abundant proteins is focusing directly to individual organelles. However, plant organelle proteomics is limited by the complexity of plant tissues, difficulty of isolating a sufficient amount of proteins and purity of individual compartments. Almost the entire plant genome is located in the nucleus. Nowadays, when it is possible to study and modify the plant genome directly, it is necessary to look for new specific transcription factors or other proteins that may act as regulators of transcription of genes involved in the plant response to water deficit[1].

Another essential organelle for the plant is the chloroplast, and drought stress is the main negative factor due to their extremely sensitivity[2]. Chloroplasts can thus be compared to sensors of environmental conditions, which coordinate the expression of proteins encoded in the nucleus with subsequent localization in plastids and thus prepare the cell to trigger a defensive response while maintaining effective photosynthesis[3].

This project is one of the first globally focused to the analysis of these two organelles and compare their response across two different tolerant wheat cultivars during short and long drought and the following recovery. Using LC-MS/MS analysis, many proteins responding differently to drought stress were detected, briefly, in the susceptible cultivar, mostly photosynthetic proteins (e.g. D1 and D2 proteins, and CP47 protein), whereas in the tolerant cultivar mainly proteins with specific role in response to drought (e.g. histone H2B, annexin and LEA 14-4) have been changed.

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MoP-08: Application of Micromanipulation and Laser Desorption Ionization Mass Spectrometry in Analysis of Pea Seed Coat

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Laser Desorption Ionization Mass Spectrometry (LDI-MS) is frequently utilized for direct surface analysis of plant samples including seeds [1]. Combination of LDI MS and multivariate statistics (MVS) has been used for investigation of physical dormancy of legumes [1,2]. Electronically controlled (EC) micro-milling is nowadays a popular method for treatment of various materials [3]. In this communication we combine EC peeling of the outermost layers of pea seed coats (PSC) in micrometer scale and consequent LDI-MS of exposed layers.

PSC of maturated and dry seeds of JI64 and JI92 genotypes differing in dormancy were selected according to the previous studies [1,2]. Small pieces of seed coats were fixed on microscope slides, the outermost (cutin) layers were peeled using micromanipulators Quick Pro (MicroSupport) under microscopic control. Outer cutin layers (OCL) were peeled off in exact depths - 2 or 5 or 10 µm. The peeled and untreated pieces of PSC were analysed by LDI-MS (Synapt G2-S, Waters). Data obtained by LDI-MS were analysed by MVS for identification of markers of particular seed coat layers and pea genotypes.

Significant decrease of normalized signals (NS) of fatty acids (FA) in peeled samples with respect to the untreated ones was observed. Removal of cutine layers exposed deeper PSC layers. NSs of polyphenols and potassium adducts of cell wall fragments (sugar chains) were higher in peeled PSCs compared to untreated ones. These results suggest that combination of EC peeling with LDI-MS is efficient tool for study of seed composition in relation with seed dormancy mysteries.

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MoP-09: Identification of procalcitonin in septic patients by affinity chips and mass spectrometry

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Sepsis is a worldwide health issue caused by a disproportionately large immunity system response for pathogen presence. Fast diagnosis can make a difference in the survival of a patient. For the clinical diagnosis of sepsis, a serum protein called procalcitonin (PCT) is often used. Procalcitonin concentration in the bloodstream correlates with sepsis severity and increases up to a thousand times in a short period. This study was aimed to develop a MALDI MS-compatible method for in-situ enrichment of PCT from patient serum.

The MALDI-compatible immunoaffinity chips were prepared. The surface modification of indium-tin-oxide coated glass slides (ITO) was performed by ambient ion soft-landing technology. The ITO surfaces were modified by an anti-PCT antibody. Developed chips were used for in-situ enrichment of PCT from serum. After sample incubation, chips were washed and spots were covered by a MALDI matrix. The enriched PCT was measured by MALDI TOF MS (Bruker Daltonics) in linear positive mode.

Recombinant PCT was used for the procedure optimization. The method sensitivity was dramatically improved with the acetonitrile precipitation protocol. The optimized method used for monitoring PCT in human serum reaches the detection limit of 10 ng/mL. The native human PCT was observed at m/z 6306 (2+) and at m/z 12620 (1+). The high-resolution mass spectrometry using MALDI FT-ICR uncovered different forms of PCT in patient samples.

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MoP-10: SPE-LC-MS/MS analysis of antibiotic residues in Olomouc wastewaters and sludge - a three-year study

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Hospital and communal wastewater treatment plants are the main sources of antibiotic residues in surface and ground waters and contribute to spread of resistant bacterial strains, which represent a global health threat. Predicted no effect concentrations (PNEC) of antibiotics anticipated for selection of resistant microorganisms are low, ng/L to µg/L [1,2]. Therefore, monitoring of antibiotic residues is important and requires sensitive and selective analytical techniques. This is the first systematic study aimed at analysis of ten selected antibiotics in surface water as well as municipal and hospital wastewaters conducted for the Olomouc agglomeration.

Analysis was performed by a validated LC-MS/MS method. Samples were spiked with isotopically labelled internal standards, preprocessed and extracted using Waters HLB SPE cartridges (wastewater) or combination of SPE on Waters MAX and QuEChERS procedures (sludge). Analytes were separated on a Kinetex XB-C18 1.7 µm column (Phenomenex) using an Acquity UPLC I-class system and detected in MRM mode using a Xevo TQ-S mass spectrometer with an ESI source (all Waters). Typical LOQ values ranged from few ng/L to 200 ng/L, and the linear calibration range usually spanned over three orders of magnitude.

While samples from the Morava river can be considered “clean”, Military and University hospital sewage as well as municipal sewage and sludge contained substantial amounts of antibiotics (especially chloramphenicol, vancomycin, clindamycin, linezolid and erythromycin), in some cases exceeding the PNEC values and thus possibly contributing to the selection of resistant bacteria. With respect to latter, vancomycin and clindamycin possess the highest potential of resistant bacteria selection in the Olomouc agglomeration.

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MoP-11: CE-ICP-MS and CE-ESI-MS: tools for sensitive determination of siderophores

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Siderophores are natural ferric ion-specific chelating agents playing a crucial role in microbial iron withdrawal. Therefore, they represent unique infectious disease biomarkers and/or novel antibiotics [1, 2].

The determination of siderophores in natural samples is challenging, because they are often present in these samples in low concentrations and exist in multi-protein complexes [2]. From these points of view, it is necessary to have a sensitive and accurate HPLC/CE-ESI-MS method.

In our work, we used CE-ESI-MS and CE-ICP-MS for separation of ferricrocin (FCR), triacetylfusarinine C (TAFC), pyoverdines (PVDs) and ferrioxamine B (FOX-B). All experiments were carried out using the Agilent CE7100 instrument connected with either Agilent ICP-MS 7700x instrument via a lab-made interface or Agilent ESI-MS 6460 instrument via a commercial interface.

Siderophores (desferri- and ferri- forms) could be separated by CE-ESI-MS using 50 mM HAc + 50 mM PFOA titrated with NH_4OH, pH 8.5 as running electrolyte, whereas CE-ICP-MS is useful to determine siderophores' metal complexes. BGE used for separation by CE-ICP-MS was 25 mM H_3PO_4/NaOH + 100 mM SDS, pH 7.0. However, those having Fe in their structure cannot be detected at ultralow concentration levels since iron has a high background signal for ICP-MS measurement due to a significant polyatomic interference ($^{40}Ar^{16}O^+$ from argon plasma gas). Hence, we exchanged Fe to Ga in their structure of to reduce the noise of the baseline. In this pilot study, we have developed a method for separation and determination of three siderophores – FCR, TAFC and PVDs, in their native (Fe containing) forms and tagged by Ga. Here, the Ga labelling provided 500fold lower LODs in comparison with the native forms without any preconcentration step.

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MoP-12: Detection of Botulinum Neurotoxin Type BoNT/A1 Using Modified MALDI Surfaces

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A group of nerve toxins produced by strains of Clostridium bacteria – botulinum neurotoxins (BoNTs) – are one of the most potent toxic substances of biological origin known. They affect neuromuscular connections by cleaving proteins of the SNARE complex, blocking neurotransmitter release, and thus stopping signal transmission. This leads to flaccid paralysis, the main symptom of a disease called botulism. Despite numerous methods available to date, the mouse bioassay remains the gold standard for the detection of BoNTs. Regardless of the ethical aspects of this assay, the required time is also inconvenient. Due to the extremely high toxicity and low lethal dose, there is a need for rapid and sensitive method for identification of BoNTs in both clinical and food specimens.

In this study, a combination of protein-modified surfaces and EndoPep-MS – detection of active BoNTs through their endopeptidase activity and mass spectrometry – is introduced. Controlled modification of indium tin oxide (ITO) surfaces is performed by Ambient Ion Soft Landing and two approaches are applied – NeutrAvidin-modified surfaces for enrichment of biotinylated peptides and antiBoNT/A1 antibody-immobilized surfaces for toxin isolation from samples prior to reaction with its substrate peptide. Peptide products of botulinum activity are then detected by MALDI-TOF MS. This method appears to be successful both in the detection of active toxin in samples with the toxin in buffer and the toxin in complex matrices, such as dry granular dog food.

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MoP-13: GC-MS analysis of chenodeoxycholic acid oxidation products

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Chenodeoxycholic acid is one of the primary bile acids. In mammals, primary bile acids are products of cholesterol metabolism in the liver. Secondary bile acids are formed by bacterial transformation of the primary bile acids in the gut [1]. Determination of primary bile acids in various biological matrices is a challenging task due to the absence of chromophores and lack of fluorescent or electrochemically active groups in their molecules. Gas or liquid chromatography coupled with mass spectrometry and enzymatic methods are commonly used to analyze bile acids [2].

The proper understanding of oxidation processes of primary bile acids, most notably the chenodeoxycholic acid, is crucial due to their relation to accurate diagnostic of numerous diseases related to metabolic defects. As the chenodeoxycholic acid itself is electrochemically inactive, dehydration step catalyzed by strong mineral acid, e.g. perchloric acid in acetonitrile [3] used in this study, must be involved before the electrochemical oxidation.

The aim of this study was to develop a fast and reliable GC-MS method suitable for analysis of products formed during the electrochemical oxidation of chenodeoxycholic acid on platinum electrode. The products of the electrochemical oxidation were derivatized by N,O-bis(trimethylsilyl)trifluoroacetamide prior to the analysis. The prevalent oxidation product was identified and its structure verified by comparison with synthetically prepared standard. Based on the NMR analysis, it was concluded that the main oxidation product arises from a transformation on the C-D ring junction forming a spiro compound.

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MoP-14: LC-MS/MS analysis of serum N-glycoproteome of breast cancer patients

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Altered N-glycosylation of cancer tissue cells as well as serum glycoproteins is one of many molecular changes associated with cancer progression. The observed changes are both in a quantity of naturally occurring glycans, and the occurrence of new highly branched glycans. Blood serum is an easily accessible biomaterial, therefore is used for N-glycoproteome characterization that can support disease diagnosis and prognosis [1, 2].

In this work, we present the development of methodology for characterization of serum N-glycoproteome of breast cancer patients using liquid chromatography mass spectrometry (LC-MS). The serum proteins were digested with trypsin using filter-aided sample preparation. Glycopeptides were then enriched and analysed using LC-MS/MS with Orbitrap FusionTM TribridTM. Enrichment efficiency of ZIC-HILIC SPE and 10 kDa centrifugal filters were compared. Also, we evaluated impact of fragmentation techniques (CID, HCD, stepped HCD, EThcD) and other instrument parameters (AGC, injection time, cycle time, etc.) on the number of glycopeptide identifications and mass spectra quality. Currently, 61 glycoproteins and 360 unique glycopeptides were confidently identified with proteomic search engine Byonic. This procedure will be applied for our next project, based on the fishing of sera protein glycoforms, suitable for the prediction of neurotoxicity of breast cancer patients treated with chemotherapeutic drug paclitaxel [3].

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MoP-15: Electron Activated Dissociation combined with Zeno Pulsing on orthogonal Q-TOF system

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Fragmentation Using Electrons with Tunable Energy on Q-TOF LC/MS/MS System delivers complementary information to CID. Electron Activated Dissociation (EAD) covers Electron Capture Dissociation (ECD), Hot ECD, and Electron Impact Excitation of Ions from Organics (EIEIO). The outcome is rich MS/MS spectra for full identification of small molecules, metabolites, position of glucuronation, full characterization of lipids including regioisomerism, double bond position, cis/trans orientation of double bond, improved peptide/protein sequence coverage, characterization of PTMs, disulfide bonds, aminoacid isomerism, etc. Zeno trap improves TOF duty cycle to more than 90% resulting in higher sensitivity of MS/MS spectra. Quantitation using XICs on specific fragments generated by CID or EAD is enabled by high speed of data acquisition.

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MoP-16: Subtyping of Breast Cancer Using N-glycan Profiling of Tumor Tissues

Iva Benešová [1 *], Lukas Uhrik [1,2], Erika Lattova [3], Filip Zavadil-Kokas [1], Ivana Ihnatova [4], Rudolf Nenutil [1], Zbynek Zdrahal [2,3], Lenka Hernychova [1]

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Breast cancer is a very heterogeneous disease that differs among different patients and each individual tumor. Subtype classification of breast carcinoma relies on presence of characteristic receptors (human epidermal growth factor receptor 2, progesterone receptor and estrogen receptor); it is assessed routinely by immunohistochemistry. Unfortunately, this classification with the other methods is not sufficient for prediction of disease prognosis and treatment efficiency. In the recent decade, N-glycans released from the cancer tissues and serum proteins of breast cancer patients were shown to be significantly altered compared to those from healthy controls.

In this work, the differences of N-glycan profiles for various breast cancer subtypes were studied. Samples of tumor tissues from 145 breast cancer patients assigned to the different subtypes were analyzed according to this workflow: Glycans were released with PNGase F, purified, reduced and permethylated. Glycan MALDI-TOF-MS spectra were acquired on UltrafleXtremeTM and automatically processed to extract glycan peak areas corresponding to glycan masses in a reference glycan library, normalized and statistically analyzed in R.

Total number of 93 signals corresponding to N-glycans listed in the glycan library were identified in the mass spectra of tumor tissue samples. Differences in N-glycan profiles were found among the most studied subtypes. For example, the area of the glycan $H_7N_6S_1$ (m/z=3345.69) was found to be significantly higher in the triple negative and HER2+ subtypes compared to luminal subtypes ($p<0.001$). Group of N-glycans with significantly different intensities between HER2+ and triple negative subtypes includes high mannose glycans H_8N_2 (m/z=2208.11, $p=0.001$) and H_7N_2 (m/z=2004.01).

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MoP-17: Development of an LC-MS/MS method for diagnosis of selected inherited metabolic disorders

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The diagnosis of inherited metabolic disorders (IMDs) is currently performed using a variety of methods. However, in order to simplify and facilitate early diagnosis, the implementation of a "universal" method for the determination of a wide range of biomarkers is proposed. For this purpose, the HILIC-MS/MS method allowing the quantification of a total of 76 biomarkers that are involved in metabolism of purines, pyrimidines, BCAA, urea cycle or β-oxidation was developed. The method is characterized by a short analysis time (10 min) and easy and fast sample preparation (dilution of urine to a creatinine concentration of 1 mmol/l). The distribution of concentrations of analytes in the urine of patients and healthy controls was evaluated by modified Z-score, which was further used to create metabolic maps in Cytoscape software for individual patients. In addition to diagnostic purposes, metabolic maps are used to monitor metabolic imbalances.

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MoP-18: Physiological Tau Interactome in Brain and Its Link to Tauopathies

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Background: Tau pathology is the common hallmark of Alzheimer's disease (AD) and most of the other tauopathies, but their connection with disease progress has not been completely understood so far. Therefore, uncovering novel tau interacting partners and pathology-affected molecular pathways can reveal the causes of diseases as well as potential targets for the development of AD treatment.

Methods: We applied an in vivo crosslinking - mass spectrometry approach to a transgenic rat model of progressive tau pathology similar to human AD. Identified proteins were annotated as novel or previously detected/predicted tau partners and pathway enrichment analysis was performed using pathDIP. Several of the most promising candidates for possible drug development were selected for validation by coimmunoprecipitation and colocalization experiments in animal and cellular models. Additionally, the identification of crosslinked sites between tau and its partners was performed and 3D visualized.

Results: We have identified 175 potential novel and known tau interacting proteins by mass spectrometry. From these proteins, 39 were annotated to Alzheimer's associated pathways, 10 to Parkinson's pathways and 22 to both diseases. Through coimmunoprecipitation and colocalization we have validated three novel proteins interacting with tau protein: BAIAP2, GPR37L1 and NPTX1; and confirmed two recently found tau partners: PSMD2 and RAN.

Conclusion: The identified novel tau interacting proteins represents contribution to the knowledge of in vivo tau interactome in brain with AD. Furthermore, our bioinformatic analysis depicted the interesting candidates possible playing important roles in pathogenesis of tauopathies.

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MoP-19: Study of interaction between monoclonal antibody NCD1.2 and peptide CD20 using HDX-MS

Lukáš Uhrík [1,2 *], Adam Krejci [1], Marta Nekulova [1], Petr Muller [1], Borivoj Vojtesek [1], Ted Hupp [3], Lenka Hernychova [1]

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Hydrogen-deuterium exchange coupled with mass spectrometry (HDX-MS) is the ideal tool for the study of protein interactions. This technique used in the study of protein structure has many advantages like easy sample preparation and analysis of proteins in their native state without size limitations. CD20 is a cell surface antigen of B-cells that is responsible for their activation and proliferation. This antigen is targeted by therapeutic monoclonal antibodies (mAb) like Rituximab, Ofatumumab, and Ocrelizumab in the treatment of human cancers and autoimmune diseases. Recently, NCD1.2 mAb was developed that specifically binds canine CD20 and can be used in veterinary medicine as a diagnostic and potentially therapeutic tool [1].

This work was focused on the investigation of NCD1.2 mAb specific interaction with CD20 canine peptide using the HDX-MS approach [2]. According to obtained results was found that the residues from CDR2 (complementarity determining region 2) of the light chain, CDR2 and CDR3 of the heavy chain were the main mediators of CD20 binding. Increased deuteration levels in NCD1.2 peptides which did not correspond to the interaction interface, showed that the antibody relaxes some regions to enable CDRs to bind antigen more tightly. These findings helped us to understand the mechanism of how an antibody can bind its epitope on the antigen and they could lead to the development of more effective diagnostic and therapeutic agents in human as well as veterinary medicine

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MoP-20: Targeted metabolomic and lipidomic study of SHR24 rat models with tauopathy

Dana Dobešová [1 *], Dominika Olešová [2], Radana Brumarová [1], Štěpán Kouřil [1], Petra Majerová [2], Jozef Hanes [2], Alena Polčík Michalicová [2], Bernadeta Jurkanin [2], Andrej Kováč [2], David Friedecký [3,1]

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Alzheimer's disease (AD) belongs to a group of tauopathic neurodegenerative diseases in which extensive neuronal damage leads to the development of dementia, cognitive function loss, and many other neurological symptoms. One cause of AD is a structural change in the tau protein, which aggregates into neurofibrillary tangles that disrupt the neuronal cytoskeleton. The aim of our study was to describe the biochemical processes in model organisms with tauopathy. For this purpose, transgenic rat models SHR24 (TG) with tauopathy induced by expression of human truncated tau protein and control rat models SHR (CN) at 4, 6, 8, 10, 12, and 14 months of age were used. Four materials (plasma, CSF, brainstem back, and front) were collected from TG and CN rats. Samples were subjected to targeted metabolomic and lipidomic approaches for a complete description of the changes in metabolic pathways. The analysis was performed using ultra-performance liquid chromatography coupled with tandem mass spectrometry. The data obtained were subjected to processing and statistical analysis, including univariate and multivariate (non)supervised methods. Both targeted analyses revealed the most changes in the metabolic and lipid profile in 14-month-old TG rats compared with the same-age CN rats. For example, significantly elevated levels of the AD biomarker myoinositol [1,2], hydroxylated acylcarnitines with long chain, phospholipids of choline, serine, glycerol, and matched lysophospholipids were found in brainstem back samples. These findings are probably related to the development of tauopathy caused by neuronal loss and membrane breakdown [1,3]. The results of our study may contribute to explain the development of AD and possibly improve diagnostic methods used in routine laboratories.

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MoP-21: Combination of Fast photochemical oxidation of protein and the Top-down mass spectrometry enables structural characterization of protein/DNA complex

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Regulation of transcription activity though the interaction between proteins and nucleic acids plays crucial role in cells. To understand regulation of specific cellular pathways, the knowledge of interaction between protein-nucleic acid is necessary. Structural proteomics methods offer the possibility for structural characterization for such complexes. Here, we described the potential of Fast Photochemical Oxidation of Proteins (FPOP) coupled to the Top-down mass spectrometry analysis. Currently evolving FPOP method utilizes reactive hydroxyl radical species to oxidize solvent accessible residues. All current works employing FPOP utilized classical bottom-up approach for sample analysis. However, multiple oxidative modifications of protein molecule can alter protein surface and thus not all modified residues reflect the native conformation. Consequently, a novel top-down approach was employed and tested a structural characterization of protein-DNA complex. We subjected model protein, DNA binding domain (DBD) of FOXO4 protein to FPOP experiment in the absence and presence of its DNA binding element, DAF16. After FPOP experiment, singly oxidized ion species of both forms were isolated by quadrupole and fragmented by collision-induced dissociation (CID) and electron-captured dissociation (ECD). By comparing the intensity of fragment ions we were able to detect regions differently oxidized in the presence of DNA. Moreover, considering the reactivity of each amino acid to hydroxyl radicals enabled us to precisely detect the oxidized residues, of which most of them had tendency to be less oxidized in the presence of DNA. Such results demonstrate the potential of the Top down approach for structural analysis in FPOP experiments.

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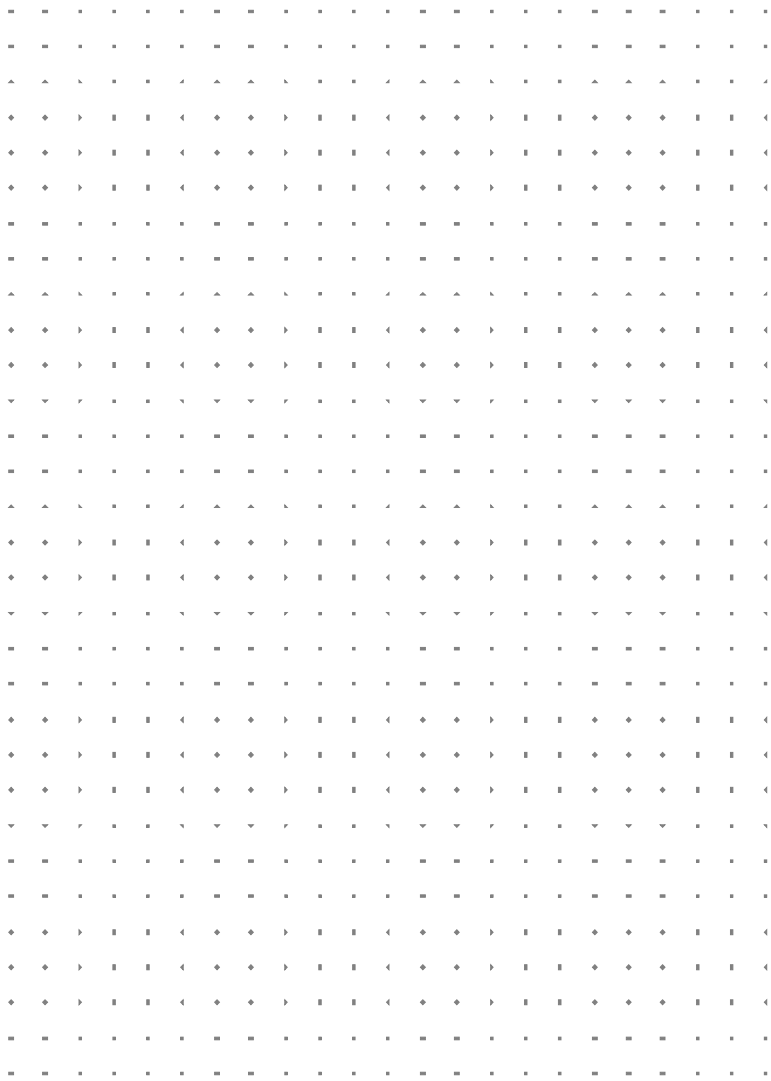

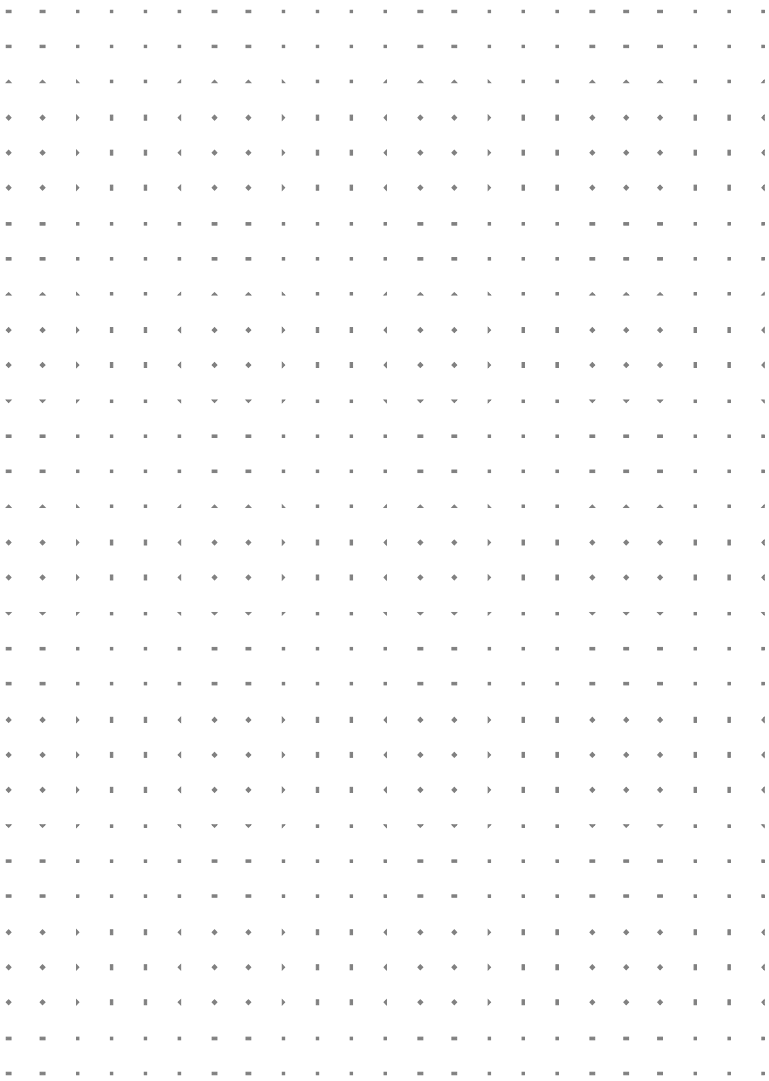

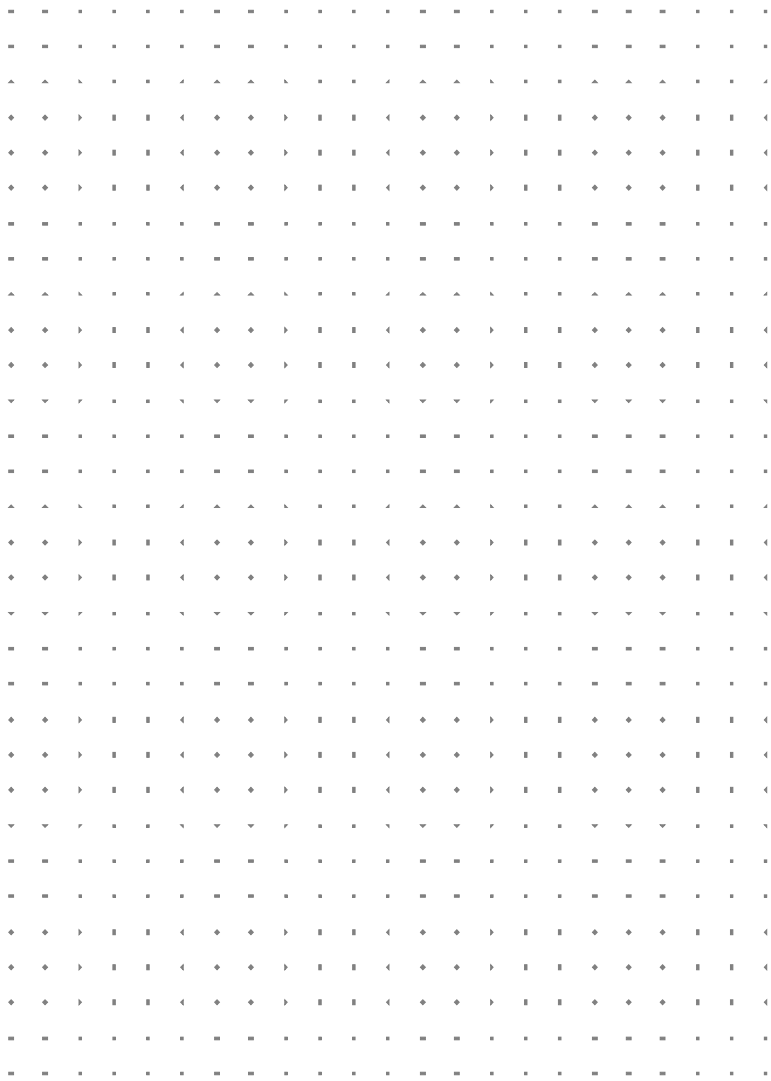

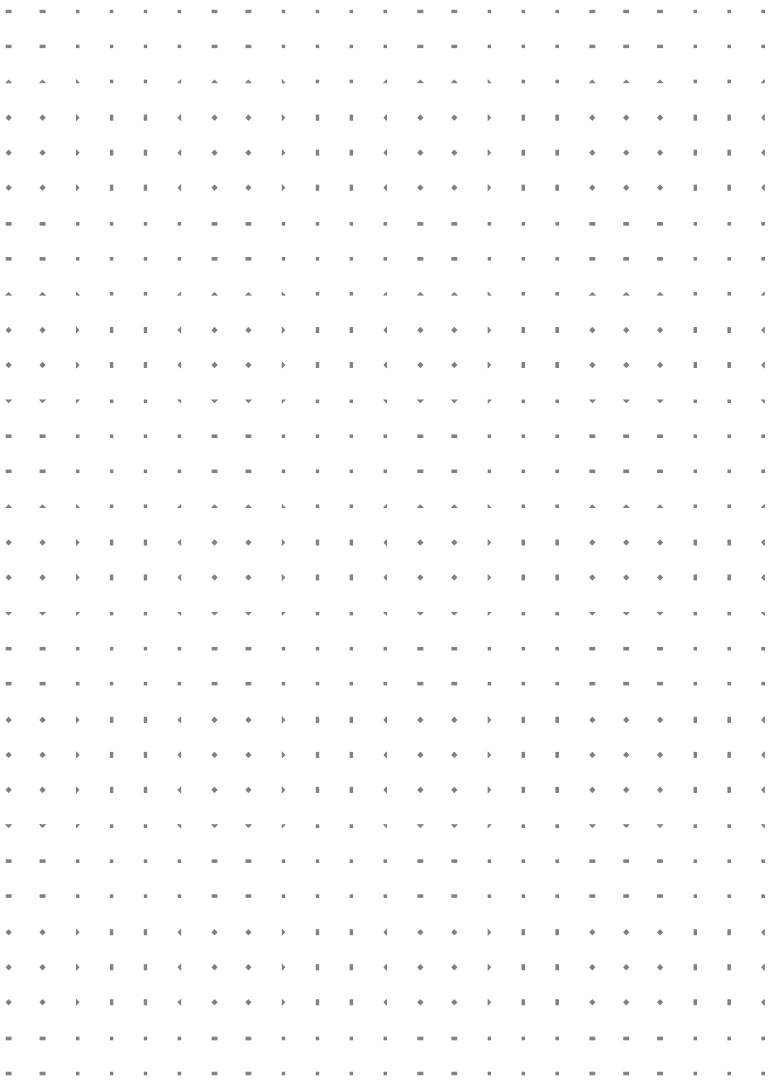

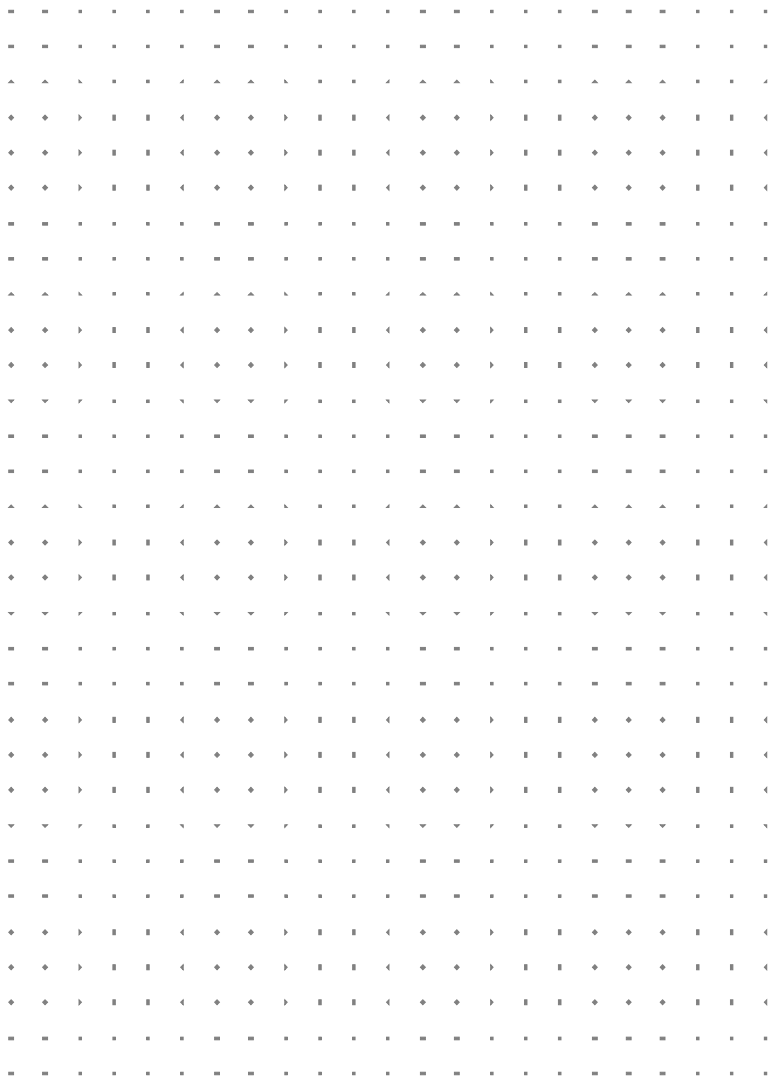

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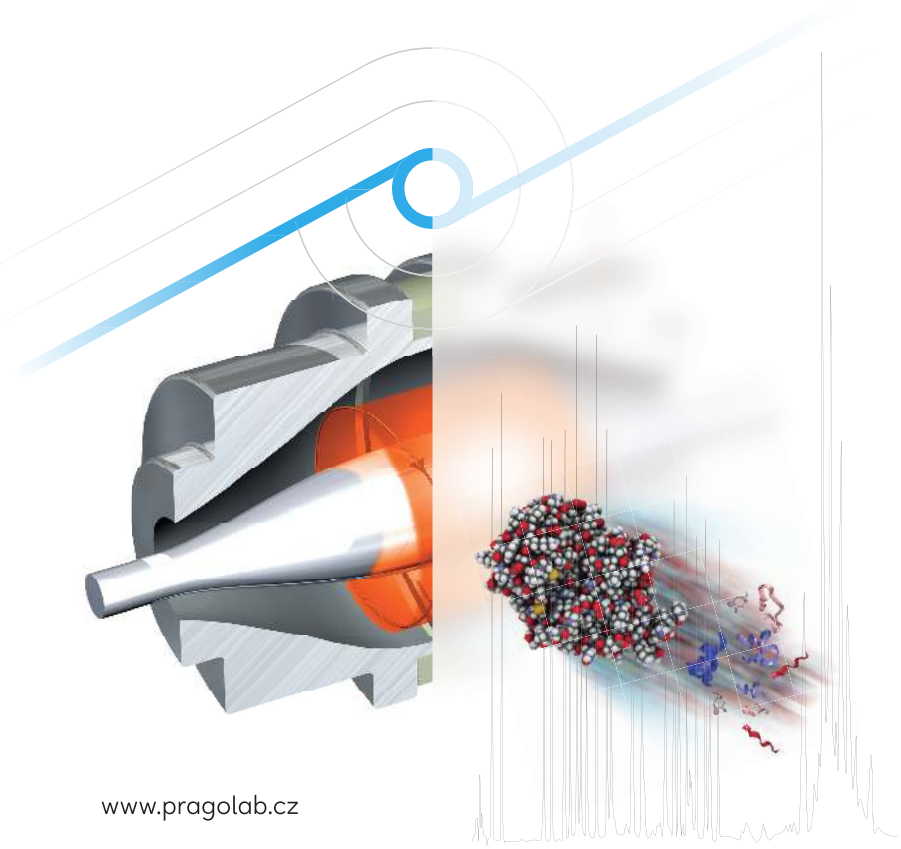

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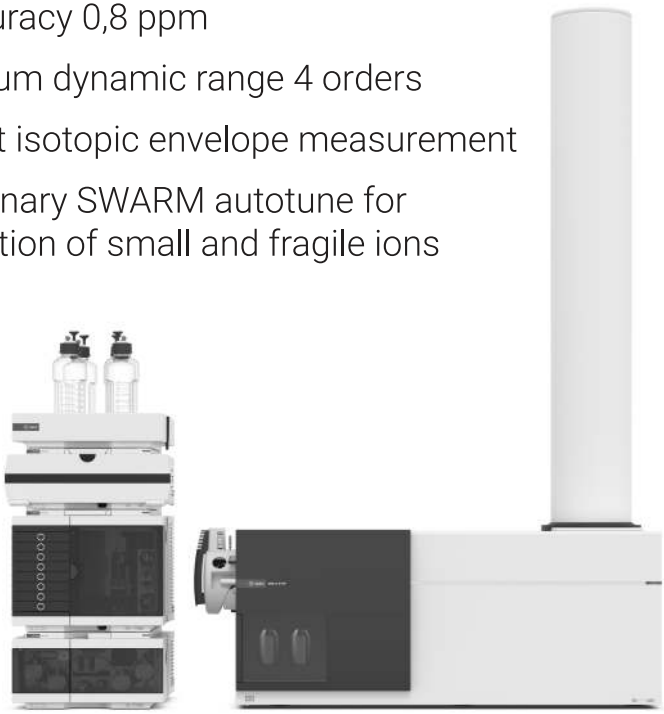

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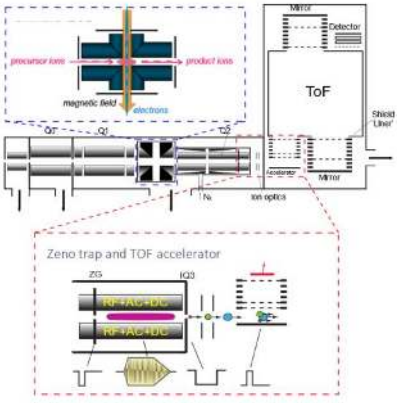

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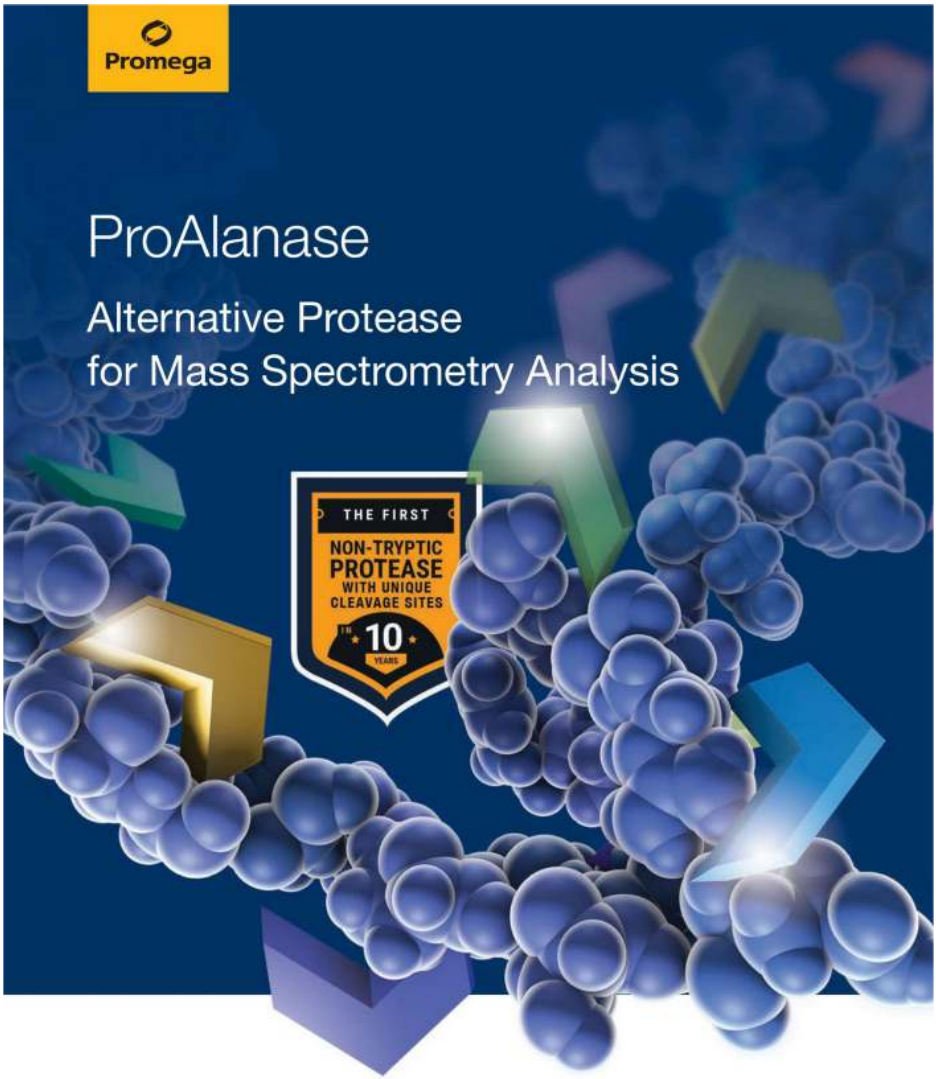

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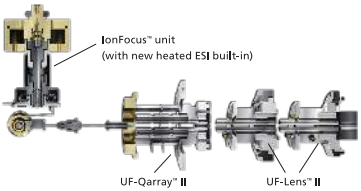

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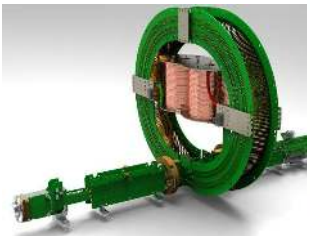

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