SEVENTH ANNUAL CONFERENCE OF THE CZECH SOCIETY FOR MASS SPECTROMETRY

Prague, April 11 – April 13, 2018

BOOK OF ABSTRACTS

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

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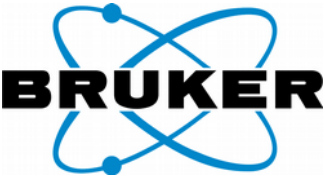

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Seventh Annual Conference of the Czech Society for Mass Spectrometry

Date

11th April – 13th April 2018

Venue

Institute of Molecular Genetics of the ASCR, v. v. i.

Vídeňská 1083

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Wednesday 11th April 2018

12:00 – 17:00	Registration
14:00 – 14:10	Opening of the CSMS Conference
14:10 – 15:10	**Plenary lecture I. (Prof. Helmut Schwarz)**
PL-1	*Ménage-à-trois: Single-atom catalysis, mass spectrometry, and computational chemistry*
15:20 – 15:50	Company Workshop – Pragolab
	Lars Kristensen, Pragolab: Speeding up Biomarker Discovery: Next Generation Technology for Reproducible and Precise Proteome Profiling
15:50 – 16:10	Coffee break
16:10 – 17:50	**Session I.**
	(Chairperson: Anton Škríba)
16:10 – 16:30	Jan Zelenka
WeO-001	*Capillary tip irradiation forms atypical flavin hydroperoxo reaction intermediate*
16:30 – 16:50	Michal Lacko
WeO-002	*Ion chemistry of glyoxal with soft chemical ionization H_3O^+, NO^+ and $O_2^{\bullet+}$ reagent ions*
16:50 – 17:10	Erik Andris
WeO-003	*Formation, chemistry and spectroscopy of gaseous terminal iron nitrides*
17:10 – 17:50	Poster talks
WeS-001	*Timotej Strmeň: Gas dynamic virtual nozzle as a sprayer for miniaturized atmospheric pressure chemical ionization source*
WeS-002	*Rafael Navrátil: Terminal iron(III)-oxo complexes*
WeS-003	*Lukáš Slavata: Gas-phase stability of protein-nucleic acid complexes*
WeS-004	*Mariarosa Anania: Reaction intermediates in palladium-catalyzed carbonylation of olefins*
18:00 – 18:30	Company Workshop – Bruker
	Daniel Vláčil, Bruker: News from Proteomics to Metabolomics with Bruker Daltonics
18:40 – 22:00	Opening party and poster session WeS-001 – WeS-004 + WeP-001 – WeP-016

Thursday 12th April, 2018

09:00 – 10:40	**Session II.**
	(Chairperson: Petr Novák)
09:00 – 09:20	Juraj Lenčo
ThO-004	*Do we still need nanospray for exploratory proteomics?*
09:20 – 09:40	Jan Urban
ThO-005	*Detection and correction of false precision in mass spectrometry*
09:40 – 10:00	Vojtěch Tambor
ThO-006	*Utilization of 3D print in separation techniques and mass spectrometry*
10:00 – 10:40	Poster talks
ThS-005	*Jiří Novák: Towards metabolite identification by CycloBranch*
ThS-006	*Petr Žáček: GCxGC/MS as a tool for study of lipogenesis in white adipose tissue*
ThS-007	*Petra Darebná: In-situ enrichment and detection of biotinylated molecules by MALDI-compatible protein chip*
ThS-008	*Karel Chalupský: Untargeted metabolomics in mouse plasma reveal new gene function*
ThS-009	*Ghazaleh Yassaghi: Hydroxyl radical footprinting of ubiquitin*
10:40 – 11:00	Coffee break
11:00 – 11:30	Company workshop – Shimadzu
	Theodor Petřík, Shimadzu: SHIMADZU introduces the Holy trinity - sensitivity, speed and robustness

11:40 – 13:20	**Session III.**
	(Chairperson: Pavel Řehulka)
11:40 – 12:00	Martin Hubálek
ThO-007	*Quantitative mass spectrometry analysis of protein corona on detonation nanodiamonds: effect of surface chemistry and size*
12:00 – 12:20	Pavel Talacko
ThO-008	*Combining untargeted – targeted proteomic strategies for analysis of differential regulation of vaginal proteome during the estrous cycle of the house mouse*
12:20 – 12:40	Arnd Ingendoh
ThO-009	*A novel approach for complex sample analysis in proteomics and metabolomics: Parallel Accumulation – Serial Fragmentation (PASEF) combining IMS and high resolution MS*
12:40 – 13:20	Poster Talks
ThS-010	*František Filandr: Combined activity of cellobiose dehydrogenase and lytic polysaccharide monooxygenase on solid substrates studied by mass spectrometry*
ThS-011	*David Jurnečka: Differential proteomics of the Bordetella pertussis strains*
ThS-012	*Pavla Vaňková: The role of hydrophobic interactions for Hsp70 allosteric switch*
ThS-013	*Štěpán Strnad: 1,5-diaminonaphthalene for MALDI imaging of lipids: optimization and application for study of neurodegeneration*
ThS-014	*Růžena Lišková: Interaction of TEAD1 transcription factor with its DNA response M-CAT motifs studied by structural mass spectrometry*
13:30 – 14:30	Lunch

Thursday 12th April, 2018

14:30 – 16:20	**Session IV.**
	(Chairperson: Martin Hubálek)
14:30 – 14:50	Anton Škríba
ThO-010	*Early and non-invasive diagnosis of aspergillosis revealed by infection kinetics monitored in a rat model*
14:50 – 15:10	Mathew Kennedy
ThO-011	*Utilising size exclusion chromatography & optimised Q-ToF instrumentation for routine native mass spectrometry*
15:10 – 15:30	Zdeněk Spáčil
ThO-012	*Profiling tryptophan metabolism and inflammatory proteins in biofluids*
15:30 – 15:50	Tomáš Gucký
ThO-013	*The therapeutic drug monitoring of psychopharmacs in routine clinical laboratory practice*
15:50 – 16:20	Poster Talks
ThS-015	*Petra Tomášová: Analysis of minor lipids in subcutaneous and epicardial fat tissue*
ThS-016	*Jiří Hausner: Improvements in membrane protein digestion in HDX-MS workflow*
ThS-017	*Ondřej Vít: Triple approach to membrane proteome profiling of human pheochromocytoma and paragangliom*
16:20 – 16:40	Coffee break
16:40 – 17:20	**Zdeněk Herman Award presented by the Resonance Foundation and oral presentation of the laureate**
17:30 – 18:00	Company workshop – ChemAxon
	David Pech, ChemAxon: ChemAxon - introduction to what we do
18:10 – 19:00	General assembly of the Society
19:10 – 22:00	Dinner and poster session
	ThS-005 – ThS-017 + ThP-017 – ThP-025

Friday 13th April 2018

09:30 – 11:10	**Session V.**
	(Chairperson: Petr Fryčák)
09:30 – 09:50	Zuzana Demianová
FrO-014	*Data independent acquisition improves metabolite coverage over traditional data dependent techniques for untargeted metabolomics*
09:50 – 10:10	Volodymyr Pauk
FrO-015	*Determination of food dyes by supercritical fluid chromatography – mass spectrometry*
10:10 – 10:30	Dominika Luptáková
FrO-016	*Identification of prognostic biomarkers in neonatal rat brain and body fluids following hypoxic-ischemic insult*
10:30 – 10:50	Ondřej Kuda
FrO-017	*Levels of palmitic acid ester of hydroxystearic acid (PAHSA) are reduced in the breast milk of obese mothers*
10:50 – 11:10	David Friedecký
FrO-018	*Importance of mass spectrometry in diagnosis of inborn errors of metabolism*
11:10 – 11:30	Coffee break
11:30 – 12:00	Company workshop – HPST
	Ondřej Lacina, HPST: Agilent Ion Mobility QTOF Update: from CCS to omic
12:10 – 13:20	**Plenary lecture II. (Prof. František Tureček)**
PL-2	*Biological radicals in the gas phase*
13:20 – 13:30	Conference closing
13:30 – 14:30	Lunch

PL-1:
Ménage-à-trois: Single-atom catalysis, mass spectrometry, and computational chemistry

Helmut Schwarz [1] *, Maria Schlangen [1]

1. Technische Universität Berlin

We shall present selected examples of gas-phase reactions which are of timely interest for the catalytic activation of small molecules. Due to the very nature of the experiments, detailed insight in the *active site* of catalysts is provided and – in combination with spectroscopic studies and computational chemistry – mechanistic aspects of as well as the elementary steps involved in the making and breaking of chemical bonds are revealed [1,2].

Examples to be discussed include *inter alia*: (i) Metal-mediated carbon-carbon bond formation; (ii) low temperature, catalytic oxidation of CO; or (iii) the coupling of NH_3 and CH_4 to produce HCN. Of particular importance are the reactions of "bare" metal-carbene complexes, when generated in the gas phase and exposed to thermal reactions under (near) single-collision conditions [3]. In addition to the well-known metathesis and cyclopropanation processes, they exhibit rather unique reactivities. For example, at room temperature the unligated $[AuCH_2]^+$ complex brings about efficient C−C coupling with methane to produce C_2H_x (x = 4, 6), or the couple $[TaCH_2]^+/CO_2$ gives rise to the generation of the acetic acid equivalent CH_2=C=O. Entirely unprecedented is the thermal carbon-atom extrusion from halobenzenes (X = F, Cl, Br, I) by $[MCH_2]^+$ (M = La, Hf, Ta, W, Re, and Os) and its coupling with the methylene ligand to deliver C_2H_2 and $[M(X)(C_5H_5)]^+$. Among the many noteworthy C−N bond forming processes, the formation of CH_3NH_2 from $[RhCH_2]^+/NH_3$, the generation of CH_2=NH_2^+ from $[MCH_2]^+/NH_3$ (M = Pt, Au), or the production of $[PtCH{=}NH_2]^+$ from $[PtCH_2]^+/NH_3$ are of particular interest. The latter species are likely to be involved as intermediates in the platinum-mediated, large-scale production of HCN from CH_4/NH_3 (DEGUSSA process). In this context, a few examples are presented that point to the operation of co-operative effects even at a molecular level. For instance, in the coupling of CH_4 with NH_3 by the heteronuclear clusters $[MPt]^+$ (M = coinage metal), platinum is crucial for the activation of methane, while the coinage metal M keeps control on the branching ratio between the C−N bond forming step and unwanted soot formation.

It will be shown that mass-spectrometry based studies on 'isolated' reactive species provide an ideal arena for probing experimentally the energetics and kinetics of a chemical reaction in an unperturbed environment at a strictly molecular level, and thus enable the characterization of crucial intermediates that have previously not been within the reach of conventional condensed-phase techniques. Clearly, these investigations open the door to a widely uncharted territory of chemistry, a field in which "each atom counts" [1,2].

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PL-2:
Biological radicals in the gas phase

František Tureček [1 *]

1. University of Washington

Mass spectrometry of radicals dates back to the pioneering studies of Fred Lossing in the early 1950's [1]. With the advent of tandem mass spectrometry, soft ionization methods, and ion spectroscopy, it has become possible to generate and study polyatomic radicals of relevance to biology. The general feature of the majority of methods used to produce gas-phase radicals for mass spectrometry studies is their reliance on oxidative or reductive electron transfer to biomolecular ions or their complexes. Femtosecond collisional electron transfer to closed-shell biomolecular ions was the first method developed and applied to this end [2]. A few examples will be presented regarding nucleobase radicals. Intramolecular oxidation of a biomolecular ligand in a ternary transition metal complex is another method that can be used to produce biological ion-radicals in the gas phase [3], as will be illustrated with nucleobase cation-radicals. Electron-transfer reduction of multiply charged biomolecular ions represents a yet different approach to ion-radicals of the hydrogen-rich type. Structure elucidation of gas-phase ion-radicals relies on the combination of ion spectroscopy, ion-molecule reactions, and quantum chemistry calculations, as will be illustrated with peptide and nucleotide cation-radicals.

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WeO-001:
Capillary tip irradiation forms atypical flavin hydroperoxo reaction intermediate

Jan Zelenka [1], Radek Cibulka [2], Jana Roithová [1 *]

1. Faculty of Science, Charles University, Prague
2. Department of Organic Chemistry, UCT Prague

Flavoproteins catalyze a broad scope of crucial reactions in biological systems such as decomposition of neurotransmiters in the brain. To mimic flavoprotein reactivity *in vitro* chemists have developed charged analogues of flavins – flavinium salts. These salts can mimic flavoproteins which react through the hydroperoxyflavin active reaction intermediate.[1] In contrast, simple flavins are not able to mimic this type of reactivity.

However, the simple flavins can oxidize some substrates upon photoexcitation. Such flavin photocatalytical system mimics flavoproteins which oxidize substrates through the electron-proton transfer cascade.[2] Only recently, Cibulka and co-workers have developed a photocatalytical system based on flavinium salts instead of flavins. But the mechanism of this photocatalysis remained unknown.

Mass spectrometry with capillary tip irradiation is a powerful technique for studying photocatalytical reactions.[3] We have used this technique in a combination with infrared photo-dissociation and quantum-mechanic calculations to determine possible reaction intermediates and the mechanism of Cibulka's flavinium salt photocatalytical system. We have observed that an atypical hydroperoxyhydroflavinium intermediate is formed during this reaction.

This is the first example of formation of any atypical flavin-hydroperoxy derivative. This intermediate differs from the usual one in the position of the hydroperoxy group. Based on the quantum-mechanic calculations this isomerism is driven by the protonation of the flavinium moiety during the photocatalytical cycle.

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WeO-002:

Ion chemistry of glyoxal with soft chemical ionization H_3O^+, NO^+ and $O_2^{\bullet+}$ reagent ions

Michal Lacko [1,2 *], Felix Piel [3,4], Andreas Mauracher [4], Patrik Španěl [1]

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4. *Institut für Ionenphysik und Angewandte Physik, Leopold-Franzens-Universität Innsbruck, Austria*

Glyoxal (m.w. 58 g/mol) is a highly reactive molecule important for atmospheric chemistry and associated with some basic biological processes. It is associated with catalytic reactions forming basic organic molecules in an inorganic environment [1] and is one of the products of oxidation of anthropogenic and biogenic volatile organic compounds (VOC) [2]. Thus, there is a need to analyse trace amounts of glyoxal vapour in ambient air and in sample headspace. A previous PTR-MS study [3] revealed a significant humidity dependence of the intensity of the m/z 59 product ion (protonated glyoxal) of the H_3O^+ reaction. Moreover, ion product at m/z 31 (probably protonated formaldehyde CH_2OH^+) resulting from this reaction correlated with the sample humidity. In the present investigation, a selected ion flow tube, SIFT, was used to investigate ion-molecule reactions of glyoxal with H_3O^+, NO^+ and $O_2^{\bullet+}$ reagent ions at variable humidities. Humidity of the helium carrier gas was varied in a controlled manner. Formation of $OC(H)C(H)O(NO)^+$ and $OC(H)C(H)O^{\bullet+}$ ions were observed in the NO^+ and $O_2^{\bullet+}$ reactions respectively. For the H_3O^+ reactions we observed formation of protonated formaldehyde at m/z 31 in addition to protonated glyoxal. However, the humidity dependence of protonated formaldehyde production has an opposite trend in comparison with PTR-MS results. The ion chemistry under thermal conditions is theoretically described using B3LYP/6-311(p,d) calculations and using numerical modelling of the ion-molecule reaction kinetics. The results indicate the important intermediate step is the formation of the $C_2H_2O_2.H^+(H_2O)$ ion, which seems to initiate a dissociation process leading into the formation of protonated formaldehyde

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WeO-003:
Formation, chemistry and spectroscopy of gaseous terminal iron nitrides

Erik Andris [1], Rafael Navrátil [1], Juraj Jašík [1], Gerard Sabenya [2], Martin Srnec [3], Miquel Costas [2], Jana Roithová [1] *

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2. Universitat de Girona
3. Ústav fyzikální chemie J. Heyrovského AV ČR

One of the few ways to study extremely reactive species is using the ion manipulation techniques, which enable their preparation and subsequent characterization. We have prepared extremely unstable terminal iron nitrides in tetragonal ligand environment by photodissociation of iron azides in the gas phase. The lack of matrix effects enabled us to unequivocally assign that the photodissociation to nitrides only proceeds from the doublet ground state of the azides [1]. Moreover, we characterized the chemical reactivity[2] and spectral features of the resulting nitrides, most importantly their Fe-N stretching frequencies [3]. We identified, why all previous attempts to measure the Fe-N frequencies in these systems failed. The spectra gave us important clues of their electronic structure that will be important in future modelling of the terminal iron nitrides, which are postulated as key intermediates in some catalytic cycles (e.g. nitrogen fixation).

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ThO-004:
Do we still need nanospray for exploratory proteomics?

Juraj Lenčo [1,2 *]

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The de facto standard LC-MS platform for proteomics operates at sub-µL/min flow rates, and requires a nanospray to introduce peptides into a mass spectrometer. Although this is almost a dogma in proteomics, this view is being reconsidered in light of introduction of exceptionally sensitive MS instruments. Our objective was to determine what are the extra costs and what optimization and adjustments to a conventional-flow LC-MS system must be undertaken to identify a comparable number of proteins as can be identified on a nanoLC-MS system. We demonstrate that the amount of a complex tryptic digest needed for comparable proteome coverage can be roughly five-fold greater, providing the column dimensions are properly chosen, extra-column peak dispersion is minimized, column temperature and flow rate are set to levels appropriate for peptide separation, and the composition of mobile phases is fine-tuned. Our results document that conventional-flow LC-MS is an attractive alternative for bottom-up exploratory proteomics.

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ThO-005:
Detection and correction of false precision in mass spectrometry

Jan Urban [1 *]

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The processing and analysis of measured mass spectra are dependent on the initial resolution and precision of the dataset. False precision is presented when the amount of significant digits for m/z value is lower than the in the actually expressed values. False precision is presented in the dataset, when discrete sampling or chosen data file format coding returns value with extra non-valid digits. Therefore, the mass profile is oversegmented.

Two approaches for detection and correction of the false precision are available: i) The estimation based on noise presence in the time domain for liquid chromatography - mass spectrometry; ii) The novel method of relative entropy. The binning problem solution is estimated via maximization of the relative entropy as a criterion parameter for objective magnitude rounding.

By both methods, the amount of dataset points could be decreased by removing false precision (where it is present) up to almost 10% of the raw measurement, without loss of information.

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ThO-006:
Utilization of 3D print in separation techniques and mass spectrometry

Michal Boháč [1], Vojtěch Tambor [1 *]

1. TriLAB Group s.r.o.

3D printing or 'personal fabrication', as a newly emergent technology, has now been widely considered as the most significant technological breakthrough of the twenty-first century. This talk considers 3D printing as a newly emerging technology and examine its translation into one specific field - laboratory deployment in scientific research and development. Novel technologies and approaches developed in scientific groups often bring along the need of specialized tools or parts needed for research projects. Due to the nature of this work, however, of-the-shelf products are mostly unavailable or unsuitable. Recent advances in additive manufacturing, the availability of desktop 3D printers in particular, now enables effortless and affordable deployment of these instruments in every laboratory and offers a straightforward option of obtaining custom designed and produced parts at extremely competitive cost. The range of use of these printers are endless, ranging from routine labware, custom tools and adaptors, over replacement parts to complex assemblies and laboratory instrument upgrades or addons. Importantly, the range of materials available in modern desktop 3D printers offers a wide spectrum of available materials, enabling exact selection based on required mechanical, chemical and thermal properties and resistance.

Some scenarios analysis of the 3D printing's general future impacts and the consequence of laboratory-based deployment will also be laid out as the conclusion.

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ThO-007:

Quantitative mass spectrometry analysis of protein corona on detonation nanodiamonds: effect of surface chemistry and size

Martin Hubálek [1 *], Iva Machová [2], Tereza Bělinová [2], Štěpán Stehlík [3], Michal Korecký [1], Bohuslav Rezek [3,4], Marie Hubálek Kalbáčová [5,6]

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Nanoparticles (NPs) from various materials provide promising structures for biomedical application as targeting carriers for drugs, genes or as imaging agents. Thanks to surface chemistry, zeta potential, and surface dipoles they can spontaneously adsorb molecules from their environment e.g. proteins, amino acids etc., which can modify properties of NPs and even cause loss of specificity in targeting. Thus, attention should be paid to the influence of NP size and surface modification for controlling their biological activity.

Detonation nanodiamonds (DNDs) are being investigated for medical applications due to their excellent biocompatibility, chemical and optical properties as well as relatively low-cost synthesis. However, molecular interactions with DNDs are not fully understood.

In this work, we characterize composition of protein corona on DNDs with different mean size (2 and 4 nm) and surface chemistry (hydrogen or oxygen groups). The experiments were focused on identification of the proteins adsorbed on the surface of DNDs in dependence on surface chemistry and size. For this purpose DNDs were incubated in complete cell culture medium with fetal bovine serum, washed by several centrifugation steps and digested either on filter or in short SDS PAGE. The quantitative differences of serum protein binding were determined by SWATH LC-MS/MS analysis.

The results clearly show different selectivity for serum proteins on either hydrogenated or oxygenated DNDs, as well as on different size.

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ThO-008:

Combining untargeted – targeted proteomic strategies for analysis of differential regulation of vaginal proteome during the estrous cycle of the house mouse

Pavel Talacko [1,2] *, Martina Černá [1,2], Barbora Kuntová [1,2], Romana Stopková [1,2], Karel Harant [1,2], Pavel Stopka [1,2]

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2. Biocev

Female house mice produce pheromone-carrying major urinary proteins (MUPs) in a cycling manner. This is thought to occur to advertise the time of ovulation via deposited urine marks. We wanted to characterize and quantify the protein content from the house mouse vaginal flushes to detect putative vaginal-advertising molecules for a direct identification of reproductive states.

Traditional approaches for specific protein detection and quantification like western blotting mostly fail in this case. The reason is that starting material is very rare, protein amount is very small and specific antibodies are not commercially available.

We employed untargeted proteomic approach in discovery phase of our study to reveal protein candidates differentially expressed during various stages of estrous cycle. We then confirmed this data using targeted approach - selected reaction monitoring (SRM). This workflow overcomes need for additional sample preparation and need for specific antibodies and fully substitutes western blotting.

Untargeted discovery experiment showed that the mouse vaginal discharge contains lipocalins including those from the odorant binding (OBP) and major urinary (MUP) protein families. OBPs were highly expressed but only slightly varied throughout the cycle, whilst several MUPs were differentially abundant.

Using SRM we confirmed that MUPs rise between proestrus and estrus, remain steady throughout metestrus, and are co-expressed with antimicrobial proteins showing them as important components of female vaginal advertising of the house mouse. We also succesfully applied untargeted – targeted proteomic strategy to reliably describe quantitative changes in vaginal proteome of the house mouse without use of antibodies and any additional quantitative methods.

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ThO-009:

A novel approach for complex sample analysis in proteomics and metabolomics: Parallel Accumulation – Serial Fragmentation (PASEF) combining IMS and high resolution MS

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In data dependent acquisition proteomics experiments only around 20% of the eluting peptide features are targeted for MS/MS due to limitations in speed, sensitivity and resolution of the mass spectrometer. Recently a novel concept called parallel accumulation – serial fragmentation (PASEF) was described to improve the sequencing speed and sensitivity of MS/MS scans significantly [1] (Meier et al., JPR 2015, PMID: 26538118). Here we show further data acquired with PASEF on a trapped ion mobility (TIMS)-QTOF in shotgun proteomics and complex metabolomics experiments.

In the timsTOF ions are accumulated for a user-defined time and released from the TIMS device depending on their mobility cross section. By applying PASEF, multiple precursors per TIMS scan are selected by sub-ms switching of the quadrupole isolation window. With this approach, 13 precursors can be fragmented within 100 ms, corresponding to 130 MS/MS spectra per sec. Since ions are “space-focused” in the TIMS device, the sensitivity of the method is dramatically improved as well.

Different accumulation and release times (25-100 ms) corresponding to median ion mobility resolutions of up to 80 were tested. The sensitivity was improved by targeting low abundant precursor ions up to 5x. As an ultimate test for the benefit of the high speed and sensitivity of PASEF, just 10-100 ng of a human cancer cell line (HeLa) protein digest were analyzed in a 30-90 min nanoLC gradients. With only 12 ng of HeLa digest, which corresponds to 50 cells only, still more than 15.000 unique peptides could be identified in a 60 min LC run. Ultimate goal of this new approach is getting closer to a complete proteome map and to enable clinical studies with very limited amounts of patient material.

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ThO-010:
Early and non-invasive diagnosis of aspergillosis revealed by infection kinetics monitored in a rat model

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Aspergillus fumigatus is a ubiquitous saprophytic airborne fungus responsible for more than one million deaths every year. Its siderophores represent important virulence factors contributing to microbiome-metabolome dialog in a host. From diagnostic point of view, the application of *Aspergillus* secondary metabolites is promising due to non-invasiveness, high speed and sensitivity.

Using a model of experimental aspergillosis in immunocompromised Lewis rats, the fungal siderophores ferricrocin (FC) and triacetylfusarinine C (TAFC) were monitored in rat urine before and after lung inoculation with *Aspergillus fumigatus* conidia [1]. Molecular biomarkers were separated in high (1×10^8)/low(1×10^4 CFU/mL) dose models by LC-MS. In current work, we supported our *in vivo* MS infection kinetics data with uPET/CT kinetics.

In high dose model, the initial FC signal reflecting the aspergillosis appeared four hours post-infection. Statistical analysis showed increasing linear to exponential metabolite profiles in three-day frame of the experiment. TAFC was shown to be less populated biomarker produced by *Aspergillus* strain exhibiting identical kinetic profiles to those of FC. In uPET/CT the first detectable signal was recorded 48 hours post-infection. Among nine biological replicates in the low dose model, three animals did not develop any infection, one experienced an exponential increase of metabolites and all remaining five animals revealed constant or random FC levels. TAFC concentration was not statistically significant and uPET/CT scan was positive as early as 6 days post-infection.

Siderophore detection in rat urine by mass spectrometry represents an early and non-invasive tool for diagnosing aspergillosis. uPET/CT scanning further determines the infection location *in vivo*.

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ThO-011:
Utilising size exclusion chromatography & optimised Q-ToF instrumentation for routine native mass spectrometry

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Since Fenn's first use of ElectroSpray Ionisation (ESI) for large molecules in 19891, many groups have developed technologies and techniques to enhance the study of proteins and protein complexes with mass spectrometry (MS) in their native state. Today, native MS is routinely used for the structural analysis of protein complexes, providing information that complements traditional methods such as NMR and X-ray crystallography.

Some confusion and controversy around the definition of native MS has always existed, likely originating from the fact, that "native" in its purest sense, would refer to the protein in its natural environment (within the cell) and not the gas phase. The term "Native MS" 2 is therefore used to describe the biological status of analytes in solution, prior to the ionisation event.

As well as introducing the technique of native MS, we will describe the main considerations and modifications required to generate the native complex in solution, required to maintain it in the gas phase based on QTof™, Synapt ™ and Vion™ platforms, as well as describe the benefits of coupling them with Size Exclusion Chromatography (SEC).

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ThO-012:
Profiling tryptophan metabolism and inflammatory proteins in biofluids

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In past decade, an important health outcomes were associated with gut microbiota, but little is known about underlying molecular mechanisms. The gut bacterial pathway metabolizes aromatic amino acids, significantly influencing tryptophan metabolism and causing lifetime immunomodulation [1]. However, concentration levels of specific microbial metabolites shaping immune response [2] and corresponding concentrations of inflammatory protein markers are largely unknown in biofluids.

We have developed MS/MS assays [3] to profile metabolites of tryptophan metabolism in biofluids (i.e. urine, amniotic fluid, serum) and targeted proteomics SRM assays to quantify acute phase proteins on triple-quadrupole mass spectrometer (6495, Agilent Technologies) and untargeted metabolomics by high resolution/accurate mass (HR/AM) mass spectrometry (Orbitrap Fusion, Thermo Scientific).

Results showed correlation between levels of certain microbial metabolites and inflammatory state evaluated on IL-6 marker in amniotic fluid. Next, we captured distribution of microbial metabolites in biofluids. For instance concentrations of indolpropionic acid (IPA), a well-established marker of colonization [2], were readily detectable in the bloodstream and amniotic fluid, but virtually absent in urine [3], which suggests tightly regulated distribution of IPA within human body. We have further explored distribution of 15 additional microbial metabolites and developed quantitative SRM assays for a panel of acute phase protein to evaluate immunomodulatory health effects in dried blood samples (DBS).

Our preliminary data demonstrate that microbiome-related metabolites of tryptophan metabolism may be useful clinical markers of microbial colonization and perhaps early indicators of pathology.

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ThO-013:
The therapeutic drug monitoring of psychopharmacs in routine clinical laboratory practice

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Therapeutic drug monitoring (TDM) of psychopharmacs becomes a key tool in rational pharmacotherapy of neurological and psychiatry disorders. We would like to discuss the most important aspects of psychopharmacs TDM indication and interpretation as well as selected methodological problems. The clinicians general awareness of evidence based and rational indications and interpretation is still low although the guidelines for psychopharmacs TDM have been well established during past three decades. The clinical value of psychopharmacs TDM will be demonstrated on a typical cases of drugs showing significant interindividual biological availability variation, drug interactions, non-linear pharmacokinetic and frequent metabolism abnormalities. The HPLC-MS/MS is a gold standard for the determination of psychopharmacs and their metabolites in biological fluids however the most serious problem of psychopharmacs TDM in routine clinical practice seems to be the lack of reference materials standardization. The most important problems of psychofarmacs TDM quality management will be discussed.

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FrO-014:

Data independent acquisition improves metabolite coverage over traditional data dependent techniques for untargeted metabolomics

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Data independent acquisition (DIA) workflows are well adopted in quantitative discovery proteomics, but still not commonly used in discovery metabolomics. Data dependent acquisition (DDA) techniques are heavily employed in the field of metabolomics and workflows on mass spectrometers have been adapted so that as much data as possible can be captured. Researchers were limited by the speed of their QTOF mass spectrometers meaning a multiple injection workflow. Also, the stochastic nature of data dependent workflows often means MSMS of low abundant metabolites are often missed. Here, we describe how DIA enables the identification of a higher number of metabolites for untargeted metabolomics workflows compared to traditional DDA approaches thus enabling a broader profile of the metabolome.

At the DDA level, the data demonstrate a significant improvement of metabolite coverage at the MSMS level when comparing the top5 to the top25 DDA method. We show over 100% increase of metabolite coverage in plasma extracts by increasing the number of selected precursor ions for DDA acquisition from top5 to top25. This result highlights the capability of the QqTOF mass analyzer for fast MSMS acquisition, which allows for the fragmentation of a large number of precursors in a single DDA cycle, leading to a larger number of metabolites identified.

In the second part of this study, we evaluated the DIA strategy with various fixed (fw) and variable window (vw) sizes with similar cycle time in a plasma extract. Using the variable window method resulted in a ~70% gain in metabolite coverage.

More confident MSMS based identifications lead to higher quantifiable metabolites in a metabolite expression experiment, which at the end allows better understanding of the biology.

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FrO-015:
Determination of food dyes by supercritical fluid chromatography – mass spectrometry

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As food safety considerations become stricter, adequate response from analytical laboratories is expected. Supercritical fluid chromatography – mass spectrometry (SFC-MS) is a tool capable of addressing such challenging issues. EC Regulation 1333/2008 provides a list of allowed food additives and establishes their limits in various food categories. Fifteen food dyes, including sulfonated azo and triphenylmethane dyes, covering a wide polarity range (LogD from -8 to 3.5) were selected as model analytes. Analysis was carried out using an Acquity UPC2 system coupled to a Xevo TQS triple quadrupole mass spectrometer. A steep gradient rising from 5 to 100% of a modifier (10 mM NH_4OH in methanol) extended the polarity range of a mobile phase and allowed separation of lower- and higher-polarity analytes in a single run without utilizing ion-pairing agents. Therefore, MS compatibility and good sensitivity was maintained (LOD<10 ng/mL for 12 compounds). Transition between supercritical and subcritical phase was continuous and did not affect the method performance, which is confirmed by partial validation. Brilliant blue dye was determined in samples of candies and energy drinks.

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FrO-016:
Identification of prognostic biomarkers in neonatal rat brain and body fluids following hypoxic-ischemic insult

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Neonatal hypoxic-ischemic encephalopathy (HIE) is among the most serious complications in neonatology. Despite adaptation of the hypothermia into standard care, adjuvant treatment is needed. A six-hour time period of the HI insult (HII) is critical for diagnosis and effective therapeutic intervention.

Using Rice-Vannucci´s model, comprising the permanent ligation of left common carotid artery in 7-day-old rats and their exposure to 90 min hypoxia at 34°C (8% oxygen), we studied the immediate, subacute and late responses of the neonatal brain by in vivo diffusion-weighted MRI, *in situ* molecular MALDI MS imaging (MSI), elemental LA-ICP-MSI and light microscopy. Cerebrospinal fluid, plasma and urine samples were collected from HI affected rats at 36 hours after the HII and analyzed by LC-MS.

In vivo experiments showed that the immediate edema response due to HII was of cytotoxic origin. The MSI revealed an aberrant plasma membrane distribution of Na^+/K^+ ions in the edema-affected areas also apparent in the MRI measurements, demonstrating intracellular water accumulation. During the subacute phase, an incipient accumulation of an array of N-acyl-phosphatidylethanolamine molecules was detected in the HI brains, and both the cytotoxic and vasogenic types of edema. Moreover, abnormal distributions of the monosialogangliosides GM2 and GM3 were observed. During the late stage, a partial restoration of the brain tissue was observed in the *in vivo* and *ex vivo* studies.

Specific molecular changes may be further utilized in neonatology in proposing and testing novel therapeutic strategies for the treatment of neonatal HIE. Detection of promising biomarkers in body fluids can help to intervene in time and to individualize care by enabling assessment of the treatment efficacy.

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FrO-017:

Levels of palmitic acid ester of hydroxystearic acid (PAHSA) are reduced in the breast milk of obese mothers

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To achieve optimal development of a newborn, breastfeeding is extensively recommended, but little is known about the role of non-nutritive bioactive milk components. We aimed to characterize the fatty acid esters of hydroxy fatty acids (FAHFAs), namely palmitic acid hydroxystearic acids (PAHSAs)-endogenous lipids with anti-inflammatory and anti-diabetic properties, in human breast milk. Breast milk samples from 30 lean (BMI=19-23) and 23 obese (BMI>30) women were collected 72h postpartum. Adipose tissue and milk samples were harvested from C57BL/6J mice. FAHFA lipid profiles were measured using reverse phase and chiral liquid chromatography-mass spectrometry method. PAHSA regioisomers as well as other FAHFAs were present in both human and murine milk. Unexpectedly, the levels of 5-PAHSA were higher relative to other regioisomers. The separation of both regioisomers and enantiomers of PAHSAs revealed that both R- and S-enantiomers were present in the biological samples, and that the majority of the 5-PAHSA signal is of R configuration. Total PAHSA levels were positively associated with weight gain during pregnancy, and 5-PAHSA as well as total PAHSA levels were significantly lower in the milk of the obese compared to the lean mothers. Our results document for the first time the presence of lipid mediators from the FAHFA family in breast milk, while giving an insight into the stereochemistry of PAHSAs. They also indicate the negative effect of obesity on 5-PAHSA levels. Future studies will be needed to explore the role and mechanism of action of FAHFAs in breast milk.

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FrO-018:
Importance of mass spectrometry in diagnosis of inborn errors of metabolism

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Inborn errors of metabolism are a group of hundreds of diseases characterised by deficient specific enzyme activity and elevated levels of corresponding substrates. Analytical methods especially chromatography has been widely used for diagnosing of IEMs. In recent decades, mass spectrometry has been greatly improved and has become an essential part of laboratory methods.

The presentation will be aimed at routine methods, as well as new scientific approaches based on mass spectrometry technology. The routine GC / MS method for urine organic acid analysis is a milestone in diagnosing of more than 50 different IEMs. Similarly, a number of LC / MS methods have been developed over the last ten years for selected IEMs (eg. Nucleotide Metabolism, Creatine Metabolism). Newborn IEM screening based on tandem mass spectrometry is one of the main ways to diagnose rare diseases in time. It is performed in many countries around the world.

Many routine laboratories are focused on research in this area. Over the past few years, omics approaches have been successfully used to find new biomarkers for known IEM, and even new IEMs have been discovered in this way. In the presentation, metabolomics of selected IEMs will introduce new findings that can be useful in diagnosis and better understanding of the disease.

Finally, these days, we are no longer able to imagine carrying a diagnosis without using a mass spectrometry.

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WeS-001:
Gas dynamic virtual nozzle as a sprayer for miniaturized atmospheric pressure chemical ionization source

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An atmospheric pressure chemical ionization (APCI) source for nanoliter-per-minute flow rates was constructed. The source was inspired by the gas dynamic virtual nozzle (GDVN) technology, which made it possible to generate steady capillary microjets [1,2]. The sprayer was manufactured from a borosilicate glass TLC capillary tube (I.D. 1.15 mm, O.D. 1.55 mm) and two fused silica capillaries: (i) a tapered-tip capillary for sample delivery and (ii) a common polyimide-coated fused-silica capillary for gas delivery. Both capillaries were glued to a fixed position The tapered-tip capillary was centered with a custom-made spacer inside the borosilicate tube. The desired shape of the borosilicate tube was fabricated similarly as in [2] using an alcohol burner. The resulting orifice of the borosilicate tube had 220 microns in diameter and was conical in shape. The sprayer has been tested in a miniaturized APCI source having a heater and corona discharge needle. Various analytes including cholesterol, acridine, and cis-11-hexadecenol dissolved in toluene or acetonitrile were successfully detected at the sample flow rates ranging from 200 to 600 nl/min. The performance of GDVN-APCI source was compared to our previously developed miniaturized source, containing a sprayer based on a tube nebulizer [3].

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WeS-002:
Terminal iron(III)-oxo complexes

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Terminal iron(IV)-oxo compounds are one of the best explored systems in bioinorganic and biomimetic chemistry.[1] Conversely, terminal iron(III)-oxo compounds, products of formal one-electron reduction of iron(IV)-oxo analogs, have been studied only scarcely. Herein, we report the generation of representative tetragonal and trigonal iron(III)-oxo complexes in the gas phase. Iron(III)-oxo complexes were prepared by two methods, either by oxidative cleavage of iron(II)-nitrate precursors or by one-electron reduction of iron(IV)-oxo compounds. All studied complexes were characterized by infrared and visible photodissociation spectroscopies, which provided Fe–O vibration frequencies and allowed assignment of spin states. The Fe–O vibration of the quartet state iron(III)-oxo complexes is located at the same frequency as that of their iron(IV)-oxo analogs. The position of the Fe–O vibration in the sextet state complexes is red-shifted by approximately 70 cm^{-1}. Therefore, the Fe–O vibration frequency reflects the spin state of iron center in terminal iron(III)-oxo compounds in contrast to iron(IV)-oxo compounds. Furthermore, we studied the effect of water coordination to the iron-oxo unit and found out that quartet state complexes undergo spin change upon water coordination. Our spectroscopic data provide a solid starting point for the identification and investigation of these reactive intermediates in the condensed phase.

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WeS-003:
Gas-phase stability of protein-nucleic acid complexes

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Interactions between proteins and nucleic acids play a crucial role in all kinds of cell regulations. Understanding of this part of regulation pathways depends on the knowledge of the protein-nucleic acid complex structure and its dynamics. Field of structural mass spectrometry has undergone a remarkable development in recent years. This include a huge increase of commercial availability of instruments coupling ion-mobility to mass-spectrometry. Native mass spectrometry is capable of gently transferring macromolecules into the gas phase and so preserving their tertiary and quaternary structure. Stability of macromolecules can thus be studied under different conditions by observing the shifts of a drift-time caused by changes of the shape during the unfolding event. We demonstrate here the power of this technique to observe significant increase in stability of the protein when interacting with its specifically recognized ligand counterpart – nucleic acid. Further we support these results by collision-induced dissociation measurements in high-resolution (FT-ICR) mass spectrometer, that reveals an involvement of low molecular weight adducts in protein-nucleic acid interaction stability in gas-phase. Well characterized complex of the DNA-binding domain of Forkhead-box transcription factor (FOXO4), with its target DNA sequence - DAF-16 DNA native response element was used as an object of this study.

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WeS-004:
Reaction intermediates in palladium-catalyzed carbonylation of olefins

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The addition of carbon monoxide to organic molecules is an important industrial process as CO is a convenient one-carbon feedstock and the resulting metal acyl complexes can be converted into ketones, aldehydes and acids with easy access to their derivatives. [1] Palladium catalyzed carbonylation of terminal olefins in alcohol selectively affords α,β-unsaturated esters under specific conditions.[2]

We set out to study this reaction by Electrospray Ionization Mass Spectrometry (ESI-MS). A viable species that could be the starting point for the catalytic cycle is a palladium(II) hydride species. Proposed mechanisms [3] suggest that this species undergoes reaction with CO and sequential coordination with styrene, leading to an acyl complex. Subsequent nucleophilic attack by the methanol gives the final product.

We were able to study different palladium species and to detect ligand exchanges and solvent effects. In particular, we detected a species identified as $[PdHCl_2(styrene)(CO)]^-$, where styrene is not π-coordinated to palladium, as showed in Collision Induced Dissociation (CID) experiments. Upon CID, the first channel identified is the loss of CO with an appearance energy (AE) of 1.6 eV, followed by reductive elimination of H-Cl (AE: 1.8 eV) and finally styrene elimination, forming a Pd(0) chloride species. The presence of methanol is essential for the formation of the Pd-H species, as demonstrated in labeling experiments with CD_3OD. Methanol also influences the speciation of palladium species in solution. Labeling experiments allowed us to identify a reductive elimination step that leads to the loss of styrene-d_1.

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ThS-005:
Towards metabolite identification by CycloBranch

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The identification of metabolites is a computationally challenging problem in mass spectrometry-based metabolomics due to their structural diversity and non-specific fragmentation. CycloBranch (http://ms.biomed.cas.cz/cyclobranch/) is our open-source and cross-platform tool allowing comparisons of conventional and fragmentation mass spectra with databases of theoretical spectra of natural products (non-ribosomal peptides, microbial secondary metabolites, siderophores, lipids, etc.) [1-3]. On standard siderophores we will show (1) how to confirm the presence of a compound in a mass spectrum utilizing the benefits of fine isotope structure and (2) how to annotate isotopic patterns of fragment ions of a compound with non-specific fragmentation. Currently, the native file formats of Bruker, Thermo, and Waters are supported as well as the plain text and standard XML-based file formats.

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ThS-006:
GCxGC/MS as a tool for study of lipogenesis in white adipose tissue

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A comprehensive two-dimensional gas chromatography with mass detection (GCxGC/TOFMS) was introduced as a novel analytical tool less than twenty years ago. Since then the technique has become well established due to its sensitivity, peak resolution, enhanced peak capacity and reproducibility [1].

Here we present methods that involve GCxGC/TOFMS in a biosynthesis study of fatty acids in white adipose tissue. The research strategy was based on feeding rats or mice with deuterated water. After a certain time of incubation the animals were killed and the white adipose tissue was dissected and introduced to a transesterification reaction. Products of the transesterification – fatty acids methyl esters (FAME) and glycerol (silylated prior analysis) - were introduced to GCxGC/TOFMS. We used the combination of the resolving power of the GCxGC and mass detection for determination of the fatty acids profile in the tissue and deuterium incorporation in both metabolites.

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ThS-007:
In-situ enrichment and detection of biotinylated molecules by MALDI-compatible protein chips

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Immobilization of proteins is of fundamental interest in biochemistry, biosensor, and material science as well as bioanalytical chemistry. The presented technology allows preparation of MALDI compatible protein chips by ambient ion landing. The electrosprayed proteins immobilized on conductive surfaces allow a wide range of bioanalytical assays. The reaction takes place directly on the protein chip and is followed by in-situ analysis by MALDI mass spectrometry. The conductive surfaces were modified with biotin-binding proteins streptavidin, avidin and neutravidin by ambient ion landing utilizing a nanospray to produce protein ions and heated tube to dry them. The beam of charged species was deposited on the vertically mounted ITO slides that were kept at high voltage of the opposite polarity with respect to the spray. The ion landing of electrosprayed protein molecules was performed under atmospheric pressure by automated ion landing apparatus on dry metal and metal oxide surfaces. Compare to other materials, non-reactive surfaces suffer minimal nonspecific interactions with chemical species in the investigated sample and are thus an ideal substrate for selective protein chips. In this study, the performance of MALDI chips functionalized by avidin, neutravidin, and streptavidin for enrichment and detection of biotinylated proteins and peptides is demonstrated. The functionality of surfaces modified with biotin-binding molecules could help to drive the MALDI-TOF mass spectrometry toward its implementation into routine clinical practice.

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ThS-008:
Untargeted metabolomics in mouse plasma reveal new gene function

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Metabolomics deals with studies of metabolites in biological samples. The metabolome - fingerprint of small compounds is the result of all metabolic processes in the body at a specific time. The metabolome therefore contains information about the metabolic state of the organism. It reflects changes in gene expression, translation, protein modification, and environmental influences. The metabolome, therefore, stands on the top of the pyramid, which includes all these processes and takes place in the body as a whole, and provides an overview of the interaction of metabolic pathways. We generated a mouse model deficient for transmembrane protein 150b (Tmem150b). When we phenotype this mouse model in our phenotyping pipeline, we observed more dark spots in fundus (signs of retinopathy) and lower levels of calcium, albumin and high-density lipoproteins (HDL) in plasma than in corresponding controls. Therefore, 40ul of plasma from seven control C57Bl/6NCrl males and seven Tmem150b males was used for our metabolomics screen. We analyzed the metabolome from six C57BL6N male mice on Agilent 6545 iFunnel Q-TOF using C18 Zorbax column with increasing gradient of 5 % methanol to 100% over 25 minutes with 50 mmol/l NH4F. We found about 7000-8000 molecular features (mzRT pairs) per mouse. Data sets were analyzed by principal component analysis (PCA) and the difference between experimental groups was analyzed by t-test. 13 molecular features were detected as the major difference between wt and Tmem150b -/- mice. Four of them were identified by MS/MS spectra and linked to Tmem150b -/- mice phenotype to vitamin D deficiency. This observation could explain and interconnect observed phenotypes and reveal gene function in more details.

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ThS-009:
Hydroxyl radical footprinting of ubiquitin

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Hydroxyl radical (OH), the most reactive form of reactive oxygen species, can react with any amino acid side chains that are solvent accessible. Therefore, the reaction of reactive oxygen with proteins has been used to probe the structure of proteins and protein complexes. To introduce hydroxyl radicals to biological systems, several methods such as radiolysis [1], photolysis [2] and Fenton chemistry [3] can be utilized.

In this study, Top Down mass spectrometry (FT-ICR) analysis has been applied to the structural characterization of oxidized ubiquitin induced by metal-mediated hydroxyl radicals. Oxidation of ubiquitin was achieved using the Fenton oxidant consisting of $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$, H_2O_2 and EDTA. Two parameters in the Fenton process were optimized in order to obtain higher yield of oxidized products - concentration of - concentration of the Fe(II) and the H_2O_2. When reasonable oxidation of ubiquitin was observed, Top Down analysis - collision induced dissociation and electron transfer dissociation - of intact and oxidized ubiquitin was performed in order to find the modification sites. Detailed interpretation of the MS/MS spectra from isolation of the +11, +10 and +9 charge states was used to identify the amino acid side chains on the protein surface that are the most susceptible to oxidation. Our results show that the Fenton reaction is a simple and an inexpensive way of radical generation for protein structural foot-printing and Top Down strategy is the most promising technology for such a comprehensive analysis.

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ThS-010:
Combined activity of cellobiose dehydrogenase and lytic polysaccharide monooxygenase on solid substrates studied by mass spectrometry

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Cellobiose Dehydrogenase (CDH) and its redox partner Lytic Polysaccharide Monooxygenase (LPMO) are essential parts of cellulolytic system in wood degrading fungi. Their combined activity allows for oxidative decomposition of recalcitrant cellulose structures. The function of CDH depends on intramolecular electron transfer (IET) between CDH's disaccharide oxidizing flavin domain and electron transferring cytochrome domain, which then transfers electrons to copper-dependent cellulose oxidizing LPMO by not yet clear mechanism. We used H/D exchange, MALDI-MS and LC-MSMS analysis, to obtain information about the protein-protein and protein-substrate interaction, product formation and protein stability under oxidative conditions. Using H/D-MS, changes were observed on CDH induced by lactose oxidation. Interestingly, no differences were observed on CDH when it interacted with LPMO both in reduced or oxidized state. On the other hand, structural perturbance occurred on LPMO. In the fully functional system, when CDH or small molecular reductants are fueling LPMO with electrons, we saw increase in deuterium exchange around the active site and we attribute it to oxidative damage and protein unfolding. Several oxidative modifications of amino acids in peptides surrounding the active site were indeed identified. These adverse effects can be significantly reduced in the presence of substrate (PASC or cellulose). So far, our findings indicate that the interaction between CDH and LPMO may only be as small as direct cofactor-cofactor interaction between the propionates of the heme group in cytochrome domain of CDH and copper ion of LPMO. MALDI-MS product formation analysis also demonstrated, that LPMO "primed" by small amount of reductant can utilize hydrogen peroxide as a co-substrate.

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ThS-011:
Differential proteomics of the *Bordetella pertussis* strains

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Pertussis, is a highly contagious respiratory disease that is caused by the Gram-negative bacterium *Bordetella pertussis*. Despite high vaccine coverage, pertussis is the most frequent vaccine-preventable disease reported in many European countries, USA and Australia. The resurgence of pertussis in vaccinated children appears to be related to the lesser potency of acellular pertussis (Pa) vaccines, improvement of clinical diagnostics, or genetic adaptation of *B. pertussis* under the vaccination pressure, resulting in suppression of expression of pertactin and filamentous hemagglutinin, the key components of the Pa vaccine. The aim of this project is to analyze protein composition of two different *B. pertussis* strains grown under different cultivation conditions. The proteomic analysis of the culture supernantant ('secretome'), outer-membrane vesicles (OMV) and the cell-associated proteins of the lab-adapted (Tohama I) and recent clinical isolate (B1917) strains were analyzed by high-throughput mass spectrometry approach. Changes in proteomic profiles of the bacterial strains grown under in vitro and in vivo conditions were used to identify proteins that can play important roles in the virulence of *B. pertussis*. These proteins could be used as potential protective antigens in a next-generation Pa vaccine.

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ThS-012:
The role of hydrophobic interactions for Hsp70 allosteric switch

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The ubiquitous ATP-dependent heat shock proteins Hsp70 are in cooperation with their co-chaperones central components of the complex cellular chaperone network maintaining stable protein homeostatsis. The Hsp70s consist of the N-terminal nucleotide binding domain (NBD) and C-terminal substrate binding domain (SBD) which are connected by a conservered flexible linker. Our previous work revealed destabilizing effect of D529A point mutation on Hsp70 SBD. Specifically on the region where L399 residue representing a central part of substrate binding hydrofobic pocket is located. This pocket is closed in ATP-hydrolysis-dependent manner by a C-terminal helical lid containing residues L510, I515, M518 and V519. Moreover, these hydrophobic residues form another hydrophobic cavity with I164 from NBD in ATP-induced state of Hsp70. We speculate that L399 and I164 have a role in stabilization of ADP and ATP bound conformations through the hydrophobic interactions with L510, I515, M518 and V519.

Hsp70 and 11 allosteric mutants (L399A/D/F; L510A/D; I515A/D; M518A/D and V519A/D) were expressed in *E. coli* and purified. Their capacity to bind and release model fluorescently labeled peptide substrate in ATP-dependent manner was evaluated by fluorescence anisotropy. The peptide binding kinetics of all mutant proteins were slower compared to the wild-type which confirms that these residues play a role in the process of subtsrate accomodation to SBD. All mutant proteins had also impaired ATP-induced substrate release further highlighting involvement of the residues L399, L510, I515, M518 and V519 in regulation of Hsp70 conformational transition. Their importance for hydrophobic allosteric switch was also confirmed by H/D exchange in presence or absence of ATP.

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ThS-013:
1,5-diaminonaphthalene for MALDI imaging of lipids: optimization and application for study of neurodegeneration

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Alzheimer's disease is a neurodegenerative disorder and it is characterized by the accumulation of hyperphosphorylated tau neurofibrillary tangles and β-amyloid plaques in the brain [1]. MALDI MSI was used for the study of lipids distribution during accumulation β-amyloid plaques using sublimation of 1,5-diaminonaphthalene (DAN) [2,3]. Significant plaque associated alterations of lipids were observed. In present work, we compared three techniques of matrix deposition - sublimation and spraying with two automatic sprayers based on different principles (ImagePrep, iMatrixSpray). The methods were optimized and evaluated for the analysis of lipids in the mouse brain. The most suitable method was applied for the study of lipid distribution changes in the brain of THY-Tau22 mouse model. Experiments were performed with UltrafleXtreme MALDI-TOF.

The sublimation method for DAN was found highly irreproducible because of the matrix sublimation in the high vacuum and heating by the laser in the ion source [3]. ImagePrep and iMatrixSpray instruments provided homogenous coating of the sample that was stable during the measurement and provided reproducible datasets. The optimized solution for spraying was 10 mg/ml DAN in 70% acetonitrile. Both sprayer methods yielded datasets with about the same number of detected compounds and at the similar signal intensity. However, iMatrixSpray has several advantages: faster matrix deposition and a formation of smaller matrix crystals, which allow measurement with better spatial resolution.

We did not find any significant changes between THY-Tau22 mice and their controls in positive and negative ion modes. The results indicate that lipid changes are not associated with the accumulation of hyperphosphorylated tau in THY-Tau22 mouse model.

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ThS-014:
Interaction of TEAD1 transcription factor with its DNA response M-CAT motifs studied by structural mass spectrometry

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TEAD transcription factors mediate gene expression regulation through interactions with their DNA response M-CAT motif. They are active mainly during growth and development and induce gene expression of proteins involved in cell proliferation, differentiation or apoptosis prevention. TEADs and many of their target proteins are also known to be upregulated in several types of cancers. Thus TEADs are considered a possible target for anti-cancer therapy.

To study the interaction of DNA binding domain (DBD) of TEAD1 with M-CAT motifs originating from regulatory regions of human genes (CTGF, SRF, C-MYC and GLUT1), dissociation constant of each complex was determined using fluorescence anisotropy-based binding assay. Subsequently, structural mass spectrometry techniques, such as H/D exchange (HDX-MS) and quantitative chemical cross-linking, were utilized to gain distance constrains and additional experimental data for structural characterization.

According to K_D assay results, tested M-CATs could be divided into two groups: one with approximately ten times higher affinity to TEAD1-DBD than the other. HDX-MS revealed differences in deuterium uptake in helix H3, part of helix H2 and in the loop connecting them, identifying such region as DNA-binding pocket. This observation was also confirmed by quantitative chemical cross-linking as cross-link formation ability of helix H3 lysines significantly decreased in complexed state. Minor protection from deuteration, probably caused by stabilization in complex state, was also observed at helix H1. Quantitative chemical cross-linking resulted in 14 distance restrains that were, together with HDX-MS results, used for molecular docking to explain the structural basis of different affinities of TEAD1-DBD to each M-CAT motif.

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ThS-015:
Analysis of minor lipids in subcutaneous and epicardial fat tissue

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An analysis of bioactive lipids in adipose tissues reckoned to lead to better understanding of pathogenesis of obesity and its complications. Existing MS methods are limited by high content of triacylglycerols (TAG), which suppress the ionization of minor lipids. The aim of our study was to optimize preanalytical phase of lipid analysis by removing of TAG from adipose tissue extract. Next, the method was used to describe differences between epicardial and subcutaneous adipose tissues obtained from patients with cardiovascular diseases.

Lipids were extracted using modified Folch method with subsequent detachment of TAG by TLC [1]. The repeatability of the presented method expressed by median of coefficients of variation was 12%. The difference in relative abundance of TAG gained with/without TLC was in average 19%.

Our novel preanalytical step allowed us to detect significant changes in low abundant lipids classes which was previous unavailable namely: glycero-phosphatidylcholines, glycerophosphatidylserines, glycerophosphatidylinositol, and ceramides. Moreover, significant changes in specific TAG level correspond with study of Jové et.al [2]describing differences between omental and subcutaneous adipose tissue.

The implementation of TLC step for selective elimination of TAG was crucial for enhancement of the MS detection limit of minor lipids in adipose tissue. The differences between subcutaneous and epicardial lipid profiles reflect their different endocrine functions arising from their location.

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ThS-016:
Improvements in membrane protein digestion in HDX-MS workflow

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Protein digestion in hydrogen/deuterium exchange mass spectrometry is a key step that provides spatial resolution. For majority of the studied soluble proteins the digestion is not problematic and full sequence coverage is easily reached. However, in case of membrane proteins this step represents the biggest bottle neck. Membrane proteins are during production extracted with high concentrations of detergents (mainly decylmaltoside or dodecylmaltoside). Detergent micelles around the protein render it inaccessible for the proteases which results in significantly worse digestion efficiency. If the protein gets digested, another issue arises from the extreme hydrophobicity of the generated peptides that are sticking to the trap or analytical column. Typical setup that routinely provided the best but still not optimal results relied on the in solution digestion by pepsin. Attempts to utilize more commonly used *on column* digestion resulted in very low coverage. Our recent research shows, that these problems can be solved by exchanging the detergent for DMNG (decyl maltose neopentyl glycol) that has much lower critical micelle concentration. Also, tuning of the digestion temperature and pressure, while maintaining minimal exchange conditions to prevent deuterium loss, helps to increase sequence coverage and enables the use or online digestion. We demonstrate these improvements on two selected membrane transporters – chloride transporter from *E. coli* and Na^+/Ca^{2+} transporter from *M. jannaschii*.

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ThS-017:
Triple approach to membrane proteome profiling of human pheochromocytoma and paraganglioma

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About a quarter of human genes encode integral membrane proteins (IMPs), which perform various key biological functions, such as receptor signaling, transport of molecules, cell-cell interactions, etc. Proteomic studies of IMPs are, however, made difficult by their amphipathy, the lack of trypsin cleavage sites and low levels of their expression.

Among the most effective approaches for identification of low-abundant IMPs seem to be those that aim to enrich either soluble (extramembrane) or hydrophobic (transmembrane) parts of IMPs. Our method named hpTC (high pH, trypsin, CNBr) allows identification of IMPs based on their hydrophobic alpha-helices protected from trypsin by the phospholipid bilayer after re-cleavage with CNBr. To maximize the membrane proteome coverage, we combine this approach with standard tryptic digest and two methods based on glycopeptide enrichment, one that uses lectin entrapment on ultrafilters (lectin-FASP) and the second using hydrazide chemistry with solid phase extraction (SPEG).

The combination of these parallel approaches targeting both hydrophilic and hydrophobic segments of IMPs allows us to identify a broader range of IMPs than the classical proteomic strategies. We apply our strategy to membrane proteome profiling of human pheochromocytoma and paraganglioma (PHEO & PGL). These neuroendocrine tumors are extremely rare, and there are no known effective therapeutic approaches. Our goal is to gather data for a detailed description of membrane proteome of human PHEO & PGL that could be used for the search of new drug targets and diagnostic markers. So far we were able to identify over 1200 IMPs in each patient sample, nearly 2000 IMPs in total. In each sample, we identified over 20 so-called missing proteins.

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WeP-001:
Mass spectrometry in the analysis of organic residues in archaeological findings

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High resolution mass spectrometry with (matrix assisted) laser desorption ionization ((MA)LDI-MS), Atmospheric Solids Analysis Probe (ASAP-MS) and gas chromatography/mass spectrometry (GC/MS) was used for the characterization of organic materials present in various archaeological findings of wide time range.

Triterpenoid milliacin occuring in broomcorn millet was detected with GC/MS in soil infills taken from excavated vessels (ceramic beakers) belonging to Moravian Corded Ware culture (based in Eneolithic period). Subsequent ASAP-MS experiment confirmed the identity of this compound through high resolution tandem mass spectra (being the first utilization of ASAP-MS in archaeology). This finding of milliacin can be considered the earliest evidence of broomcorn millet usage in Central Europe. MALDI-MS combined with Principal Component Analysis and Orthogonal Projections to Latent Structures revealed presence of fat residues in some of the above mentioned excavated vessels - signals belonging to di- and triacylglycerols were found. Those results were confirmed by ELISA assay that proved the presence of a dairy product.

Besides, LDI-MS was used for analysis of unknown fibre pieces captured in an eyelet of S-shaped-end ring jewels found in proximity of female skulls in early medieval tombs. Signals of sodium adducts of oligosaccharide chains suggest the presence of plant fibre material (presumably linen, hemp or nettle) used for the fixation of the jewels. Detailed research concerning the origin of these materials is now in progress.

A database combining archaeological information with data from chemical analyses (including mass spectra) was established and is being continuously filled and improved for the needs of the current archaeological cases solved by our team.

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WeP-002:

Degradation of hydroxylated PCBs by *Pleurotus ostreatus* with GC/MS identification of a reaction product

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Polychlorinated biphenyls (PCBs) are anthropogenic contaminants which, due to their slow degradation in nature, still represent an environmental burden despite their ban in the 1980s. The group of ligninolytic (white-rot) fungi and especially *Pleurotus ostreatus* (the oyster mushroom), was found to have a high degradation potential towards PCBs with various metabolites detected *in vivo*, e.g. hydroxylated polychlorinated biphenyls (OH-PCBs). To study the degradation of OH-PCBs, a mixture of five mono- and dichlorinated OH-PCBs was tested *in vitro* with three laccase containing parts of *P. ostreatus* liquid culture: concentrated extracellular liquid, mycelium and semi-purified laccase. The *in vitro* samples were extracted with ethylacetate, derivatized by 1-iodopropane and analysed by GC/MS. All *in vitro* experiments resulted in a similar degradation pattern. A dependency on the position and number of chlorine atoms and also on the addition of laccase mediators, syringaldehyde (SA) or hydroxybenzotriazole (HBT) was observed. 2-hydroxy-5-chlorobiphenyl, 4 hydroxy-4'-chlorobiphenyl and 4-hydroxy-3,5-dichlorobiphenyl were degraded below 20% within one hour even without a mediator. On the contrary, 3-hydroxy-2',5'-dichlorobiphenyl and 4-hydroxy-2',5'-dichlorobiphenyl were degraded only in the presence of SA or HBT. A degradation product (not present in any of the controls) was identified as a monochlorobenzoic acid by MS and the retention time was compared with standards of three possible isomers. The results prove the involvement of laccase in the transformation of OH-PCBs to chlorobenzoic acids.

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WeP-003:
MALDI-TOF MS and MS/MS analysis of permethylated N-glycans purified and fractionated with microgradient separation technique

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The MALDI-TOF MS profile of N-glycans from patient's sera or other clinical samples may contain an important information about the patient disease and its statistical analysis may substantially assist in the diagnostic process. The sample is usually processed by enzymatic deglycosylation of proteins, followed by glycan purification and their permethylation that significantly increases the detection sensitivity, especially of higher and labile glycan species. In this work, the permethylated glycans were purified and fractionated from certain polymeric contaminants introduced to glycan sample during its preparation. For removal of these compounds, a simple microgradient setup with reversed-phase chromatography was used. It consists of a gastight microsyringe connected to a home-made reversed phase capillary column. Sample elution was carried out in two steps: the first step used gradient of acetonitrile up to 32% for removal of more hydrophilic contaminants and the second gradient elution step (32-49.5% acetonitrile) may be used either for direct N-glycan profiling (eluent is collected into the tube) or for N-glycan fractionation on the MALDI target. Compared to the common sample analysis without fractionation, the higher mass N-glycans (up to m/z 6300) were detected and some of them also successfully analyzed using MALDI-TOF/TOF MS/MS. These results confirmed both applicability of microgradient separation for permethylated N-glycans and presence of some previously not observed multiantennary N-glycans in the patients' sera samples.

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WeP-004:
Formose reaction – towards the detection of products

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Formose reaction is a very important method for synthesis of monosaccharides and sugar-like substances from a very simple molecule – formaldehyde [1]. First findings date back to 1861 when Buterlow noticed the formation of sugar-like substances after treatment of formaldehyde solution with a base [2]. In 1959 Breslow proposed a mechanism involving two different processes for condensation of formaldehyde [3]. The first process, formation of glycoladehyde from two molecules of formaldehyde, is a very slow process. Once glycolaldehyde is formed, a fast autocatalytic process is initiated to produce more glycolaldehyde and various straight and branched carbohydrates.

Formose reaction, due to its autocatalytic nature, possible retro aldol reactions in alkaline medium and side reactions (e.g. Cannizzaro reaction), is still not fully revealed and understood. We would like to contribute to the elucidation of the reaction mechanism and kinetics of formose reaction by mass spectrometry. However, the detection of sugar-like substances is not straightforward. We have tested electrospray ionization (ESI) and atmospheric-pressure chemical ionization (APCI) in both positive and negative mode. We were able to detect sugar-like substances $(CH_2O)_n$ as deprotonated in negative mode. Better ionization was observed using APCI.

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WeP-005:
Testing of new custom-made inlet for nanoDESI ionization

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Desorption nanoelectrospray (nanoDESI) was designed in 2006 [1]. NanoDESI uses a narrower spray tip (2 μm I.D.) and generates primary charged droplets of smaller sizes than DESI. Analytes are ionized without assistance of a nebulizing gas [1]. It can be applied to direct analysis of samples from surface. Its applicability was demonstrated e.g. by analysis of anthocyanins in red wine [2], pharmaceuticals in whole blood [3] or identification of dyes in markers. In this work new technical modification (a custom-made inlet) of nanoDESI ionization was tested and compared for two instruments.

Experiments were carried out using a Q-TOF Premier mass spectrometer and Xevo TQD triple quadrupole mass spectrometer (all Waters, Manchester, UK). The commercial inlet of instrument was replaced by a custom-made inlet with a sample cone and a heated capillary allowing for the regulation of pressure drop. Our home-made ion source (nanoDESI) consisted of a nanoelectrospray tip (2 ± 1μm I.D., PicoTips emitter, New Objective, Woburn, USA) and a motorized stage for positioning of a sample. Methanol/water (3/1, v/v) acidified by 2 % formic acid was used as a spray liquid to desorb analytes, e.g. caffeine, ultramark 1621.

Technical details of inlet modification will be presented describing differences for used mass spectrometers. Commercial inlet can be replaced easily without loss of vacuum. Pressure drop influenced dramatically ion signal for both mass spectrometers. Desorption/ionization was also successfully evaluated for various surfaces, e.g. rough glass, teflon. The modification can support wider applicability of nanoDESI.

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WeP-006:
Pesticide analysis in tea

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It is estimated that the entire human population on Earth consumed over 150 billion servings of tea, which is just over 28.6 billion liters per year. It makes tea the most consumed traditional natural beverage worldwide. As any plant, tea production has to be protected from pest and diseases, therefore pesticides are used. Recent decades there is pressure to control the level of pesticides in human beverages like a tea. New pesticides are developed and some of them are no longer approved. Here we analyzed tea samples from different geographical locations for pesticide content. We used extraction method named QEUCHERS which is based on an acetonitrile extraction from solution of boiled tea, followed by drying and dissolving in methanol. We validated and calibrated the method for detection of 150 pesticides using Pesticides Comprehensive Test Mix Kit (Agilent). Samples were analyzed by Agilent 6545 iFunnel Q-TOF with UHLPC. We analyzed 20 samples from different region and found not approved pesticide substance or concetration above legal limit in 17 samples according to EU directive 2009/128/EC. Methoprotryne was the most present pesticide followed by dioxacarb. Both pesticides are not approved for use in EU countries. All together, we established pesticide assay and found not aproved substances in 17 tea samples.

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WeP-007:
Subfractionation of *Cronobacter sakazakii* membranes and proteins identification by mass spectrometry

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Cronobacter spp. are opportunistic foodborne pathogens, which could cause severe necrotizing enterocolitis and meningitis, especially to immunosuppressed people and newborns with low birth weight. The *Cronobacter* pathogenesis process remains unclear, even though several virulence factors such as outer membrane protein A (ompA), filamentous hemagglutinin and *Cronobacter* plasminogen activator (Cpa) have been already described. Identification of potentially virulent proteins could help to clarify this process.

In this work, we used the method for membrane fractions isolation (periplasm, outer and inner membrane) of a virulent strain *Cronobacter sakazakii*. We tested six detergents and different conditions for accurate dissolution of outer membrane. We choose N-Lauroylsarcosine with moderate vortex, which provided the best result. Protein concentration for each fraction was determined by Pierce™ BCA Protein Assay Kit with minor differences in workflow. Membrane fraction proteins were identified using mass spectrometry coupled with liquid chromatography (LC-MS). Subcellular localization of all identified proteins was determined by in silico analysis using four different softwares (CELLO, PSORTb, SignalP and UniProtKB database).

Altogether, over 1000 proteins were identified and divided into ten groups based on their potential virulent function.

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WeP-008:
Hydrogen-deuterium exchange usage for the DO-1 antibody epitope characterization

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Tumor suppressor p53 is the most commonly mutated protein in cancer [1]. It is a homotetrameric transcription factor composed of N-terminal transactivation domain, central DNA binding domain, tetramerization domain and regulatory C-terminal domain. Wild-type p53 is activated by various genotoxic stresses that causes its stabilization and increased DNA binding capacity. The aim of this project is to analyze molecular mechanisms responsible for conformational changes and activation of DNA binding capacity of p53. The monoclonal antibodies and structural proteomics (hydrogen-deuterium exchange – HDX) are used to detect protein epitopes and reveal potential conformational changes. We used a well characterized monoclonal antibody DO-1 as a proof of concept.

In our experiment the protein was either alone or in interaction with antibody deuterated at two time intervals. It was digested on-line with aspartic protease nepenthesin-1, followed by separation of the peptides in liquid chromatography system and measurement with a mass spectrometer Orbitrap Elite ETD (Thermo Fisher Scientific). The obtained data were evaluated by HDExaminer 1.4 (Sierra Analytics).

In MS/MS analysis of p53, 184 peptides were identified, which characterized over 85 % of the protein sequence. HDX results showed significant decrease of deuterium uptake only in transactivation domain (close to N-terminus) after interaction with DO-1. This result was in agreement with published data and phage display analysis [2]. The HDX epitope analysis is therefore suitable for the detection of protein allosteric and/or oligomerization changes after interaction with antibody.

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WeP-009:
Quo vadis glycine? Tracking the fate of isotope-labeled substrates by metabolomics and genomics

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Metabolomics using labelled compounds provide insight into cellular biochemistry under physiological conditions without the need for genetic engineering. Previous experiments showed that the chromerid alga *Chromera velia* is able to take advantage of external sources of glycine, a key metabolic compound of many anabolic reactions. Chemical-analytical techniques and bioinformatic tools were used to track the metabolism of glycine-1-^{13}C in *C. velia*. We propose a methodology to evaluate concurrently the speed of labelled glycine consumption, the ratio of ^{13}C incorporation to metabolic products, and hence the fate of glycine-derived ^{13}C. Our analyses were applied on photosynthesis-related compounds, namely the quantitation of chlorophyll a by HPLC-UV-ESI-MS, free amino acids and lipids by HPLC-ESI-MS, and monosaccharides by GC-MS. Based on the obtained profiles, *C. velia* genomic sequence data, and KEGG database data, we created glycine metabolic pathway maps along with the involved enzymes. Our results show that labelled glycine was almost entirely consumed during 15 hours after administration. The primary targets of glycine-derived ^{13}C were other amino acids and lipids, where artificial ^{13}C appeared within 7 hours after administration. Incorporation of ^{13}C to chlorophyll a was significant after 48 hours. Detailed investigation revealed insignificant enrichment of the artificial ^{13}C atoms in monosaccharides even after a month of cultivation. These findings support the hypothesis that the main source of carbon for monosaccharide synthesis is the CO_2 fixation. Furthermore, the ability to utilize organic compounds such as glycine points underlines the importance of mixotrophy in *C. velia*.

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WeP-010:
Mitochondrial ATP synthase disorders studied by quantitative proteomics of CRISPR-Cas9 knockout cell lines

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Mitochondria are cellular organelles producing almost 90% of ATP, the key energetic molecule, and mitochondrial proteome consists of approx. 1500 proteins. ATP is synthesized by the mitochondrial multi-subunit ATP synthase that together with the respiratory chain constitutes the oxidative phosphorylation (OXPHOS) system. Mutations in proteins affecting OXPHOS function comprise broad group of mitochondrial diseases and among them, disorders of ATP synthase belong to the most severe. The majority of ATP synthase disorders are caused by the mutations in the nuclear TMEM70 gene encoding mitochondrial TMEM70 transmembrane protein localized in the inner mitochondrial membrane and involved in the biogenesis of the eukaryotic ATP synthase. TMEM70 is expressed at very low levels as compared with structural subunits of ATP synthase and its molecular function is not yet known. To better understand the role of this factor, we generated TMEM70 knockout HEK293 cell lines by CRISPR-Cas9 technology. Several biochemical methods, including blue native electrophoresis, ATPase enzyme activity assay, and mitochondrial oxygen consumption revealed a defect of ATP synthase, which was fully restored upon expression of wt TMEM70. Label-free (LFQ) as well as SILAC quantitative MS analysis of knockout cell lines showed an isolated decrease of all subunits of mitochondrial ATP synthase. Co-immunoprecipitation performed as an affinity enrichment LFQ MS analysis of cellular lysates identified a few mitochondrial OXPHOS complex I proteins as interaction partners of TMEM70. Collectively, TMEM70 knockout cell lines recapitulate the pathology observed in fibroblasts from human patients and demonstrate that CRISPR-Cas9 knockouts and quantitative MS are promising tools in studying human pathologies.

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WeP-011:
A liquid chromatography/mass spectrometry method for determination of blood-brain barrier shuttle peptides

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Delivery to the brain is a major challenge in central nervous system (CNS) drug development. The blood-brain barrier (BBB) prevents access of biotherapeutics to their targets in the CNS and therefore prohibit the effective treatment of many neurological disorders. Nowadays, there is increasing interest within the biofarmaceutical industry in the development of BBB- shuttles. One of the major challenges is to find BBB-shuttle that is easy to produce, biologically stable and easy to conjugate with potential „cargo“. Immunohistochemistry or biochemical-based techniques were traditionally used for the quantification of proteins and peptides. However, LC-MS methodologies are being increasingly adopted and they offer a lot of advantages. Ultra-performance liquid chromatography – tandem mass spectrometry coupled to a triple-quadrupole mass spectrometer (UHPLC-MS/MS) provides improved separation speed, throughput, and sensitivity by employing stationary phase particles of around 2 µm or smaller. Here, we developed UHPLC-MS/MS method for quantification of several BBB-shuttle peptides. With its use we analyzed BBB permeability and plasma protein binding of candidate compounds.

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WeP-012:
C-H activation of 2-phenylpyridine and *N*-methylindole

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C-H activation reactions are useful instruments for synthesis of variable organic compounds. These reactions are catalyzed by different transition metals.The most widely used catalytic metal for C-H activation is palladium. Palladium complexes are used in combination with other reactants such as bases or reoxidation substances. Among others, palladium catalysis is often performed in presence of Ag salts in stoichiometric amounts. It is assumed, that silver compounds play a role of oxidizing agents in Pd-catalyzed cycles and a role of halogen traps in reactions with electrophilic halides. However, some recent studies suggest an active role of silver cations in the C-H activation step [1, 2]. In this work, we investigated appearance and existence of Pd-Ag intermediates in C-H activation of 2-phenylpyridine and *N*-methylindole. We studied various conditions of electrospray and various approaches to preparation of reaction mixtures.

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WeP-013:
Fecal bile acid profile in patients with short bowel syndrome

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Short bowel syndrome (SBS) occurs in patients who underwent extensive resection. The aim of the study was to verify that the changed conditions in the gut of SBS type I (jejuno/ileostomy), SBS type II (jejuno-colic anastomosis) and PN-independent SBS type II patients lead to a change in the composition of the bile acid spectrum in feces. Age- and sex-matched healthy subjects (HC) served as controls.

Bile acids (BAs) were extracted from feces by methanol extraction. Liquid chromatography was performed with a gradient elution from a column. MS analysis was performed using Q-TOF LC/MS in negative-ion mode. Data were analyzed using a database search software functions.

The BAs profile was different in HC and SBS patients. As far as primary BAs are concerned, chenodeoxycholic acid (CDCA) was elevated in all SBS groups compared to HC while cholic acid (CA) content was not different among the groups. Two secondary BAs, deoxycholic acid (DCA) and ursodeoxycholic acid (UDCA), were elevated in all SBS subjects compared to healthy controls. In contrast, litocholic acid (LCA) fecal content was reduced in all SBS groups.

In this study we describe a specific BAs signature in feces associated with SBS. The BAs profile associated with SBS is characterized by elevation of CDCA, reduction of LCA and increase in DCA and UDCA content in stool samples. Reduction of LCA in the remaining intestine may have adverse impact on the intestinal health as LCA at physiological concentration exerts anti-inflammatory properties and limits the overgrowth of some pathogens. Therefore, the restoration of normal bile acid spectrum in SBS patients may represent a promising therapeutic option.

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WeP-014:
UHPLC-MS/MS method for determination of metabolomic markers for autism spectrum disorder in human urine

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Autism spectrum disorder (ASD) is a heterogeneous neurodevelopmental disorder. Currently, the diagnosis of ASD is performed through observation, standardized behavioral scales and interviews with parents which in practice leads to a diagnosis at an average age of 4 years. Laboratory method which could detect earlier stages of the disease is of great need, as this could help patients to start with treatment prior to the appearence of the behavioral symptoms. Also, metabolomic markers could help with further differentiation and characterization of various subgroups of the autism spectrum.

In this study, we propose an ultra-high performance liquid chromatography-tandem triple quadrupole (UHPLC-QqQ) mass spectrometry method for the simultaneous determination of nine metabolites in human urine. These metabolites, namely xanthurenic acid, xanthosine, inosine, indoxyl-sulphate, indoxyl-3-acetyl acid, indolyl lactate, N-acetylarginine, methylguanidine and glucuronic acid were selected as potential biomarkers for ASD according to prior metabolomic studies. Analysis was carried out by means of reversed phase liquid chromatography with gradient elution. Separation of the metabolites was performed on a Phenomenex Luna® Omega 1.6µm Polar C18 (100 mm x 1.0 mm) column at flow rate of 0.15 ml/min with acetonitrile/water 0.1% formic acid aqueous as the mobile phase. Detection was carried out on a triple quadrupole mass spectrometer via both positive and negative ion electrospray ionization in multiple reactions monitoring (MRM) mode.

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WeP-015:
The study of perifosine in colorectal carcinoma spheroids by MALDI TOF MSI and confocal microscopy

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Before any newly developed drug reaches the market, it is first verified in vitro on cell lines and only later on laboratory animals and humans. Many cancerostatics work perfectly in 2D cell models, however they fail to maintain their functionality in body tumours because of their poor penetration to deeper cell layers; or close linkage to cell metabolism, proliferation etc. The biological gap between cell lines and animals is very wide and therefore, 3D cell cultures (spheroids) were developed to bridge it. These spatial objects of size approximately 1 mm are composed from standard 2D cancer cells, but they much precisely mimic the behaviour of cells in body tumours, both physically and with respect to their microenvironment.[1]

In our study, the spheroids derived from colorectal carcinoma and potential cancerostatic perifosine were studied. The cellular objects were treated by perifosine for selected time interval (2, 8 and 24 hours) and stained by two viability markers (calcein, propidium iodide) one hour before processing for matrix-assisted laser desorption ionization mass spectrometry imaging (MALDI MSI), according to previously published protocol [2]. The analysis of sections under confocal microscope was carried out before matrix application (in our case DHB sublimation). The examination of selected fluorescent molecules provided information about cell viability across the section, which was then related to perifosine distribution detected by MALDI MSI. Interestingly, both perifosine and the live cell marker calcein were found to diffuse from spheroid to gelatine during sample preparation.

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WeP-016:
Analysis of fatty acid methyl esters using temperature-programmed HPLC and micro-APCI-MS detection

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Atmospheric pressure chemical ionization (APCI) is a useful technique for nonpolar and low-polar compounds [1]. The analytes are ionized by a sequence of charge and proton transfer reactions involving nebulizing gas and solvent molecules. APCI can be easily used in normal-phase or non-aqueous reversed-phase (NAPR) HPLC for detecting fatty acid methyl esters (FAMEs) [2]. Analytical HPLC separations are increasingly carried out at low flow rates, especially when high sensitivity is required for limited sample amounts. Currently, nano-, capillary-, or micro-flow HPLC/MS rely on electrospray ionization, which offers sensitive detection for many analytes. Unfortunately, commercial ion sources are designed for high sample flow rates. In this project, a micro-APCI source with a heated nebulizing chip [3] was assembled. The applicability of the ion source for FAMEs was studied using direct infusion experiments and micro-flow HPLC. In micro-APCI, FAMEs provided abundant molecular adducts $[M+H]^+$ as in the case of conventional high-flow APCI. The sensitivity of the detection in micro-APCI was tested for methyl laurate dissolved in various solvents appropriate for HPLC. Toluene was found to be the best solvent regarding the signal intensity. The detection limit of FAMEs ionized in micro-APCI was two orders of magnitude lower than for conventional APCI. The NAPR-HPLC/micro-APCI was optimized for FAME mixtures. An isocratic micro-HPLC with C18 stationary phase and acetonitrile were used. Good separation of FAMEs according to the number of aliphatic carbon carbons and cis/trans isomerism has been achieved using column temperature gradient from 5°C to 50°C. The increase of column temperature was beneficial for lowering retention of long-chain FAMEs.

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ThP-017:
Identification and quantification of cell membrane proteins regulated by interferons

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Membrane proteins represent one third of the proteins encoded by human genome. They participate mainly in cell-cell and cell-extracellular matrix interactions and in regulation of cell growth and differentiation. They also have an important role in anti-cancer immune response together with secreted proteins such as interferons. Interferons (IFNs) are cytokines produced by immune cells. They regulate expression of many types of genes in normal and cancer cells and have an impact on cancer cell behavior including metastatic spread and interactions between immune system and cancer. The aim of our study is to discover proteins localized on the surface of cancer cells that are regulated by type I and type II interferons and could be important as potential targets of biological therapy.

We treated SiHa cells with IFN-α14 and IFN-γ for 24 hours. Pulsed stable isotope labeling method was used for quantification of newly synthetized proteins after IFN treatment. The proteins on the surface of live cells were biotinylated and isolated with streptavidin beads. Protein fraction enriched by membrane proteins was measured with Orbitrap Elite ETD and the obtained results were evaluated with Proteome Discoverer 1.4 and DAVID database.

We successfully isolated a protein fraction highly enriched by membrane and cell surface proteins. In this fraction we identified and quantified cell surface and other proteins that are regulated with type I and/or type II IFNs, including HLA-A, HLA-B, HLA-C, STAT1, IFIT2, CD47, ICAM1, SLC12A7, NOTCH3, ITGB8 and other potentially interesting proteins. These proteins are important for regulation of immune response and cell interactions and could help us to understand the role of IFNs in tumor growth and progression.

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ThP-018:
Identification of molecular pathways involved in the etiology of neurofibrillary degeneration of tauopathies

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Tauopathies represent a group of neurodegenerative disorders characterized by accumulation of neurofibrillary tangles which are composed of pathological tau protein. Alzheimer's disease, the most prevalent form of tauopathy, is the leading cause of dementia. Until today, there is no cure and no early preclinical diagnostic assay available for this neurodegenerative disease. Mechanism leading to the formation of pathological tau in diseased brain is still not fully understood. Identification of proteins interacting with pathological and physiological forms of tau protein may lead to identification of novel targets for therapy, diagnostic biomarkers and molecular pathways which play role in tau pathology.

We applied a novel strategy to identify molecular pathways that are involved in the etiology of pathology of neurofibrillary formation. Tau proteins were *in vivo* crosslinked in brains of transgenic rat models for human tauopathies and control animals. Isolated and purified complexes containing tau proteins were analyzed by MALDI-ToF/ToF tandem mass spectrometry. After statistical evaluation of data, we identified over 20 novel putative tau interacting partners, which may play role in neurofibrillary degeneration.

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ThP-019:
Proteomic analysis of Mason-Pfizer monkey virus virions

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Mason-Pfizer monkey virus (M-PMV) belongs to the *Retroviridae* family, similarly to the human immunodeficiency virus (HIV). Although, M-PMV causes AIDS-like disease in its host organism, it differs from HIV in the genome and virion structure, as well as in its morphogenesis. Recent studies also points to the fact that M-PMV most likely uses other cellular pathways during its life cycle than HIV.

LC-MS/MS-based proteomic approaches has already been used to analyze the composition of purified HIV-1 virions. In this study, we performed proteomic analysis of M-PMV virions produced in human embryonic kidney (HEK) cells to identify host cell proteins that are incorporated into newly formed particles. The potential contaminating proteins coming from non-virion particles co-purifying with virions were exclude from the analysis by the use of control samples obtained from uninfected cells, as well as by measuring the virion samples in repetitions. Additionally, label free quantification was used to reveal proteins reproducibly incorporated into the virions, since their reliable identification could help to clarify the mechanisms of morphogenesis or virulence of M-PMV.

About sixty of host cell proteins were stably identified in whole M-PMV particles produced in HEK 293T cells. From those, the proteins participating in clathrin-mediated endocytosis or proteins of the ESCRT complex were identified. Those proteins were already found to be used also by HIV-1 when entering or leaving the host cell. Additionally, another proteins, such as proteins involved in COPI and COPII mediated endosomal transport, were identified exclusively in M-PMV virions. This suggests that M-PMV uses different transport pathways of its proteins within the host cell.

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ThP-020:
Proteomic analysis of rat brain capillaries reveals differential protein expression in response to neurofibrillary pathology

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The blood brain barrier (BBB) is often regarded as a passive barrier that protects brain parenchyma from toxic substances, circulating leukocytes, while allowing the passage of selected molecules. Structural and functional changes of BBB with subsequent increased vascular permeability are associated with numerous diseases and conditions that affect central nervous system, including tauopathies. Tauopathies represent a heterogeneous group of neurodegenerative disorders characterized by abnormal depositions of hyperphosphorylated microtubule associated protein- tau into intracellular neurofibrillary tangles.

In this study, proteomics analyses of brain capillaries isolated from transgenic rat model for tauopathies were performed. Proteins found to be differentially expressed were identified with matrix-assisted laser desorption/ionization time-of-flight/time-of-flight mass spectrometry (MALDI TOF/TOF MS) analysis using ICPL™ technology for comparative quantification of proteins. Rat brain capillaries from transgenic model for tauopathies reveals differential protein expression in response to neurofibrillary pathology. 8 proteins were significantly upregulated, and 10 proteins were downregulated. We showed decreased expression of fatty acid-binding protein that coordinate lipid response in cells, plasma membrane calcium-transporting ATPase, glutamine and serine-rich protein, ubiquitin carboxyl-terminal hydrolase and annexin V.

On the other hand, we detected increased expression of glutamate dehydrogenase and heat shock protein HSP 90.

These findings build up a comprehensive profile of the endothelial proteome and provide a useful basis for further analysis of the pathogenic mechanism that underlies neurodegeneration in tauopathies.

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ThP-021:

Fractionation of complex peptide mixtures using basic pH reversed phase chromatography with microgradient separation technique

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Complex peptide mixtures often require multidimensional chromatography separation prior their mass spectrometry analysis in proteomic studies. Because (nano)ESI-MS/MS is commonly coupled with reversed phase liquid chromatography in acidic pH (e.g. in 0.1% formic acid), another sufficiently orthogonal liquid chromatography technique has to be used for fractionation of complex peptide mixtures. Next to ion exchange chromatography techniques, a reversed phase liquid chromatography of peptides in basic pH mobile phases can also be used [1]. In this work, the microgradient separation technique consisting of gastight microsyringe connected to a home-made reversed phase capillary column was applied for fractionation of low microgram amounts (5-60 micrograms) of complex peptide mixtures. This type of microgradient separation uses a gradient of acetonitrile in water containing ammonium formate (pH 10). Peptides may be fractionated into e.g. 17 or 33 fractions. These fractions were first dried in a vacuum centrifuge and then analyzed using nanoLC-MS/MS (QExactive) with concurrent UV detection. In the final setup, the number of identified proteins from a cell lysate sample increased about three times and similarly the number of identified peptide sequences was increased approximately four times. This approach is applicable in general for shotgun proteomics experiments, including e.g. SILAC and iTRAQ based relative protein quantitation. Finally, this technique is cost effective and fast, because the fractionation takes only about 45 min per sample.

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ThP-022:
Proteomic analysis of exosomes from porcine seminal plasma

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Exosomes are 30-150 nm sized vesicles released by cells to the extracellular space. They are formed by a lipid bilayer membrane with an internal cargo of proteins, RNA or even DNA. Exosomes may serve to monitor cellular processes and to search for biomarkers of diseases in body fluids.

Huntington's disease (HD) is a hereditary neurodegenerative disorder caused by mutation in huntingtin (HTT) gene resulting in prolonged polyglutamine sequence at N-terminus, leading to progressive motor, behavioural and cognitive decline and death. The Libechov transgenic HD (TgHD) minipig model expressing N-terminal part (548aa with 124Q) of human mutated huntingtin exhibits mild neurological impairment. Moreover, TgHD boars show decreased fertility after 1st year of age.

We aimed to develop techniques for isolation and proteomic characterization of exosomes from seminal plasma of the minipig HD model.

We have isolated exosomes by differential centrifugation and ultracentrifugation. Size of vesicles was determined by electron microscopy and flow cytometry. Successful isolation of exosomes was confirmed by western blot for positive and negative markers (Alix, Lamin, nucleophosmin, ATPalpha, UQCRC1 and Beta-tubulin). Presence of (mutated) huntingtin was detected using EPR5526 and 1C2 antibodies.

For proteomic analysis of exosomes we employed Filter Aided Sample Preparation (FASP) method using different detergents (RIPA buffer, Triton X-100 and SDS) and LC-MS/MS (Eksigent nanoLC 425 coupled to Sciex TripleTOF 5600+). A total of 800 proteins were identified with 1% FDR, including 69 from the 100 most frequently identified exosomal proteins (ExoCarta).

In conclusion, we have established a proteomic platform for HD biomarker discovery in porcine body fluid exosomes.

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ThP-023:
Identification of potential deubiquitination enzymes in pathogenic bacteria *Francisella tularensis*

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Ubiquitination process is an essential regulatory mechanism in cell homeostasis. Part of the mechanism is driven by deubiquitination enzymes (DUBs) cleaving ubiquitin (Ub) molecule from ubiquitinated proteins. DUBs play a very important role in retention of ubiquitin's homeostasis and correction of Ub-protein's conjugates. Activities of several host DUBs are known to be modulated by some of pathogenic microorganisms during the infection process. Some of them possess their own DUB(s) that may be involved in the host-pathogen interaction during infection [1]. The aim of this work is to identify potential DUB(s) in the intracellular bacteria *Francisella tularensis*. The cell lysate of *F. tularensis* was incubated with specific probe designed for isolation of cysteine protease like DUBs [2]. Isolated proteins were digested with trypsin, identified by nanoLC-MS/MS analysis using QExactive instrument and the obtained data were processed in Proteome Discoverer software connected to Mascot database searching engine. The workflow was tested for two different cultivation media (Chamberlain and BHI medium) and the results were compared using label-free quantitation approach. Several identified proteins were submitted to a bioinformatics tool Phyre2 for a structural comparison and identification of potential DUBs among them. The next work will focus on label-free comparison of isolated sample and corresponding control isolated without the specific-probe.

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ThP-024:
Silencing of carbonic anhydrase I enhance a malignant potential of prostatic (PC_3) cancer cells

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Carbonic anhydrases (CAs) are zinc metalloenzymes that catalyze the reversible hydration of carbon dioxide to a bicarbonate ion and proton. Besides a role in pH homeostasis and respiration, the isoenzyme CA I can be also involved in erythroid differentiation and some pathological processes as anemia, chronical acidosis, diabetic macular edema, proliferative diabetic retinopathy, and vasogenic edema. It was found that variation in expression of CA I can be associated even with some malignancies. The low level of CA I in the colonic epithelial cells might serve as an indicative and specific marker for prediction of colorectal cancer. On the other hand, patients with prostate cancer contained an increased level of CA I peptide fragments in the plasma compared to the healthy controls.

In our previous work, we reported that some patients who relapsed after high-dose therapy (HDT) and autologous stem cell transplantation (ASCT) were positive for autoantibodies against CA I, and the tumors in these patients spontaneously regressed. These patients' sera, which were positive for autoantibodies against CA I, were further used to examine their effect on the biology of tumor cells grown *in vitro*. We have shown that the expression of the CA1 mRNA was up-regulated in the tumor cells during the treatment. The mRNAs coding for proteins associated with basal lamina assembly, cytoskeleton, WNT7B and collagen triple helix repeat containing 1 (CTHRC1) were downregulated. To examine the effect of the opposite phenomenon, the CA1 mRNA was silenced by RNA interference system in PC3 cells. Our preliminary proteomic result suggests that knock-down of the CA1 mRNA in PC3 cells alters the proteome and secretome of cancer cells to enhances their malignant potential.

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ThP-025:
mAssistent: converter of mass spec file formats

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Mass spectrometry is a sophisticated tool for proteomics and metabolomics analysis. Unfortunately, there is available wide range of different types of measurement devices from different producers. Therefore, there is also various file formats specification from ASCII files to binary formats. On the other hand, the data processing software have limited access to read only some of the formats. For comparison of different approaches to data analysis, it is necessary to be able to convert the file formats from each other. We developed an easy file format converter platform between most typical file formats, with possible extension for other required specifications.

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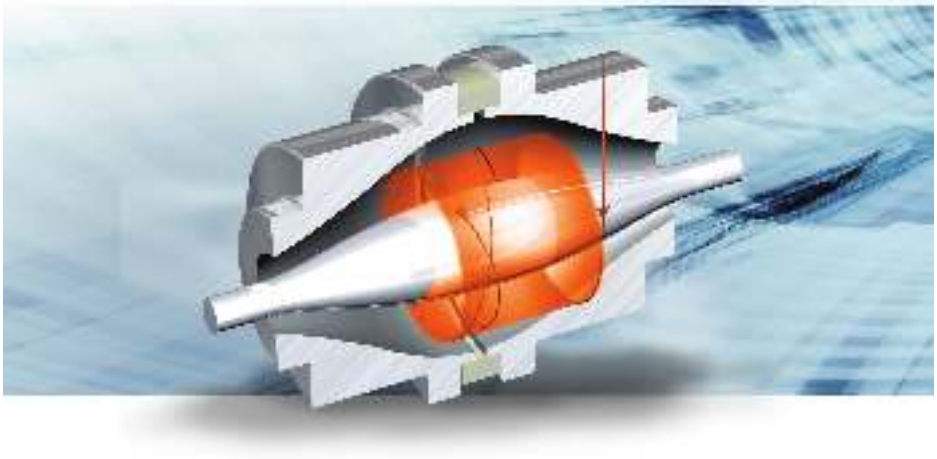

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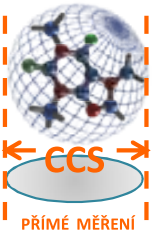

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