# EIGHTH ANNUAL CONFERENCE OF THE CZECH SOCIETY FOR MASS SPECTROMETRY

Olomouc, March 27 – March 29, 2019

BOOK OF ABSTRACTS

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

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*Czech Society for Mass Spectrometry*

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

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# Eighth Annual Conference of the Czech Society for Mass Spectrometry

## Date

*27th March – 29th March 2019*

## Venue

*BEA campus Olomouc*

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*Czech Society for Mass Spectrometry, Olomouc*

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*Petr Verner (freelancer)*

## Wednesday 27th March 2019

| | |
|---|---|
| 12:00 – 19:30 | Registration |
| 12:30 – 14:50 | Company Workshop – Pragolab |
| | *Lukáš Plaček, Pragolab; Michaela Ščigelová, Thermo Fisher Scientific: Proteome Discoverer v. 2.3* |
| 15:00 – 15:10 | Opening of the CSMS Conference |
| **15:10 – 16:00** | **Plenary lecture I. (Prof. Aleš Svatoš)** |
| *PL-1* | *Interpretation of mass spectrometry in the metabolomics: GNPS and Sirius* |
| **16:00 – 17:00** | **Session I.** |
| | *(Chairperson: Petr Fryčák)* |
| 16:00 – 16:20 | Zdeněk Spáčil |
| *WeO-001* | *Exposome research: towards functional characterization of gut microbiota and the role of inflammaging* |
| 16:20 – 16:40 | Kamila Kalachová |
| *WeO-002* | *GC/MS Ion Sources – Possibilities & differences in real life applications* |
| 16:40 – 17:00 | Zdeněk Kameník |
| *WeO-003* | *Molecular networking/GNPS for untargeted mass spectrometry-based metabolomics* |
| 17:00 – 17:20 | Coffee break |

17:20 – 18:20 *Poster talks I (Chairperson: Lenka Hernychová)*

*WeS-001* *Violetta Shestivska: SIFT-MS for real-time quantification of hydrazine and methanol as target products of electrochemical $CO_2$ reduction*

*WeS-002* *Aleš Horna: Long term monitoring of 8-hydroxy-2´-deoxyguanosine and 8-hydroxyguanosine in urine as indicators of oxidative stress using UHPLC-QqQ*

*WeS-003* *Matyáš Krijt: Determination of reference values for de novo purine synthesis intermediates in urine and dry blood drop by LC-MS/MS*

*WeS-004* *Julie Rendlová: Compositional analysis of untargeted metabolomic data using multiple Bayesian hypotheses testing*

*WeS-005* *Jan Václavík: New biomarkers of 3-hydroxy-3-methylglutaryl CoA lyase deficiency*

*WeS-006* *Štěpán Kouřil: Removing false features in metabolomics data using correlations*

*WeS-007* *Gabriela Rylová: Comparison of plasma metabolites identification by liquid chromatography /MS and direct infusion /MS*

*WeS-008* *Dominika Luptáková: Identification and classification of melanoma cancer tissue using REIMS analysis and localization of melanoma lipid remodeling by MALDI mass spectrometry imaging*

18:30 – 19:00 Company Workshop – Bruker

*Martina Macht, Bruker Daltonik GmbH, Bremen: Not only for Proteomics - so far unreached throughput, analytical depth and sensitivity using timsTOF Pro featuring PASEF*

19:00 – 23:00 Welcome mixer and poster session I (from 20:00 to 21:00)
WeS-001 – WeS-008 + WeP-001 – WeP-015

# Thursday 28th March, 2019

| | |
|---|---|
| **9:00 – 9:50** | **Plenary lecture II. (Prof. David Goodlett)** |
| *PL-2* | *Lipid A as a therapeutic and diagnostic* |
| **09:50 – 10:50** | **Session II.** |
| | *(Chairperson: David Friedecký)* |
| 09:50 – 10:10 | Petr Bednář |
| *ThO-004* | *Solids Analysis Probe Mass Spectrometry – possibilities and recent applications* |
| 10:10 – 10:30 | Soeren-Oliver Deininger |
| *ThO-005* | *Advances in MALDI imaging mass spectrometry provided by latest TOF and MRMS technology* |
| 10:30 – 10:50 | Jan Petr |
| *ThO-006* | *Potential of capillary electrophoresis connected to inductively coupled plasma mass spectrometry* |
| 10:50 – 11:10 | Coffee break |
| 11:10 – 12:10 | Poster talks II (Chairperson: Petr Pompach) |
| *ThS-009* | *Jana Václavková: Proteomic analysis of exhaled breath condensates* |
| *ThS-010* | *Růžena Lišková: Native nanoelectrospray-MS as a tool for quick evaluation of protein-DNA complex formation* |
| *ThS-011* | *Tomáš Oždian: Proteomic profiling reveals DNA damage, nucleolar and ribosomal stress are the main responses to oxaliplatin treatment in cancer cells* |
| *ThS-012* | *Hynek Mácha: Ion-mobility Mass Spectrometry of Cyclosporines* |
| *ThS-013* | *Rudolf Kupčík: Application of two amorphous $TiO_2$ nanotubes-based materials for highly selective phosphopeptide enrichment followed by nanoLC-MS/MS analysis* |
| *ThS-014* | *Jiří Houšť: Fragmentation Mechanism in Echinocandins* |
| *ThS-015* | *David Jurnečka: The hybrid molecule of adenylate cyclase toxin and α-hemolysin translocates its cell-invasive enzyme into cell cytosol and targets CD11a-positive cells* |
| *ThS-016* | *Ghazaleh Yassaghi: Top down mass spectrometry and hydroxyl radical footprinting* |

| | |
|---|---|
| 12:20 – 12:50 | Company workshop – HPST |
| | *Jitka Zrostlíková, Ondřej Lacina: Seznamte se s budoucností: Nový Agilent LC/MS s vysokým rozlišením* |
| 12:50 – 14:00 | Lunch |
| **14:00 – 15:20** | **Session III.** |
| | *(Chairperson: Martin Hubálek)* |
| 14:00 – 14:20 | Alice Sosic |
| *ThO-007* | *Mass spectrometry-based approaches in drug development: elucidation of protein-nucleic acids-ligand interactions for the investigation of small molecules mechanism of action* |
| 14:20 – 14:40 | Zdeněk Perutka |
| *ThO-008* | *Wine proteins – hidden markers of the winemaking process quality?* |
| 14:40 – 15:00 | František Filandr |
| *ThO-009* | *Detailed analysis of Lytic Polysaccharide Monooxygenase auto-oxidative degradation during catalysis* |
| 15:00 – 15:20 | Jan Fiala |
| *ThO-010* | *Native mass spectrometry of protein/nucleic acid complexes: the case of FOXO4/DAF16 complex* |
| 15:30 – 16:00 | Company workshop – Merck |
| | *Stanislav Kukla: Welcome to our new Merck universe – hot news from Sigma-Aldrich, Millipore, Supelco and Milli-Q portfolio brands* |
| 16:00 – 16:20 | Coffee break |
| **16:20 – 17:00** | **Zdeněk Herman Award presented by the Resonance Foundation and presentation of the winning thesis** |
| 17:00 – 17:15 | Josef Cvačka |
| *ThO-011* | *The Czech Museum of Mass Spectrometry: An Update* |
| 17:15 – 18:30 | General assembly of the Society |
| 18:30 – 23:00 | Party and poster session II (from 20:00 to 21:00)<br>ThS-009 – ThS-015 + ThP-016 – ThP-031 |

## Friday 29th March 2019

| | |
|---|---|
| **09:00 – 10:20** | **Session IV.** |
| | *(Chairperson: Petr Verner)* |
| 09:00 – 09:20 | David Friedecký |
| *FrO-012* | *Metabolomics in inherited metabolic diseases* |
| 09:20 – 9:40 | Sanja Cavar Zeljkovic |
| *FrO-013* | *An overview of mass spectrometry implementation in quality control of crops and products* |
| 9:40 – 10:00 | Jaroslava Jáčová |
| *FrO-014* | *Diagnosing of organic acidurias and beta-oxidation defects by LC-MS/MS* |
| 10:00 – 10:20 | Petr Žáček |
| *FrO-015* | *Headspace SPME in connection with GCxGC-TOF/MS – a powerful tool for analysis of volatiles in complex biological samples* |
| 10:30 – 11:00 | Company workshop – Pragolab |
| | *Dave Wanless, Thermo Fisher Scientific: Discover the world of isotopes* |
| 11:00 – 11:20 | Coffee break |
| **11:20 – 12:10** | **Plenary lecture III. (Prof. Magnus Palmblad)** |
| *PL-3* | *Mass Spectrometry Data, Analyses and Tools in Context* |
| 12:10 – 12:30 | Poster prize, Final remarks |
| 12:30 – 13:30 | Lunch |

## PL-01:
# Interpretation of mass spectrometry in the metabolomics: GNPS and Sirius programs

Aleš Svatoš [1] *, Riya Menezes [1]

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In the lecture I compare two ways of in silico interpretation of CID spectra obtained from extracts from different *Arabidopsis thaliana* mutants measured on a UPLC-MS/MS system using a DDA acquisition on a Q-Exactive HF-X spectrometer. For intensity normalization, retention time correction, and peak-picking/integration, XCMS was used in the R-environment. The data was interpreted in GNPS [1] (https://gnps.ucsd.edu) created by Pietro Dorrestein. GNPS is a living system that is dependent on supporting contributors/curators of the spectrum. GNPS aggregates the data available in all open mass spectra databases. In contrast to the previous approaches, the CID spectra are clustered upon similarity and thus new metabolites homologues of known substances are considered as well. Spectra of known and new metabolites are part of the same structural cluster, thereby facilitating the manual interpretation of new compounds.

Sirius [2] and CSI: FingerID [3] created in Jena are going even further. Their aim is *ad hoc* interpretation of MS and MS/MS data leading to the design of possible chemical structures. Information on the exact mass and intensities of isotopic peaks obtained from LC-MS spectra are used to design a possible molecular composition. This is refined in the second step, when CID spectra are converted to oriented graphs, where the nodes constitute *m/z* values, and the edges are neutral/radical losses. The individual graphs are evaluated according to the number of allowed/forbidden losses and the best used for selecting the correct molecular composition. Data Structure PubChem is used to interpret data. Structures are converted to vectors that contain information about functional groups and structural motifs. Similar vectors are also created from oriented graphs and both vectors are compared.

In the paper I will evaluate the success of both methods on the same samples.

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**REFERENCES:**

1. *Wang M. et al.: Nat. Biotech. 34(8), 828-837 (2016).*
2. *Rasche F. et al:. Anal. Chem. 83(4), 1243–1251 (2011).*
3. *Duhrkop K. et al.: Proc. Natl. Acad. Sci. U. S. A. 112(41), 12580-12585 (2015).*

## PL-02:
# Lipid A as a Therapeutic and Diagnostic

David Goodlett [1 *]

*1. University of Maryland, Baltimore*

Lipid A is the membrane anchor for Gram-negative bacteria that holds the much larger lipopolysaccharide (LPS) molecule in place in the outer membrane. Importantly in mammals, Toll-like receptor 4 (TLR4) recognizes lipid A the result of which is activation of a cytokine cascade that can aid the host in clearing the infection or if unchecked lead to a deadly cytokine storm. There are a range of activities from agonistic to antagonistic that are directly related to structure. To exploit this we are working to better define the lipid A structure activity relationship for use as a vaccine adjuvants and antisepsis therapeutics (e.g. [1]). We are also using lipid A and related Gram-positive molecules to identify bacteria direct from source in under an hour [2]. At the ICCVS in Gdansk we are interested in investigating the classic use of bacterial extracts as an immunotherapy (i.e. Coley's toxins late 1800s NYC) that have been recently revived [3]. We are also working to define protein antigens that can be used as imaging agents, therapeutics and diagnostics in point of care devices [4]. I'll present the above topics from the perspective of how mass spectrometry is helping to solve these problems.

* *Correspondence: david.goodlett@gmail.com*

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2. *Leung L.M. et al.: Sci. Rep. 7, 6403 (2017).*
3. *Kim O.Y. et al.: Nat. Commun. 8, 626 (2017).*
4. *Freiberg J.A. et al.: mSystems 1, e00149-16 (2016).*
5. *Yoon S.H. et al.: Rapid Commun. Mass Spectrom. 30, 2555-2560 (2016).*

## PL-03:
# Mass Spectrometry Data, Analyses and Tools in Context

Magnus Palmblad [1 *]

*1. Leiden University Medical Center*

Mass spectrometry has been used across many omics domains, primarily proteomics and metabolomics with their specializations, such as glycomics or lipidomics, but also to targeted SNP analyses and epigenetics. Despite being a Jack of many trades, understanding complex biological problem requires additional information and context not provided by mass spectrometry measurements alone. Here I will give a brief and personal history of integrating mass spectrometry data with genome-wide SNP, gene expression and small-molecule data, across time and spatial dimensions [1].

Much information is also held in databases, repositories and the scientific literature. Though considerable text mining effort have been spent on building gene-disease and protein-protein interaction networks, we can also probe the literature for mass spectrometry measurements. This information in turn tells us something about bias in data, or the general applicability of separation, ionization, fragmentation and detection methods. In this talk, I will show how to combine text mining and machine learning to visualize patterns in the literature and databases in ways familiar to practitioners of mass spectrometry [2].

Just as one type of measurement cannot answer all questions, no one tool can execute all data analysis tasks. Complex questions require combination of software tools. For reproducible and scalable analyses, these tools need to be combined in automated, documented workflows, guiding the analysis from raw data all the way to final statistical analysis and visualization. I will report on recent efforts [3] to simplify finding tools fit-for-purpose and automatically assemble them into workflows, both with emphasis on mass spectrometry data analysis.

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2. *Palmblad M.: Anal. Chem. in press (2019).*
3. *Palmblad M. et al.: Bioinformatics 35(4), 656-664 (2019).*

## WeO-001:
# Exposome research: towards functional characterization of gut microbiota and the role of inflammaging

Veronika Vidová [1], Gabriela Dovrtělová [1], Kateřina Coufalíková [1], Eliška Stuchlíková [1], Anne-Christine Aust [1], Jana Klánová [1], Zdeněk Spáčil [1] *

*1. Masarykova univerzita, Centrum RECETOX*

Exposome paradigm presents an opportunity to understand the environmental basis for human health and disease. The human gut microbiota represents a key external factor affecting the metabolism and overall health of the host through immunomodulation. A similar mechanism is relevant for inflammaging, the chronic physiological stimulation of the innate immune system, associated with the development of diseases. An appropriate multi-omics toolbox is required to explore immune-metabolic viewpoint for age-related diseases.

We applied tandem mass spectrometry assays using a triple quadrupole mass analyzer (selected reaction monitoring – SRM) for metabolic profiling of tryptophan and kynurenine pathway and membrane lipids in biofluids and cell culture. SRM-proteomics was used for determination of inflammatory markers. The untargeted high resolution/accurate mass (HR/AM) mass spectrometry (Orbitrap Fusion, Thermo Scientific) was used for metabolic screening.

Results demonstrated certain microbial metabolites to be clinically relevant markers of inflammation or a disease condition, documenting a tight connection between microbial colonization and the host immune system. We have mapped the distribution of microbiota-associated metabolites within biofluids in adults, pregnant women, and neonates and linked them to levels of acute phase proteins. We have completed a study on age-dependent alterations of neuronal membrane lipids in rodents with translational importance for neurodegenerative diseases.

The study is pioneering the functional characterization of microbiota, mediated via stimulation of the immune system and essential to understanding its role in human health. This is consistent with age-dependent changes, underlying inflammaging and neurodegeneration.

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## WeO-002:
# GC/MS ion sources – possibilities & differences in real life applications

Kamila Kalachová [1 *]

*1. HPST, s.r.o.*

Since the first mass spectrometer (MS) for gas chromatography (GC) was introduced in 1982, the development of GC/MS technique made a significant step forward. In this presentation, comparison of two different ion sources, currently available in Agilent GC/MS instruments, will be shown. More sensitive version of traditional ion source with extractor lens and newly developed high efficiency ion source with completely new design will be introduced. Real life results will be presented, using two different applications focusing especially on sensitivity differences between these two ion sources. The first study shows results of the steroids analysis using Agilent GC/MS triple quadrupole. In the second study, GC/MS single quadrupole connected to static headspace autosampler for volatile organic analysis (VOCs) is employed. Besides the sensitivity, the repeatability and long-term stability of the systems will be demonstrated. Additionally, different ways of instrument sensitivity usage will be revealed.

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## WeO-003:
## Molecular networking/GNPS for untargeted mass spectrometry-based metabolomics

Zdenek Kamenik [1 *]

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

My talk will introduce molecular networking/GNPS that was developed by the research group of Pieter Dorrestein at UCSD (San Diego, USA) as a workflow to process and analyze data for mass spectrometry-based untargeted metabolomics. The talk will cover principle, options, and several application examples of the approach. I will specifically show how metadata or molecular cartography can be used for the data analysis; how novel compounds were discovered; how you can give a context to your data within other data sets.

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## ThO-004:
# Atmospheric Solids Analysis Probe Mass Spectrometry – possibilities and recent applications

Petr Bednář [1] *, Lukáš Kučera [1], Monika Cechová [1], Petr Barták [1], Petr Smýkal [2], Jaroslav Peška [3], Karel Lemr [1]

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Atmospheric Solids Analysis Probe Mass Spectrometry (ASAP-MS) is a tool for direct and fast analysis of solid and liquid materials without complicated sample preparation. It gained popularity especially in petroleomics and characterization of polymers but has been used also for study of plant materials [1]. This communication deals with application of ASAP-MS in archaeological, art and botanical research.

This technique allowed detection of triterpenoid miliacin in soil content of vessels dated to Eneolithic period found during excavation in Central Moravia (Czech Republic). Miliacin is a chemical marker of broomcorn millet. The proof of this cereal by the presence of miliacin appears to be possible even if grain residues are not found in sample for paleobotanical expertise due to decomposition. The identification of millet in Eneolithic context is extraordinary and can be considered as the first direct evidence of millet in Central Europe [2]. Besides, recently we utilized ASAP-MS for characterization of oils used as binders in paintings. ASAP-MS was for the first time used also for analysis of legume seeds. It can be readily used to study the fatty acids (FA) profile present in seed coats of whole and intact pea seeds. Classification of pea genotypes with respect to physical dormancy and investigation of related biological markers were possible by multivariate statistics. ASAP-MS showed better selectivity and signal of FA compared to Laser Desorption Ionization Mass Spectrometry providing on the other hand spatial distribution information. Nevertheless, results obtained by both techniques are in mutual agreement [3]. Presently, the possibilities of both techniques to analyze small parts of plant tissues (e.g. cell layers) are studied.

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## ThO-005:
# Advances in MALDI imaging mass spectrometry provided by latest TOF and MRMS technology

Soeren-Oliver Deininger [1 *]

*1. Bruker Daltonik GmbH, Bremen*

Over the last two decades, mass spectrometric imaging (MSI) has evolved into a powerful tool being utilized in various branches of lifescience research. MALDI imaging in particular has benefitted a lot from the continued implementation of new advanced mass spectrometric technologies as well as the availability of vastly improved software required for in-depth analysis of imaging MS data.

Various mass spectrometry platforms are well suited for MALDI imaging, for example MALDI-TOF and MALDI-MRMS instruments. Preference for the one or the other of these MS platforms depends on the final aim of analysis as well as on various practical aspects , f.e. sample characteristics, sample throughput required etc..

MALDI-TOF instruments are particularly versatile with regard to accessible m/z range, allow for highest possible data acquisition speed, and, due to latest developments in laser technology, enable an ultimate level of pixel definition. MALDI-MRMS, being highly complementary to MALDI-TOF, has its key merits in the extremely high resolution it offers together with unparalleled mass accuracy.

The talk will briefly highlight the most important key technology features that have been implemented rather recently. A selection of application examples will be presented to illustrate the particular strengths of MALDI imaging methods based on latest MALDI-TOF and MALDI–MRMS technology, respectively.

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## ThO-006:
# Potential of capillary electrophoresis connected to inductively coupled plasma mass spectrometry

Jan Petr [1] *, Daniel Baron [1], Andrea Šebestová [1], Petra Švecová [1], Radka Pechancová [1], Tomáš Pluháček [1]

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Capillary electrophoresis (CE), well-known separation technique, connected with inductively coupled plasma mass spectrometry (ICP-MS), an element-specific detector, represents an interesting and beneficial tool in the field of analytical chemistry. CE allows easy manipulation with the separation selectivity; it has low electrolyte and sample consumption. ICP-MS can detect and quantify elements, metalloids and metals, with excellent limits of detection and a wide linear dynamic range. In our work, the CE 7100 Agilent instrument was hyphenated to the 7700x Agilent ICP-MS instrument using a lab-made cross-piece interface. The interface was further optimized on the separation of Cd(II) and Pb(II). Then, the connection of CE-ICP-MS was used for the study of interaction of carboxylic magnetite core-shell nanoparticles (NPs) with polymyxin B. Here, two different interaction regions were identified and analyzed (interaction constants of 15 (4) x $10^6$ $M^{-1}$ and 16 (2) x $10^3$ $M^{-1}$ and stoichiometry of 0.7 (0.3) and 3.5 (0.5), respectively). CE-ICP-MS was also applied for chiral separation of (R,R)- and (S,S)-oxaliplatin. Under optimal conditions, 40 mM sodium borate buffer pH 9.5 with 60 $mg.mL^{-1}$ sulfated β-cyclodextrin, the separation was obtained in 10 minutes with a resolution of 2.0. LOD was 64 $ng \cdot mL^{-1}$ what represents detection of 38 femtograms of oxaliplatin in the CE sample zone. Finally, CE-ICP-MS was used for the Taylor dispersion analysis of carboxylic magnetite NPs in electrolytes containing Au NPs. Interesting behaviour of both NPs including dispersion as well as their interaction was observed due to the use of element specific detection ($^{56}Fe$, $^{197}Au$).

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## ThO-007:

# Mass spectrometry-based approaches in drug development: elucidation of protein-nucleic acids-ligand interactions for the investigation of small molecules mechanism of action

Alice Sosic [1 *], Richard Goettlich [2], Dan Fabris [3], Barbara Gatto [1]

*1. University of Padova*
*2. Justus Liebig University Giessen*
*3. University at Albany-SUNY*

The HIV-1 nucleocapsid (NC) protein is a nucleic acid chaperone playing a pivotal role in essential steps of the viral life cycle and represents an excellent molecular target for drug development. Even if different classes of molecules have been proposed as anti-NC agents, drug-candidates interfering with NC functions are still missing in the therapeutic arsenal against HIV. [1]

Searching for new inhibitors, we proposed a novel anti-NC strategy that draws the spotlight on nucleic acid substrates of NC. Two classes of compounds, differing in their mode of interaction with nucleic acids, have been identified as potent *in vitro* NC inhibitors. 2,6-dipeptidyl anthraquinones [2] are able to interact with nucleic acids in a non-covalent manner, whereas bis-3-chloropiperidines (B-CePS) [3] are explored as RNA cross-linking agents.

Binding details have been widely investigated by direct infusion electrospray ionization mass spectrometry (ESI-MS). The analyses were performed in nanoflow ESI mode by using quartz emitters produced in-house. Our MS-based approach allowed us 1) to unambiguously elucidate at the molecular level the reactions of small molecules with the protein and the nucleic acid substrates, and 2) to demonstrate the actual mechanisms of NC inhibition *in vitro* for both classes of chemical entities.

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## ThO-008:
# Wine proteins – hidden markers of the winemaking process quality?

Zdeněk Perutka [1,2 *], Miloslav Šufeisl [3], Miroslav Strnad [4,5], Marek Šebela [1,2]

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*2. Faculty of Science, Palacký University, Olomouc, Czech Republic*
*3. Šufeisl Winery, Oslavany, Czech Republic*
*4. Laboratory of Growth Regulators, Palacký University, Olomouc, Czech Republic*
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Grape proteins are important components of wine contributing to its chemical and organoleptic properties. Alterations in their quantity and quality are caused by the enviromental and biotic stress events during grape development and maturation. The most abundant are PR proteins, which are highly resistant to proteolysis and stable under acidic conditions: chitinases and thaumatin-like proteins. The presence of yeast and mold proteins in wine may reflect also the winemaking process with other factors such as the amount of accessible nutrients, decreasing pH during the fermentation period or applied fining procedure.

We analyzed proteins in experimental Moravian white wines characterized by their instability and haze formation in bottles during storage despite prior bentonite treatment. To study the relationship of wine proteins and haze we carried out proteomics of hazy and clear white wines produced with partly or largely botrytized grapes and standard reference wines. Wine proteins were identified after their extraction, electrophoresis and tryptic digestion by reversed-phase liquid chromatography of peptides coupled with tandem mass spectrometry. The use of a simple membrane separation process (dialysis, ultrafiltration) and gel permeation chromatography allowed us to fractionate proteins under native conditions. Plant defense proteins, yeast glycoproteins and various enzymes from *Botrytis*, particularly hydrolases, were found [1].

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## ThO-009:
# Detailed analysis of lytic polysaccharide monooxygenase auto-oxidative degradation during catalysis

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Lytic polysaccharide monooxygenases (LPMOs) are industrially important cellulolytic enzymes used in cellulose saccharification enzyme cocktails and are promising enzymes to use in mass-production of second generation biofuels. Unlike standard cellulases and glycosidases, they degrade polysaccharides oxidatively instead of hydrolytically. Their active site harbours copper ion, which upon its reduction from $Cu^{2+}$ to $Cu^{1+}$ generates reactive oxygen species (ROS). Active site is located on a flat surface surrounded with aromatic amino acids facilitating substrate binding enabling created ROS to precisely attack β-1-4 glyosidic bond in polysaccharides. Owing to the shape of the catalytic site, they have the ability to depolymerize recalcitrant crystalline cellulose structures not degradable by standard glycosidases, which is a bottleneck in current biofuel production. LPMOs are however notoriously known for their low stability during reaction caused by auto-oxidative damage occurring on enzyme in solution in absence of suitable amount of substrate binding sites. We used mass spectrometry to analyse oxidative amino acid side chain modifications, as well as oxidative peptide bond cleavages in time dependant manner in LPMO9c incubated under reducing conditions with the aim to provide information for potential rational engineering of the enzyme to increase its stability during catalysis.

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## ThO-010:
# Native mass spectrometry of protein/nucleic acid complexes: the case of FOXO4/DAF16 complex.

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Formation and dissociation of protein/nucleic acid complexes play important role in many kinds of cell processes and their regulations. Since native mass spectrometry has been previously used to gain structural information of proteins and protein complexes, we initiated a study to utilize ion mobility spectrometry (IMS) for structural characterization of protein/nucleic acid complexes. Although very little is known about the behavior of protein/nucleic acid complexes in gas phase, we started to investigate the influence of ionization and activation on stability of protein/nucleic acid complexes. Forkhead-box transcription factor (FOXO4), dsDNA sequence (DAF16) and their complex served as model system where different conformers were identified according to their collisional cross-sections (CCS).

Stability of FOXO4, DAF16 and FOXO4/DAF16 complexes were investigated in the liquid, liquid-gas and gas phase. Solution activation was performed by placing nanoemitter filled with sample into temperature controlled copper block. Liquid-gas phase stability was studied transferring droplets through thermostated stainless-steel capillary placed in-front of instrument gas cone. Gas phase activation was performed either by ramping voltage of sampling cone element situated behind ion source entrance orifice or increasing collision voltage in collision cell. According to our results, the increasing solution temperature led not surprisingly to complex melting. Different behavior was observed in the gas phase. When FOXO4/DAF16 complex was formed it is hard to dissociate it. Surprisingly, binding of DAF16 on FOXO4 even increased the FOXO4 stability in the gas phase when comparing CCS values for apo- and holo-forms of FOXO4.

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## ThO-011:
# The Czech Museum of Mass Spectrometry: An Update

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The history of the study of matter known as mass spectrometry is longer than one and a half century. The foundations of mass spectrometry were laid by physicists who explored discharges in gasses. Later it has become an excellent tool for chemical analysis, and in recent years, we are witnessing its expansion into the biological sciences. Mass spectrometry technology has evolved over time.

Key technologies of mass spectrometers are often hidden under a cover that does not allow the user to see them. The Czech Museum of Mass Spectrometry displays components of such technologies. The visitors have the opportunity to see technical solutions used in the past and present instruments. The exhibition encompasses devices for generation of ions, manipulation with them in electric and magnetic fields, and detecting them. The museum also aims at collecting historical documents, photographs, and artifacts that relate to the history of mass spectrometry in the Czech Republic and former Czechoslovakia.

This lecture is an update on the current situation and future development of the Czech Museum of Mass Spectrometry.

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## FrO-012:
# Metabolomics in inherited metabolic diseases

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Inherited metabolic diseases (IMD) are genetic defects compromising enzymatic or transport protein functions, resulting in abnormalities in human metabolism. Accumulations of toxic substrates of the affected enzymes are the predominant cause of clinical symptoms and the key to the disease diagnosis. Historically, new biomarkers were found either accidentally and/or following biochemical logic. Effective biomarkers (high sensitivity and specificity) are especially important in population-based screening programmes, where false results have very serious consequences.

Untargeted metabolomics was applied to identify new potential biomarkers of several IMDs and multiple stage LC-HRMS (Orbitrap technology) was used for their structural elucidation. We analyzed biofluids from patients suffering from diseases in amino acid (e.g. phenylketonuria) and organic acid metabolism (e.g. 3-hydroxy-3-methylglutaryl-CoA lyase deficiency). In studied diseases new biomarkers were found. As an example, we were able to find four new biomarkers in phenylketonuria, a disease known since 1934, heavily investigated and population screened worldwide. All the newly identified biomarkers contain phenylalanine moiety (eg. Phe-Glc, Phe-Phe, N-lacPhe, Glu-Phe) and can be explained in the biochemical context. In all of the diseases studied we were able to perform fragmentation to third up to the sixth stage in biofluids without purification or preconcentration providing unequivocal chemical structure mostly to the MSI 2 level.

Untargeted metabolomics and HRMS fragmentation are an effective and promising tool in discovering new biomarkers and diagnosing of IMDs.

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## FrO-013:
# An overview of mass spectrometry implementation in quality control of crops and products

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The quality and safety of crops, including medicinal plants and vegetables, are in high demand nowadays, which is extremely dependent on their complex phytochemical analysis. Due to the different environmental and genetic factors, there are wide varieties of a single plant species available on the market, and the knowledge of their chemical composition is extremely important, both from nutritional and medical points of view. Among all techniques for identification of compounds currently existing, the mass spectrometry and its hyphenated techniques become the major platform for chemical profiling, metabolomics and quality control of plant-based foods [1].

Here we present the brief overview of mass spectrometry techniques utilized in quantification of several different classes of low-molecular weight natural products, from polyamines which are present in minute concentrations in plant tissue, to cannabinoids, terpenoids, phenolic and fatty acids that are the major compounds in some plant-based products [2]. The advantages and drawbacks of each particular hyphenated method, coupled with electron ionization or electrospray triple quadrupole mass spectrometer are discussed, from sample preparation to data processing [3].

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## FrO-014:
# Diagnosing of organic acidurias and beta-oxidation defects by LC-MS/MS

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Analysis of organic acids offers an important insight into central metabolism. It has traditionally been performed using routine GC-MS platform that requires lengthy sample preparation and allows analysis of just small batches of samples. The GC-MS under common conditions is not capable to analyze acylcarnitines (together with organic acids) and acylglycines as polar compounds suffer from poor detection.

We developed the LC-MS/MS method that allows complex analysis of organic acids, acylcarnitines and acylglycines in biological matrices (e.g. urine, plasma). Under acidic conditions on a non-polar C18 column, detection and quantification of 124 diagnostically important biomarkers for more than 60 disorders covering organic acidurias, aminoacidopathias, fatty acid beta-oxidation disorders, and others was enabled. Analytes showed good linearity and reproducibility of results. All diseases tested (e.g. methylmalonic, glutaric and isovaleric aciduria) were unambiguously approved.

The method that minimizes GC-MS drawbacks was approved using samples of patients with inherited metabolic disorders. Alternatively, it can be applied as a sensitive, simple and robust approach for targeted metabolomics of clinical samples.

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## FrO-015:
# Headspace SPME in connection with GCxGC-TOF/MS - a powerful tool for analysis of volatiles in complex biological samples

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Biological matrixes are usually challenging analytical materials mainly due to its complexity and possible interferences [1]. Number of detectable compounds can reach thousands, even more. Traditional one-dimensional separation techniques usually do not have the capacity to handle so many analytes. In the case of analysis of volatiles in difficult matrices we can take advantage of a relatively novel analytical approach of two-dimensional comprehensive gas chromatography hyphenated with mass spectrometric detection (GCxGC-TOF/MS). Connecting of this analytical technique with headspace solid phase microextraction (SPME) sampling technique we obtain a powerful tool for analysis of volatiles [2].

We employed this tool for analysis of urine volatiles of three mice strains (BALB/c, C57BL/6, WILD). In each sample we observed around five hundred compounds. Using a software for peaks alignment we selected those which are responsible for making differences either among strains, or sexes. Some of those compounds may serve for communication purposes (semiochemicals) [3]. Moreover, we compared their mass spectra with those available in commercial mass libraries.

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## WeS-001:
# SIFT-MS for real-time quantification of hydrazine and methanol as target products of electrochemical $CO_2$ reduction

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The increase of $CO_2$ in the atmosphere has stimulated thinking on how to reduce it. The concentration of $CO_2$ in the atmosphere has recently reached the alarming limit of 400 ppm [1]. An inexpensive and broadly applicable technology capable of reducing $CO_2$ highly desired. The goal of the current project is to develop a method of the electrochemical reduction of atmospheric $CO_2$. The objective of this work is to identify and quantify volatiles produced during the $CO_2$ reduction in real time using selected ion flow tube mass spectrometry, SIFT-MS [2]. These volatile products include methanol ($CH_3OH$) and hydrazine ($N_2H_4$) that have the same nominal molecular mass (32 Da).

A detailed study of the rate coefficients and product ions of the reactions of the SIFT-MS reagent ions $HCO_3O^+$, $NO^+$ and $O_2^+$ with these two compounds has shown that protonated analyte molecules at *m/z* 33 are formed in the analyte reactions of $HCO_3O^+$ with $CHCO_3OH$ and $NCO_2HCO_4$, which complicates analysis. However, by virtue of the ionisation energies of methanol (10.84 eV) and hydrazine (8.1 eV), charge transfer rapidly occurs between $NO^+$ and $N_2H_4$ but not between $NO^+$ and $CH_3OH$. This allows these two compounds to be separately identified and quantified by SIFT-MS if both are present in a mixture such as the headspace of an electrochemical cell.

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## WeS-002:
# Long-term monitoring of 8-hydroxy-2´-deoxyguanosine and 8-hydroxyguanosine in urine as indicators of oxidative stress using UHPLC-QqQ

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The oxidative stress resulting from either overproduction of the hydroxyl free radicals or inadequate antioxidant defenses is thought to precede a number of diseases including cancer, cardiovascular, diabetes and neurodegenerative disorders. Currently urinary levels of 8-hydroxy-2´-deoxyguanosine are being studied as a biomarker for estimating oxidative stress.

Samples of urine of one volunteer were stored at -18 °C. Samples of 0.5 ml of urine were diluted by 0.5 ml of 0.2 mM formic acid in methanol, centrifuged at 10000 rpm for 10 minutes, filtrated through 0.22 µm Nylon filter and 20 µl was injected.

UHPLC-QqQ analyses were carried out in Ultimate 3000 system combined with TSQ Acces Max. Kinetex F5 column (50 x 2.1mm, 1.7 µm) with Zorbax Eclipse XDB-C18 precolumn (4.6 x 5 mm, 1.8 µm) was used for chromatography separation. Mobile phase A consisted of formic acid (0.1 % v/v). Mobile phase B consisted of methanol (0.1% v/v). Mass spectrometric detection was based on MRM transitions and HESI; collision gas pressure 1.5 mTorr; cycle time 0.5 s; capillary temperature in source 325 °C; vaporizer temperature 350 °C; auxiliary gas pressure 45 arb. units; ion sweep gas pressure 2 arb. units; positive polarity spray voltage 3500 V; discharge current 4 µA. MRM transitions of 8-hydroxy-2´-deoxyguanosine: 284.122 (parent ion), 168.000 (quantifier ion), 140.000 (qualifier ion). MRM transitions of 8-hydroxyguanosine: 300.240 (parent ion), 168.200 (quantifier ion), 140.300 (qualifier ion).

The resulted concentrations of biomarkers were standardized at urine creatinine values. Medians of 8-hydroxy-2´-deoxyguanosine, 8-hydroxyguanosine were 12.07 ng/g, 50.45 ng/g, respectively.

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## WeS-003:
# LC-MS/MS detection of intermediates of *de novo* purine synthesis in urine and dry blood spot

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*De novo* purine synthesis (DNPS) is a biochemical pathway converting phosphoribosylpyrophosphate (PRPP) into inosine monophosphate (IMP) within ten steps. There are already two known disorders of DNPS caused by mutations in genes for individual enzymes. But the data based on exome sequencing (ExAC database) reveal the possibility of mutations in other genes coding enzymes of this pathway. We assume, that defects of these enzymes remain unseen owing to the lack of diagnostic methods. In order to develop diagnostic methods for hidden genetically determined DNPS disorders is essential to prepare DNPS intermediates, which are not commercially accessible.

We introduced constructs coding individual enzymes of DNPS into *E. coli*. Expressed recombinant enzymes were isolated by affinity chromatography and used in biochemical assays to synthesise DNPS intermediates in their ribotide form. Riboside forms were prepared by dephosphorylation catalysed by a calf intestine phosphatase. Generated products were purified by column chromatography and then used as standards for LC-MS/MS methods. The analytical methods are based on reverse phase separation and selected reaction monitoring detection on triple quadrupole.

Patients suffering from neurological symptoms may possess metabolic disorder. Limits of specialized laboratory screening are set by diagnostic methods, which require standards for their development. We validated LC-MS/MS methods for detection of all plausible genetically determined DNPS disorders and determined physiological values of detectable DNPS intermediates in samples of urine and dried blood spot (DBS).

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## WeS-004:
# Compositional analysis of untargeted metabolomic data using multiple Bayesian hypotheses testing

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Currently, both clinical targeted and untargeted metabolomic approaches aim to find statistically significant differences in chemical fingerprints of patients with some disease and a control group and to identify biological markers allowing a prediction of the disease. Traditionally, the differences between controls and patients are evaluated by both univariate and multivariate statistical methods. The univariate approach relies merely on t-tests (or their nonparametric version) where the results from multiple testing are compared by p-values and fold-changes using a so-called volcano plot. As a counterpart, a multiple Bayesian hypotheses testing is proposed, introducing a concept of b-values as well as a Bayesian version of the volcano plot incorporating distance levels of the posterior highest density intervals from zero. Moreover, since each metabolome is a collection of some small-molecule metabolites in a biological material, relative structure of metabolomic data is of the main interest. A proper choice of orthonormal coordinates w.r.t. Aitchison geometry considering the compositional character of a metabolome is, therefore, an essential step in any statistical analysis of such data. The theoretical background is accompanied by an analysis of a data set containing plasma of patients suffering from an inherited metabolic disorder of ketone body synthesis and leucine degradation – 3-hydroxy-3-methylglutaryl-coA lyase deficiency (HMGCLD).

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## WeS-005:
# New biomarkers of 3-hydroxy-3-methylglutaryl CoA lyase deficiency

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3-OH-3-Me-glutaryl-coenzyme A lyase deficiency (HMGCLD) is a rare inherited metabolic disorder caused by mutations in HMGCL gene. The mitochondrial enzyme is responsible for catalysing the cleavage of HMG-CoA to acetyl-CoA and acetoacetic acid. Diagnosis is established by tandem MS based newborn selective metabolic screening that contains SRM transition for C5-OH carnitine (262 → 85) which represents four different compounds (Me-malonylcarnitine, 3-OH-isovalerylcarnitine, succinylcarnitine and 2-Me-3-OH-butyrylcarnitine) pointing to different inborn errors of metabolism (IEM). In order to distinguish between these IEM organic acid profiles of urine samples must be acquired. We run untargeted metabolomic analysis of five plasma samples from HMGCLD patients together with a group of controls with the aim to find new diagnostic biomarkers that would be detectable even via newborn screening. Apart from known biomarkers such as 3-OH-isovalerylcarnitine and 3-Me-glutarylcarnitine, other acylcarnitine (AC) species derived from intermediates in the leucine degradation pathway were observed. Furthermore, organic acid counterparts of these AC were also significantly increased compared to controls. Newly found elevated AC could hypothetically be also expected in one other condition, 3-Me-glutaconyl-CoA hydratase deficiency (MGCA). HMGCLD could be distinguished from MGCA by measuring of 3-OH-3-Me-glutarylcarnitine or 3-OH-3-Me-glutarate. The result suggests that 3-OH-3-Me-glutarylcarnitine and 3-OH-3-Me-glutarate could be specific biomarkers of HMGCLD in dried blood spots by FIA-MS screening and thus speed up differential diagnoses among all the diseases screened by C5-OH transition. That would allow for earlier introduction of dietary intervention directly from first screening sample.

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## WeS-006:
# Removing false features in metabolomics data using correlations

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Untargeted LC-HRMS analysis produces a large number of features (measured ions with unique m/z and retention time) which corresponds to the potential compounds in the analyzed sample. During the data processing, it is necessary to merge associated features belonging to one metabolite to prevent multiplicities and possible misidentification. There are many LC-MS processing tools available (open source – XCMS and CAMERA, MZmine2, vendor software Compound Discoverer and many others). All of them use complex algorithms to merge features belonging to one compound like isotopic peaks, adducts and multiply charged features but none of them can do it without mistakes and none can deal with fragments formed in ion source during the analysis.

There are some independent post-processing tools, which can improve the merging of associated features like Ion Fusion or MS-FLO – however, they cannot detect fragments yet. Our approach is more comprehensive, revealing all correlated features in the data. It combines graphical tool called correlation network with Pearson's pairwise correlations and retention time range to group all required features to one compound and then uses the largest sample mean of the peak area as a reference. We assume that some biological correlated compounds will be also accidentally merged during the process – but it will not affect further statistical analysis considerably since it is rather rare.

Our approach was tested on the metabolomics data from patients with phenylketonuria and healthy controls. Twenty percent of features, which could be falsely considered as potential biomarkers, were removed using this tool.

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## WeS-007:
# Comparism of plasma metabolites identification by liquid chromatography /MS and direct infusion /MS

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Metabolomics has shown a great potential in several biological applications. Discovery of diagnostic biomarkers, drug metabolization, their effects on whole metabolome and progression of diseases are examples where studying of metabolome is mainly focused. One of the biggest tasks in metabolomics is to obtain reliable and reproducible data in reasonable time. Metabolome consists of chemically very different and distinct entities and due to this complexity, different separation techniques, including LC and GC are used. Direct mass spectrometry analysis, by direct infusion is used as an alternative in metabolomics. The main advantage of this method is high-throughput screening of samples. The present work explores difference between liquid chromatography (LC) / MS separation and direct infusion in the same plasma samples. Firstly, plasma was measured on C18 column by LC/MS (6550 iFunnel Q-TOF, Agilent). Further direct infusion on the same instrument was performed. We compared these approaches, using three different analysis methods, i.e. ProFIA (R package), xcms, and MALDIquant. We have detected unique m/z values per each dataset: 9387 for C18 separation, 2944 using ProFIA, 1497 using MALDIquant, and only 57 using xcms for direct infusion. We have found 16.8 % overlap between classical LC/MS and proFIA processing. In conclusion, direct infusion is very fast method for metabolite profiling but in our experimental setup major information about features present in plasma were lost.

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## WeS-008:
# Identification and classification of melanoma cancer tissue using REIMS analysis and localization of melanoma lipid remodelling by MALDI mass spectrometry imaging

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Malignant melanoma, characterized by the high ability to metastasize to the deep organs, is one of the most aggressive form of skin cancer worldwide. Although it alone accounts for 1% of skin cancer cases, it is responsible for 90% of death [1]. Understanding of the metabolic profile of cancer cells reprogramming, growth and dissemination is a crucial step for affective and targeted drug development.

Classification of the animal melanoma model B16/F10 (ATCC CRL-6475) and melanoma related alterations in lipid profile were investigated upon subcutaneous injection of metastatic cancer cell line to C57BL/6 mice. Melanoma, surrounding skin and lungs tissues were analysed by REIMS and MALDI mass spectrometry imaging (MSI). Acquired data were statistically evaluated using PCA–LDA analysis. Changes in lipid distribution were correlated with light microscopy upon hematoxylin and eosin staining.

REIMS analysis yielded characteristic spectral profiles with the most dominant glycerophospholipids in a mass range 600–900 Da, especially mono- and poly-unsaturated PE and PA with C34 – C36 long fatty acid chains as characteristic melanoma markers. MALDI MSI showed the most significant changes at the level of PC. Mono-unsaturated PC with long fatty acid chains revealed the highest intensity in vital part of the tumour (PC (32:1); PC (34:1)), whereas poly-unsaturated PC with higher number of double bounds (PC (34:2); PC (36:4)) were represented mainly in necrotic areas of the tumour. The most significant marker of the tumour lipids from ganglioside sub class was especially monosialoganglioside GM3. Key lipids molecules participating in tumour growth and microenvironment processes that may serve as biomarkers of malignant melanoma.

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## ThS-009:
# Proteomic Analysis of Exhaled Breath Condensates

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Exhaled breath condensate (EBC) represents a source of biomarkers such as proteins, arachidonic acid metabolites, vasoactive peptides amines, DNA, RNA, microRNA and small molecules. These biomarkers can provide valuable information about respiratory as well as systemic diseases. Finding non-invasive methods for early detection of lung diseases would be highly beneficial. Proteomic analysis of EBC has the potential to detect early changes in the status of the respiratory system. It could complement some invasive sampling methods in future and provide non-invasive lung diseases screening technique. Our study is focused on children's asthma. The aim of this study is to identify biomarkers of asthma bronchiale in children's exhaled breath condensate.

Exhaled breath condensate proteins in the sample are solubilized and denatured, reduced by DTT, digested by trypsin and concentration of peptides is measured. Samples are purified and diluted for HPLC/MS analysis which is performed in three technical replicates using high resolution LTQ Orbitrap Elite mass spectrometer (Thermo Scientific). Measured spectra are analyzed by Proteome Discoverer software (Thermo Scientific). Data are further statistically evaluated by Statistica and Bioconductor R – package.

In our study we collected and analyzed from 62 pediatric asthma patients. We have successfully analyzed the exhaled breath condensate of diagnosed and treated asthma and compared them with the samples of 62 healthy controls. We have found proteomic biomarkers of asthma and asthma treatment prediction. Discovered biomarkers will be validated by MRM mass spectrometry analysis and immunochemical methods.

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## ThS-010:
# Native nanoelectrospray-MS as a tool for quick evaluation of protein-DNA complex formation

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Transcription factors mediate gene expression regulation through interactions with DNA and other regulatory proteins. Therefore, they play a crucial role in many biological processes including growth and development of organisms, various metabolic pathways or tumorigenesis. Due to their aforementioned properties, strict regulation of transcription factor's activity is required and one of the possible ways how they are regulated, is through differences in their affinity to different DNA sequence motives. In our work, we have studied the effect of orientation and surrounding sequence of the DNA response motif on interaction of DNA binding domain (DBD) of transcription factor TEAD1 with its DNA response M-CAT motifs originating from regulatory regions of human genes.

First of all, we needed to check the recombinantly prepared TEAD1-DBD's ability to form complexes with all the selected DNA oligonucleotides and to estimate Kd of each complex. Since methods usually used for protein-DNA Kd determination (such as thermophoresis, fluorescence anisotropy or gel shifts) are tedious and sample consuming or need one of the complex components to be labelled, they are not suitable for evaluation of multiple complexes in short time. Thus, we have tested the potential of native nanoelectrospray ionization coupled to FT-ICR MS for Kd determination.

Using six TEAD1-DBD•M-CAT complexes with different Kd, that were previously determined by fluorescence anisotropy-based binding assay, we have observed all components (free DNA, free protein and the complex) in the obtained spectrum. Furthermore, the ratio of signal intensities of TEAD1-DBD in its free and complexed form was used to calculate bound fraction of TEAD1-DBD which was in agreement with known dissociation constants.

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## ThS-011:
# Proteomic profiling reveals DNA damage, nucleolar and ribosomal stress are the main responses to oxaliplatin treatment in cancer cells.

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Oxaliplatin is widely used to treat colorectal cancer in both palliative and adjuvant settings. It is also being tested for use in treating hematological, esophageal, biliary tract, and other gastric cancers. Despite its routinely use, little is known about cellular response to oxaliplatin. To address this issue, whole-cell proteomics study was performed. Chemosensitive CCRF-CEM cells were treated with oxaliplatin at 29.3 μM (5x IC50) for 240 minutes (half-time to caspase activation). The proteomes of treated and control cells were then compared by high-resolution mass spectrometry with SILAC isotope labeling. This experiment revealed 4049 proteins expressed over 3 biological replicates. Among these proteins, 76 were significantly downregulated and 31 significantly upregulated in at least two of three replicates. In agreement with the DNA-damaging effects of platinum drugs, proteins involved in DNA damage responses were present in both the upregulated and downregulated groups. The downregulated proteins were divided into three subgroups; i) centrosomal proteins, ii) RNA processing and iii) ribosomal proteins, which indicates nucleolar and ribosomal stress. In conclusion, our data was supported by further validation experiments indicate the initial cellular response to oxaliplatin is the activation of DNA damage response, which in turn or in parallel triggers nucleolar and ribosomal stress. This effect was further verified on other cancer cell lines.

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## ThS-012:
# Ion-mobility Mass Spectrometry of Cyclosporines

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Ion-mobility separates ions in the gas phase according to their spatial arrangement and charge state; furthermore it is used for collision cross section determination [1]. Cyclosporines are hydrophobic cyclic undecapeptides of microbial origin. Cyclosporine A is used as immunosuppressive drug and due the presence of β-hydroxyl group in side-chain of MeBmt residue it can undergo an acid catalyzed rearrangement to the corresponding isocyclosporine. This rearrangement takes place in protonated molecules in the gas phase in mass analyzers with longer duty cycle, e.g. ion trap [2]. This work reports on a similar rearrangement in Synapt G2-Si Q-IM-TOF and SolariX (12T) FTICR mass spectrometers. Characteristic fragmentation ions were characterized by high mass resolution FTICR MS. Cationization with alkali-metal leads to stabilization of cyclosporines' forms in which the rearrangement is blocked as shown by $MS^2$ spectra. In mobilograms individual isoforms had slightly different mobilities according to their collision cross sections (CCS) and could be distinguished. CCSs of sodium adducts of isocycloforms revealed higher mobility in comparison with the cycloforms. Isoforms of cyclosporines exhibited more peaks, which were not completely separated. It may indicate more conformations in the gas phase similarly to situation in solution [3].

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## ThS-013:

# Application of two amorphous $TiO_2$ nanotubes-based materials for highly selective phosphopeptide enrichment followed by nanoLC-MS/MS analysis

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Phosphorylation is a reversible covalent modification and plays a critical role in many biological processes. Mass spectrometry as an important tool for phosphoproteome analysis needs preceding phosphopeptide enrichment step for analysis of complex peptide mixtures due to low abundance and low ionization efficiency of phosphopeptides. Here we demonstrate the enrichment of phosphopeptides with substantially higher selectivity by introducing of metal oxides-based nanotubes. Two developed nanomaterials, the amorphous $TiO_2$ nanotubes ($TiO_2$NTs) and the same material decorated by $Fe_3O_4$ nanoparticles ($TiO_2$NTs@$Fe_3O_4$NPs) with superparamagnetic properties, were tested for phosphopeptides enrichment and compared with commercial $TiO_2$ microspheres. To compare the aforementioned materials, digest of BSA and alpha-casein as a simple (phospho)peptide mixture and the whole cell lysate digest of Jurkat T-cells representing a real complex peptide sample were tested for phosphopeptide enrichment. The simple mixture was analyzed with MALDI-IT-Orbitrap MS and the cell lysate digest was measured using nanoLC-MS/MS (Q-Exactive). The obtained results revealed that $TiO_2$NTs and $TiO_2$NTs@$Fe_3O_4$NPs used for phosphopeptides enrichment from complex biological samples perform similarly as $TiO_2$ microspheres in the number of identified phosphopeptides. However, the presented nanomaterials showed a significantly enhanced enrichment selectivity which enables reduction of ionization suppression of phosphopeptides within nanoLC-MS analysis. The numbers of identified non-phosphorylated peptides were approximately 4-fold lower as compared to $TiO_2$ microspheres. Moreover, further modifications in the enrichment protocol led to increase in the overall phosphoproteome coverage of the cell lysate digest.

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## ThS-014:
# Fragmentation mechanism in echinocandins

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Echinocandins represent the class of antifungal agents currently used in the treatment of candidiasis and/or aspergillosis. These semisynthetic drugs are composed of a cyclohexapeptide ring and a specific lipophilic side chain. Echinocandins interact as non-competitive inhibitors of 1,3-β-D-glucan synthase. This enzyme is responsible for the biosynthesis of β-glucans, the major building part of fungal cell wall, and its inhibition leads to fungal cell wall disintegration [1, 2].

In this work, we show the fragmentation behaviour of echinocandins. The MS and MS/MS spectra were collected on 12T FTICR MS SolariX using ESI+ and MALDI+ ion sources. Before collision-induced dissociation (CID), the loss of several water molecules was typical for all echinocandins. Moreover, caspofungin showed the loss of ethylenediamine, whereas the $SO_3$ group loss was associated with micafungin. During the CID, the common mechanism of fragmentation was observed – the ring was opened in the isopeptide bond between N-acetylated ornithine and modified proline resulting in detection of typical $b_i$ ion series in the spectra. Furthermore, proline hypercleveage was observed in caspofungin and micafungin. In addition, isolation and CID of the sodium adduct of anidulafungin showed loss of acetaldehyde, the side chain of threonine. The mechanism of this type of loss is consistent with literature [3].

In conclusion, echinocandins showed typical neutral losses of water, ammonia, ethylenediamine, and $SO_3$ group, fragmentations resulting in the presence of $b_i$ ion series, and loss of threonine side chain in case of sodium adduct of anidulafungin.

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## ThS-015:
# The hybrid molecule of adenylate cyclase toxin and α-hemolysin translocates its cell-invasive enzyme into cell cytosol and targets CD11a-positive cells

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The *Bordetella pertussis* adenylate cyclase toxin (CyaA) and *Escherichia coli* α-hemolysin (HlyA) are cytolytic leukotoxins of the Repeat in toxin (RTX) family. CyaA is capable to directly penetrate target cells across their cytoplasmic membrane without the need of endocytosis and delivers into the cell cytosol its adenylyl cyclase (AC) enzyme domain. The Hly portion of CyaA mediates toxin binding to the integrin CD11b/CD18 of myeloid phagocytic cells and translocates the AC domain into target cells. Chimeras of the cyaA and hlyA genes were constructed and the acylation status of the produced CyaA-HlyA chimeras were inspected by FT-ICR mass spectrometry. Membrane binding, cell invasive and hemolytic activities were further determined using sheep erythrocytes. We show that the AC domain of CyaA have to be fused to the adjacent AC-to-Hly-linker segment and the pore-forming domain of CyaA in order to be efficiently translocated across the cell membrane. The C-terminal acylated and RTX domains of CyaA then can be replaced with the corresponding domains of the HlyA. Further, the myristoyl-activated hybrid molecule targeted and penetrated cells expressing the integrin CD11a/CD18. This suggests that the key element of the cell specificity of the hybrid is located in the C-terminal portion derived from HlyA.

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## ThS-016:
# Top Down Mass Spectrometry and Hydroxyl Radical Footprinting

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Protein footprinting coupled with mass spectrometry is commonly applied to study protein structures and interaction. Traditional approaches including HDX, stable chemical labeling and hydroxyl radical footprinting. Among these are hydroxyl radicals considered as a perspective reactive reagent. Even the utilization of hydroxyl radicals for footprinting was introduced two decades ago and there are several groups generating radicals by different methods including Fenton chemistry, electrochemistry, synchrotron radiolysis of water and FPOP; the bottom up mass spectrometry is the only method to identify modified residues and determine the solvent accessible area of proteins.

Here, we present the application of top down sequencing, high resolution mass spectrometry, in order to localize modified residues within the protein structure. In FPOP labeling experiments, an excimer laser (248nm KrF) was used to generate •OH dissociating hydrogen peroxide for half a second. When a significant oxidation of Ubiquitin was observed, intact, singly and doubly modified proteins were isolated and fragmented by a broad repertoire of dissociation techniques. To annotate fragment ions, ms2links algorithm [1] was used and the home-built software was forced to quantify the extend of modification. Careful analysis of fragment spectra revealed the sequence information at residue level and unambiguously identified the site of oxidation. Different ion types can provide complementary information for the structural characterization of protein. Quantitation of oxidation was calculated using the fragment ion intensities from CID, ECD, ETD and IRMPD. All measurements were carried out in triplicate. Thus, such comprehensive the top down experiment determined oxidized residues of singly modified protein.

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## WeP-001:
# Metabolic profiling of tryptophan and kynurenine pathway in dried blood spots

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The mode of delivery, caesarian section (CS) or normal delivery (ND) determines the diversity of the bacterial microflora. The different microbial composition, has an influence to the health of the newborn. Tryptophan and its metabolites, produced by many different bacterial species, have an important role in the mammalian gut immune homeostasis [1]. Different Body fluids were taken from participants in the CELSPAC Cohort Study. In the CELSPAC Study, in total 134 samples were collected. For the group comparison, 20 samples of CS were compared to 20 matched samples of ND. We analysed a panel of tryptophan metabolites in neonatal dried blood samples (DBS) and meconium/first stool. Tryptophan metabolites were analysed using the targeted SRM metabolic profiling in DBS. The extracted tryptophan metabolites were quantified using labeled internal standards. The profiles of tryptophan and kynurenine metabolites in DBS were compared both in CS and ND groups. Analytes were tested for significance with 95% confident intervals. Statistically significant differences between CS and ND groups were indole-3-lactic acid and indole-butyric acid. These metabolites correspond to the difference in microbiota, confirmed by metagenomics analysis.

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## WeP-002:
## Proteomic analysis of plant response to drought with comparison of gel-based and gel-free proteomic approaches

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In despite of the fact that drought is the most crop influencing factors in the world, a mechanism of drought tolerance in various plants is still unclear. The aim of our study lies in an observation of changes in wheat proteome under drought conditions and in a comparison of two proteomic approaches – gel based and gel free methods. For deeper clarification of wheat response to drought two tissues of two wheat varieties with differ in drought tolerance and were analysed.

The samples were analysed by gel-based method 2D-DIGE and significant changed spots were identified by MALDI-TOF-MS/MS. Mass spectra with ambiguous identification were analysed manually by de novo sequencing. Altogether, 262 crown spots, resp. 259 leaf, were analysed, 168, resp. 192 of them were identified. By gel-free LC-MS/MS method were more than 1000 proteins in both tissues significant identified with at least 1 unique peptide or 2 peptides overall, but statistical analysis are still in process.

Focusing on late embryogenesis abundant proteins (LEA), important protective proteins, showed interesting protein profiles in wheat varieties. By 2D-DIGE of crowns we found 1 LEA 3 with faster biosynthesis and 3 isoforms of LEA1 in tolerant variety compare to sensitive variety with slower response and only 2 isoforms of LEA 1. In leaves, except slower biosynthesis of 2 isoforms of LEA 1 in both varieties, any significant differences between varieties weren't observed. Surprisingly, by LC-MS/MS were no LEA 3 neither LEA 1 found but founded 4 proteins from LEA 2 group (directly involved in plant response to drought) were differently expressed in high concentrations (some of them more in sensitive variety). Clarification of these results is interesting task of the following days and for future studies.

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## WeP-003:
# Malignancy of PC3 cells derived exosomes is affected by silencing of CA I

Radivojka Bánová [1], Martina Zdurienčíková [1], Silvia Tyčiaková [1], Oldřich Benada [2], Mária Dubrovčáková [1], Ján Lakota [1,3], Ľudovít Škultéty [1,2] *

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We report results showing that the silencing of carbonic anhydrase I (siCA1) in prostatic (PC3) tumor cells has a significant impact on exosome formation. An increased diameter,concentration, and diversity of the produced exosomes were noticed as a consequence of this knock-down. The protein composition of the exosomes' cargo was also altered. Liquid chromatography mass spectrometry (LC-MS) analyses identified 42 proteins significantly altered in PC3 siCA1 exosomes compared with controls. The affected proteins are mainly involved in metabolic processes, biogenesis, cell component organization, and defense/immunity. Interestingly, almost all of them have been described as "enhancers" of tumour development through the promotion of cell proliferation, migration, and invasion. Thus, our results indicate that the reduced expression of the CA I protein enhances the malignant potential of PC3 cells

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## WeP-004:
# Age-related changes in levels of gangliosides in selected rat brain structures

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Gangliosides (GSs), sialic acid-containing glycosphingolipids, are essential components of cellular membranes, especially in neuronal cells. Besides affecting the function of the membrane in which they are located, these amphiphilic lipids modulate a variety of biological functions through transmembrane signalling [1]. In the brain, GSs are abundant and their content and composition change during aging, which is the most important risk factor for several neurodegenerative diseases [2].

The aim of our study was to analyse the levels of selected GSs (GM1, GM2, GM3, GD1a, GD1b, GT1b, GQ1b, GD2, and GD3) in different brain structures of rats 1 and 11 months old (Wistar and Sprague-Dawley). Brains of individual rats were partitioned into sets of 11 structures from which GSs were extracted using a modified Folch method [3]. SRM assays were used for quantification of GSs in a triple-quadrupole mass spectrometer (6495, Agilent Technologies).

Our results show increasing levels of monosialic acid-containing GSs, especially GM1 and GM2, and decreasing levels of polysialic acid-containing GSs in old rats compared to young ones. However, neither decrease nor increase of GSs were observed in all tested brain structures. These data confirm earlier findings, that levels of GSs change during the aging [2]. In addition, we proved that GSs alteration are region-specific. Our findings offer a new insight important for understanding of pathophysiological mechanisms in age associated neurodegenerative diseases.

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## WeP-005:
# Monitoring of human procalcitonin by functionalized MALDI surfaces

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Increasing number of studies point out that ion soft- and reactive landing (ISL and IRL) in atmospheric pressure is a promising way for deposition of polyatomic ions on broad variety of conductive surfaces such as gold or glass coated with indium-tin-oxide (ITO) etc. Some of the surfaces are sufficiently durable for MALDI ionization, therefore combination with MALDI-TOF MS is possible. ISL and IRL are suitable techniques when working with proteins. Studies performed scanning tunneling microscopy have shown that high percentage of landed proteins retained their native structure. The combination of surfaces prepared by ISL/IRL containing intact proteins with MALDI-TOF MS can be used for many purposes e.g. protein sequencing or *in situ* protein enrichment with subsequent MS analysis. For the aim of our study, immunoaffinity surfaces prepared by ISL deposition of antibody on ITO glass were tested.

Our interest is the application of functionalized MALDI surfaces for clinical analysis of procalcitonin (PCT) in human serum. Procalcitonin is a valuable and specific biomarker of sepsis. Its concentration in bloodstream is under physiological conditions below the limit of detection of current methods. During sepsis, its concentration increases dramatically and PCT can be monitored. So far clinical determination of PCT requires expensive commercial kits and apparatures. MALDI-TOF MS in combination of functionalized surfaces could represent an alternative approach for fast and low-cost detection of PCT during sepsis.

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## WeP-006:
# Studying of viral hexameric helicase by hydrogen-deuterium exchange mass spectrometry

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The bacteriophage Φ8 packages its genome into an empty protein capsid (procapsid) to protect itself from a host cell. Procapsid consists of four virus-encoded proteins including P4. The P4 protein is a hexameric molecular motor translocating a single-stranded RNA into a viral capsid using chemical energy from ATP hydrolysis [1]. Here we study the P4 helicase in several states using hydrogen-deuterium exchange and mass spectrometry [2]. Pepsin column was used for on-line digestion of proteins incubated in deuterium prior HPLC peptide separation and mass spectrometry analysis. This approach was applied to monitor the protein structure on a peptide level. Using exchange kinetics data, changes in the structure of protein complex in the presence and the absence of single-stranded RNA have been revealed. Several regions of P4 have shown different deuterium exchange rates than protein exposed to RNA. We observed the probable binding sites of RNA and opening of hexameric ring which is an initial step in RNA loading into the hexameric central channel [3]. Hydrogen-deuterium exchange method revealed the interaction of helicase molecular motor with RNA or ATP molecules.

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## WeP-007:
## Infection with *Coxiella burnetii* triggers host defense responses in human macrophages in a time-dependent manner

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*Coxiella burnetii* cause Q fever. Humans acquire the infection by inhalation of contaminated aerosols generated by domestic livestock. *C. burnetii* is able to reconfigure the intracellular environment to evade host cell defenses [1].

We conducted a comparative label-free proteomic analysis of the THP1 derived macrophages infected with a patient isolate. Samples were collected at 3 and 7 days post infection, with their respective controls. Extracted proteins were trypsin digested using a filter-aided unit. NanoRSLC system was employed for peptide separation on a µPAC column. Mass spectrometric detection was performed on Q-Exactive Orbitrap. Relative quantification was accomplished by mean of Progenesis QI, followed by Mascot Server database search.

Extreme separation of the µPAC column, allowed identifying an average of 2249 and 2195 proteins in the early and late infection, respectively. Data from the early and late infection time points were pairwise evaluated against respective controls. Principal component analysis showed clustering of biological replicates according to the experimental variable (bacterial infection). We considered proteins as differentially abundant if their fold change ratio achieved ≥ 2 and ANOVA p-value ≤ 0.05.

121 proteins were found to be differentially regulated in the early stage of infection, in a later time point, 93 were recognized. In early time infection, 114 unique proteins were associated with the THP1 cells and 7 with *C. burnetii*. In the late stage, we discovered 60 THP1 and 33 bacterial proteins. The number of identified bacterial proteins dramatically increased in the later stage of infection, it may indicate successful colonization of the human cells due to negative regulation of host defense responses.

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## WeP-008:
# A novel MALDI-TOF MS-based method for blood meal identification in phlebotomine sand flies

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Hematophagous females of phlebotomine sand flies are vectors of human and animal diseases such as leishmaniases. Identification of their blood sources is therefore crucial for understanding transmission cycles and knowing disease reservoirs. It can also help with a setting proper control measures in endemic areas. Nevertheless, conventional methods for host blood determination are laborious, expensive and facing some limitations related to blood degradation. A promising approach employing MALDI-TOF protein profiling was reported recently, however it was applied solely for freshly engorged females until 24 hours post-bloodmeal (PBM).

Here we introduced a new method for bloodmeal identification, which is based on peptide mass mapping (PMM) using MALDI-TOF mass spectrometry. Host blood from homogenized abdomen was digested by trypsin and the resulting peptides, typically specific fragments of host hemoglobins, were detected and sequenced on MALDI-TOF MS. The approach was first tested on females experimentally fed on rabbit or different rodent species yielding 100% correct host identification until 36 h PBM. When investigating females fed on two hosts, PMM successfully revealed both bloodmeals for 60% specimens until 36 h PBM. In a blind study using field-collected females PMM method correctly identified host blood for 52 of 54 (96%) fresh females from Greece and 10 of 24 (42%) one-year old specimens from Armenia as was verified using traditional cytB gene sequencing.

MALDI-TOF peptide mass mapping was proven as an accurate and fast approach for host blood identification, especially useful during field surveys when high numbers of samples are processed. Although tested on phlebotomine sand flies, it could be applied to other blood-sucking insects as well.

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## WeP-009:
# Mass spectrometry-based proteomic analysis for subtyping of amyloid deposits from FFPE and SFA samples

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The systemic amyloidosis is a rare disorder characterized by the abnormal deposition of misfolded amyloid protein in various organs [1]. Over time, the accumulating amyloid damages the tissue microenvironment and causes organ failure. To date, there are 36 known fibril proteins in human that can cause amyloidosis [2]. Early diagnosis is critical for effective patient management. IHC is the preferred method for routine amyloid subtyping. However, it is an antibody-based method with numerous unspecificities [3]. Therefore, we have introduced mass spectrometry-based proteomic analysis for subtyping of amyloid deposits in FFPE and SFA samples.

So far we have obtained 300 FFPE and 57 SFA samples for subtyping of amyloid deposits. In FFPE samples, Congo red positive-stained amyloid deposits were dissected using laser microdissection; the proteins were extracted from excised materials and digested using trypsin. In the SFA samples, the proteins were solubilized and digested directly with trypsin. All samples were subsequently separated by liquid chromatography, and individual peptides were acquired by tandem mass spectrometry. Acquired spectra were identified and quantified using a search engine - MaxQuant. The most abundant amyloid protein determined the amyloid subtype.

The mass spectrometry-based proteomic analysis enables subtyping of different kinds of amyloid proteins (e.g. Ig kappa, Ig lambda, transthyretin, fibrinogen, serum amyloid A, semenogelin). In addition to already mentioned proteins, we also observed the presence of SAP, ApoA-IV, ApoA-I, ApoE, CLU, VIM and VTN. All those proteins are associated with the amyloid formation. Mass spectrometry-based proteomic analysis of FFPE and SFA samples offers a powerful tool for typing of systemic amyloidosis.

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## WeP-010:
# Application of Atmospheric Solids Analysis Probe Mass Spectrometry in analysis of binders used in art

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Atmospheric Solids Analysis Probe Mass Spectrometry (ASAP MS) is a method enabling direct analysis of volatile and semi-volatile compounds present in liquid and solid samples [1]. Utilization of ASAP in combination with high resolution mass spectrometry was proposed as a suitable approach for rapid analysis of selected classes of binders used in artworks i.e. oils, waxes, resins, polysaccharides, proteins etc.

To the best of our knowledge the application of ASAP MS has been used for investigation of colorants [2] but not for binders in artworks. Advantage of this technique is its minimal requirements on sample preparation. This pilot study is focused on analysis and resolution of oils used as binders in oil paintings. A small amount of oil was dissolved in acetone (approximately 0.05 g/mL) then deposited on capillary glass tube and after solvent evaporation placed into ASAP source of a high resolution tandem mass spectrometer (Synapt G2-S, Waters). Main parameters influencing the ionisation of compounds, i.e. probe temperature, corona current and flow of desolvation gas were optimized. Set of oils used in artworks was measured by the optimized ASAP-MS method. Obtained data were processed using multivariate statistics that allowed classification of oil samples (linseed oil cold-pressed, linseed oil bleached, poppy oil, clove oil, rosemary oil, lavender oil, nut oil, safflower oil). The compounds characteristic for different kinds of oils (markers) are studied in detail. A consequent study focused on the analysis of binders directly on artworks is now in progress.

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## WeP-011:
# Interactions of Azoles with Copper(II) during the Electrospray Process

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Azoles is a broad group of fungicides frequently used in agriculture and/or as pharmaceuticals. Their action lies in the inhibition of aromatase and C-14 demethylase, which serve as the key enzymes in sterols biosynthesis. The behaviour of azoles is strongly influenced by the presence of other (biologically active) substances [1]. Moreover, in the evaluation of their impact to the environment we have to count with the cocktail effect. Overall description of all the impacts is impossible. To reach the meaningful data correlating with the real behaviour the preparation of simplified systems consisting of several components only is essential. For this purpose, it is useful to use ESI-MS gas-phase studies to estimate basic reaction schemes and possible degradations of the analytes. The results received from the gas-phase experiments have to be carefully judged to generalize to the real environmental systems.

In our contribution we have studied the interactions of three different azoles in Cu(II) presence. During the electrospray process Cu(II) is easily reduced to Cu(I) [2], on the other hand its oxidation state also depends on its ligands. Additionally, the presence of copper significantly induces azoles degradation.

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## WeP-012:
## Proteomic insight into the interactions of Mason-Pfizer monkey virus G-patch domain

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*Retroviridae* family includes viruses with similar life cycle characteristic by the use of reverse transcriptase for the conversion of their viral genomic RNA to DNA. Nevertheless, the individual retroviruses more or less differ in the individual stages of the life cycle or their genetic equipment. Among others, Mason-Pfizer monkey virus (M-PMV) differ from human immunodeficiency virus (HIV-1) in the presence of G-patch domain (GPD) in its genome. Although, the role of this domain in M-PMV has not been elucidated yet, the GPDs were also found in eukaryotic proteins involved in splicing or transport of mRNA.

To reveal the possible role of GPD we performed the proteomic analysis of native M-PMV virions and M-PMV virions lacking the GPD (ΔGPD), both cultured in HEK 293T cells. Virions isolated via several subsequent gradient centrifugation steps were characterized by transmission electron microscopy and their protein profiles were examined by the use of gel electrophoresis. Finally, the LC-MS/MS analysis of isolated virions was performed to identify the host cell proteins packed in different types of virions. After the comparison of proteins presented in native and ΔGPD virions several proteins lacking in the ΔGPD virions was detected. Among them also RNA helicase DHX15, which was stably presented in native virions, was found to lack in ΔGPD virions. Interestingly, this helicase has already been reported to be implied in ribosome biogenesis and the mRNA splicing in human cells, but the both processes are regulated by the interaction with human GPD containing proteins. These findings suggest that M-PMV GPD may be associated with the regulation of viral mRNA processing or transport.

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## WeP-013:
# Mass spectrometric methods in quality control of hemp (*Cannabis sativa L.*) products

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*Cannabis sativa L.* is a very perspective plant due to its agronomical, industrial and medicinal use. According to the EU regulation [1], plants containing less than 0.2 % of tetrahydrocannabinol (THC) are called (industrial) hemp. In the food industry, seeds of hemp varieties are mainly used. Hemp seeds are edible themselves but are also used for the production of hemp oil and meal. They have high nutritional value due to their specific fatty and amino acid compositions, and they are also a good source of vitamins and minerals [2]. Mass spectrometry (MS) has become an outstanding analytical method with unequaled sensitivity, detection limits, speed and diversity of its applications [3], especially in food chemistry and quality control. Therefore, we employed several MS methods to check the quality of different hemp products available on the Czech market. The content of fatty acids was measured by GC MS, amino acids were analyzed via UHPLC-MS/MS, while minerals were quantified via ICP-MS. Moreover, the content of the main phytocannabinoids was measured by both UHPLC-MS/MS and GC-MS. The quality of products found on the Czech market showed comparable quality.

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## WeP-014:
# Degradation pathways of penconazole complex induced with essential elements in gas phase!

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In food production, protection of crops is one key factor to stimulate/sustain yield in crops. Common pathogens that influence the yield are fungi. Our research is focused on one group of compounds, which are widely used to control fungi, azole fungicides. Regardless their wide usage in agriculture and pharmaceutical industry, they have some downfall in usage. So far, they are known to affect enzymes in steroid biosynthesis, *aromatase* and *14 alpha-demethylase* [1]. In our study we aim to discover how azoles bind in plants, we put our focus on two plant essential elements, copper and zinc. Binding of penconazole with these elements yields many complex compounds with different properties and stability, which lies in their electron configuration [2].

Those properties strongly influence nature of formed complex and their behavior in the gas phase. To mimic degradation of formed complexes and to study their stability we use collision induced dissociation in ESI-MS. There is valuable presumption that in ESI-MS gas phase we can mimic behavior of compounds in solution. Simulating natural breakdown of complex compounds, we observe possible degradation pathways and their degradation products [3]. The degradation products are directly influenced by presence of essential metal(s). The experiments are provided using ESI-MS together with voltammetric approach. These two methods allows providing exact explanation on level of reaction/degradation mechanisms. Detailed explanation of these degradation pathways can be used in explanation how azoles can influence plants, animals and humans beside their primary role as fungicides.

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## WeP-015:
# Equine atypical myopathy: A metabolic study

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Equine multiple acyl-CoA dehydrogenase deficiency also known as atypical myopathy (AM), is a highly fatal muscle disease of grazing horses. This syndrome is accompanied by muscular weakness, stiffness, acute myonecrosis and myoglobinuria, which in at least 75% of cases leads to death within 72 h. It is caused by ingestion of *Acer Pseudoplatanus* seeds containing hypoglycin A, whose active metabolite, the methylenecyclopropyl acetyl-CoA, is responsible for inhibition of FAD-dependent acyl-CoA dehydrogenases.

The aim of this work was to compare the serum metabolomic profile of horses suffering from AM and controls and to confirm AM diagnosis in blood of newborn foal.

Metabolomic analysis was performed using liquid chromatography with aminopropyl column (Luna 3 µm NH2, 2 x 100 mm, Phenomenex) coupled to tandem mass spectrometry (QTRAP 5500, AB Sciex). The metabolites were detected by multiple reaction monitoring in both positive and negative mode and statistically evaluated in R programme language with statistics packages. Analysis of specific biomarker MCPA-carnitine was performed by liquid chromatography-tandem mass spectrometry using the UltiMate 3000 system with BEH C18 column (50 mm, 1.7 µm, 2.1 mm) coupled to a triple-quadrupole mass spectrometer (Triple Quad 6500; SCIEX, Framingham, MA, USA).

Significant differences were demonstrated in the concentrations of various glycine conjugates and acylcarnitines (C2–C26). Moreover, the concentrations of purine and pyrimidine metabolites, vitamins and selected organic and amino acids were altered in horses with AM. Metabolomic analysis of the foal's blood revealed increased concentrations of acylcarnitines and MCPA-carnitine consistent with metabolic profiles of blood from AM affected horses.

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## ThP-016:
# Fragmentation techniques for synthetic glycopeptides sequencing

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Carbohydrate-binding proteins have very diverse functions in all domains of life. These proteins are crucial in numerous processes, such as recognition, infection processes and immune defense. Surprisingly, the affinity of those proteins towards their carbohydrate ligands is usually quite low. This weakness is compensated by combination of several individual binding events, resulting in an increase in apparent affinity.

Combinatorial libraries of heteroglycopeptides, where a peptide backbone is decorated by up to three different saccharide units were developed. This artificial system can be used to study the complex nature of multivalent protein-carbohydrate interactions. Such a one-bead-one-compound library offers huge diversity of glycopeptides, each of them representing single binding possibility for studied biologically active protein.

We present a study that aims to select a reliable MS/MS method for synthetic glycopeptides sequencing. Using a high resolution Orbitrap instrument with a possibility of various fragmentation techniques (CID, HCD, ETD and UVPD) and chip-based ion source TriVersa NanoMate platform, we acquired the data with set of different fragmentation conditions. By the use of peptide with known sequence, the fragmentation parameters were optimized to obtain spectra suitable for glycopeptide identification. We then applied the optimized fragmentation parameters and processing approach to characterize unknown glycopeptides.

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## ThP-017:
## Changes of cerebrospinal fluid peptides due to tauopathy

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Tauopathies represents heterogeneous group of neurodegenerative diseases. The tauopathies are characterized by abnormal deposition of microtubule associated protein tau into intracellular neurofibrillary tangles (NFT) composed mainly of hyperphosphorylated form of this protein. The diagnosis of tauopathies, is based on presence of clinical features and pathological changes. There is an ongoing intensive search for biochemical diagnostic markers over the last decade, to support the clinical diagnosis. Measurements of biochemical markers in CSF are increasingly used in the diagnostic process of dementia. In present study, we used SHR72 transgenic rat model for tauopathy expressing truncated tau protein (aa151–391/4R) to analyse the peptidomic profile of cerebrospinal fluid (CSF) by liquid chromatography – MALDI mass spectrometry (LC-MALDI TOF/TOF). Out of 345 peptides, we identified a total of 175 proteins. Among them 17 proteins differed statistically significant in CSF from transgenic rats. The amount of the following proteins was higher in CSF of transgenic rats compare to control animals: neurofilament light and medium polypeptide, apolipoprotein E, gamma-synuclein, chromogranin A, reticulon-4, secretogranin-2, calsyntein-1 and -3, endothelin-3, neuroendocrine protein B72A, alpha-1-macroglobulin and augurin. Interestingly most of the identified proteins were previously linked to AD and other tauopathies, indicating the value of transgenic animals in biomarker research.

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## ThP-018:
# Proteomic analysis of phospholipid-binding proteins in the regulation of plant cell cell polarity

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Specific protein-lipid interactions are fundamental for all organisms, because they regulate e.g. reproduction, growth, morphology, and responses to pathogens. In plants, lipid-protein interactions haven't been satisfyingly described yet. Our laboratory has already proved that distribution and relative amount of anionic phospholipids, such as phosphatidylinositolphosphates, phosphatidylserin, and fosfatidic acid, on plasma membrane are affecting plant cell polarity. Various proteins are specifically bound to anionic lipids in membranes and these interactions then start various signalling processes, also involving exocytosis and endocytosis. We hypothesise that distinct combinations of anionic lipids are responsible for regulation of vesicular transport processes like exocytosis and endocytosis. Aim of this project is to identify peripheral membrane proteins interacting with anionic phospholipids, to comparatively analyse the specificity of these interactions towards distinct anionic lipids and to analyze the effect of membrane curvature on protein-phospholipid interactions. This aim will be reached by binding the proteins to prepared lipid vesicles with various composition and size. After the binding we will isolate and analyse the proteins on MALDI-TOF MS and LC-MS/MS. This research will improve our understanding of cell polarization mechanisms and signalling protein targeting in cell.

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## ThP-019:
# Preparation of human erythropoietin by expression in eukaryotic cell system and its purification

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Erythropoietin (EPO) is a glycoprotein hormone and essential growth factor responsible for erythroid differentiation, survival, and proliferation [1]. Recombinant human EPO (rhEPO) is used for the treatment of anaemia in patients with chronic kidney disease, myelodysplasia, inflammatory bowel disease (Crohn's disease and ulcerative colitis) or cancer chemotherapy [2]. For this wide range of applications, there is an enormous demand to satisfy medical needs and introduce new variants into the market.

The main objective of this work was to design a novel, highly efficient eukaryotic expression system producing “native” EPO by methods of molecular cloning, and to develop an approach for its purification as a sequence of several consecutive steps primarily based on chromatography and membrane separation processes. All these steps were selected and optimized considering their potential applicability in the industry and to fulfil requirements of the current European Pharmacopoeia.

The rhEPO was produced by cultivation of human embryonic kidney cells 293 (HEK 293) transfected with a eukaryotic expression vector carrying the gene for EPO. The supernatant was then microfiltered to remove solid particles. The filtrate was concentrated by ultrafiltration and the buffer exchanged by diafiltration. Optimal binding of rhEPO on Capto MMC was achieved. Due to the presence of multiple interactions, the protein elution was carried out by gradual adjustment of pH and ionic strength. Almost 60% of EPO was recovered by 50 mM Tris-HCl pH 9 containing 1 M NaCl.

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## ThP-020:
# Nasty facets of wastewater or things they don't tell you about antibiotic analysis by SPE-LC-MS/MS

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Overconsumption of antibiotics has led to expansion of resistant bacterial strains, which represent a global epidemiological threat. Hospital and communal wastewater treatment plants are the main sources of antimicrobials in surface and ground waters. Therefore, monitoring of antibiotic residues in wastewaters is of uppermost importance.

SPE-LC-MS/MS is the gold standard for trace determination of antibiotics. Numerous papers suggest a straightforward method development and recommend verified sample preparation procedures. However, we have revealed crucial drawbacks during generic method development and validation for multi-class antibiotic residues in surface, influent and effluent wastewater (β-lactams, macrolides, tetracyclines, glycopeptides, nitrofurans, oxazolidinones and chloramphenicol). Besides obvious precautions, such as minimizing analyte adsorption onto polar surfaces (using polypropylene filters and polypropylene or silanized glassware, avoiding pre-column filters with metallic frit), attention should be also paid to rarely discussed aspects of analysis (composition of needle wash, carryover, sample degradation etc.). Despite utilization of isotopically labelled standards and excellent instrumental sensitivity (LLOQ 0.0025–2.5 ng/mL), wide dynamic range (3 orders of magnitude, ULOQ 10–200 ng/mL), good linearity ($R^2$>0.990) and trueness (bias<15%), quantification in real samples was obstructed by varying recoveries and unpredictable matrix effects. None of the tested extraction methods provided uniform recoveries for all antibiotic classes owing to their distinct chemical properties. While analysis of surface water and effluent was amenable, influent samples demonstrated severe chemical noise and signal suppression for analytes and internal standards.

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## ThP-021:
# Proteomic analysis for finding potential plasma biomarkers of patients with pancreatic carcinomas

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Pancreatic cancer is one of the most common causes of cancer-related deaths worldwide. The prognosis of this disease is very poor, because it is often diagnosed at an advanced stage due to long-discrete symptoms. Only 5 % of patients will survive five years after diagnosis, but most of them will not survive for one year. Treatment of this disease is complicated by the fact that cancer is often diagnosed at an advanced stage. CA 19-910 is clinically-known pancreatic tumour biomarker used in diagnostics and therapeutic monitoring that is also used for the early detection of recurrent disease after treatment. However, it is not a specific biomarker for pancreatic cancer because its amount can be increased by another pathological conditions such as cholestasis.

The aim of this work is to use plasma samples of four group of patients (with pancreatic cancer, with long-term type II diabetes, with fresh produced type II diabetes and with control groups of healthy patients) to try to find new protein biomarkers with increased sensitivity and specificity that would help to detect pancreatic cancer early on. To achieve this goal the previously untested combination of proteomics techniques, particularly MALDI-TOF MS (Matrix-Assisted Laser Desorption/Ionization – Time of Flight Mass Spectrometry) with spectra analysis by PCA (Principal Component Analysis), which will allow us to find the differences between the analysed groups, and two-dimensional electrophoresis followed by identification of proteins by LC-MS/MS (Liquid Chromatography coupled to tandem Mass Spectrometry) will be used.

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## ThP-022:
# The utilization of mass spectrometry in the analysis of archaeological findings

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A new extraction method was developed for untargeted analysis of less polar analytes in archaeological samples using gas chromatography combined with mass spectrometry (GC/MS). Procedure involved treatment and simultaneous extraction of analytes from samples with acid (6 mol/L HCl in water:acetonitrile, 1:9, v/v) and base (10 mol/L NaOH in water:acetonitrile, 1:9, v/v) at increased temperature and consequent liquid-liquid extraction with hexane. The obtained extract was derivatized by silanization prior to GC/MS analysis. For GC/MS analysis, this sample preparation method provided less complicated chromatogram than previously used procedures utilizing methanol as extraction solvent (presence of methanol caused formation of methylesters besides requested silylderivatives). According to analyses of samples prepared by procedures of experimental archaeology this method is suitable for detection of organic residues in archaeological findings.

The optimized GC/MS method allowed detection of several silanized derivatives of plant phytosterols found on a crust covering an authentic bronze finding tentatively considered to be “Sun Symbol” (located in the archaeological area with number of findings from Hallstatt and La Tène culture in Kobeřice, Vyškov region, Czech Republic). Besides, direct analysis of the finding by laser desorption ionization mass spectrometry (LDI-MS) technique revealed signals of carbohydrates. These results confirmed that the crust was of plant origin. Consequent imaging LDI-MS experiment allowed visualisation of the carbohydrate distribution on the crust of the object. The developed procedures contributed to detailed description of archaeological findings and thus better understanding of our ancient history.

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## ThP-023:
# A combination of fast GC/SIFT-MS for rapid and selective quantification of atmospheric monoterpenes

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A bespoke fast GC unit has been developed and used in combination with SIFT-MS analytical technique for selective quantification of biogenic monoterpene isomers that play a critical role in atmospheric chemistry and are associated with surface ozone production and secondary aerosol formation [1]. Proper estimation of their concentration is necessary for the purpose of atmospheric photochemical modelling. SIFT-MS is known for its ability to quantify wide range of volatile organic compounds simultaneously and in real time but selectivity is still limited. SIFT-MS can only quantify the total concentration of monoterpene isomers. Thus, more traditional method of gas chromatography [2, 3] was employed.

We have successfully separated eight of the most abundant plant monoterpene isomers (α-pinene, β-pinene, camphene, myrcene, 3-carene, R-limonene, α-terpinene and γ-terpinene) in less than 180 s using an electrically heated 5 m long capillary column. The performance of fast GC/SIFT-MS was tested in situ in an 24 hours online monitoring of monoterpenes emitted from the Pinus Nigra tree.

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## ThP-024:
# Reduction of nitro compounds in atmospheric pressure chemical ionization

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Reduction of nitro to amino group is sometimes observed in both chemical ionization [1] and atmospheric pressure chemical ionization (APCI) [2]. The main requirement for the reduction to occur is the presence of a protic solvent like methanol or water. Otherwise, the reduction is not observed [2]. First, to investigate the peaks that are related to reduction, an $[M+H]^+$ ion of 1-nitronaphthalene was fragmented in high-resolution orbitrap and compared with its full-MS spectrum. Two peaks were found not to be a result of fragmentation: $[M+H–16]^+$ ($C_{10}H_8NO$) and $[M+H–30]^+$ ($C_{10}H_{10}N$). Peak $[M+H–16]^+$ is probably a nitroso intermediate of the reduction [2] while $[M+H–30]^+$ was a protonated 1-aminonaphthalene resulting from the full reduction of the nitro group. The extent of the nitroarene reduction was dependent on the solvent flow. For 1-nitronaphthalene for example, the ion $[M+H–16]^+$ didn't reach 1 % intensity of the base peak $[M+H–30]^+$ at high solvent flows (100 µl/min and more), while at the solvent flow 40 µl/min the relative intensity of $[M+H–16]^+$ rose to around 5 % and was even higher in lower solvent flows. The reason for this phenomenon is likely that at lower solvent flow rates there are fewer methanol molecules available for the complete reduction of 1-nitronaphthalene. To investigate if the catalytic reduction takes place on the APCI probe surface contaminated by soot, we also measured 4-nitrophenol in water using open-tubular µ-APCI source [3] which had no surface contaminated with the soot. The reduction of 4-nitrophenol was observed in open-tubular µ-APCI source as well suggesting that the soot in the commercial APCI probe is not required for the reduction to occur.

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## ThP-025:
# Quantification of acute phase proteins in 134 neonatal dried blood samples from CELSPAC birth cohort study

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Acute phase proteins (APPs) are generally not transported across the placenta and thus provide a measure of the activity of the innate immune system in the neonate [1]. APPs concentrations are typically determined by immunoanalytical methods. However these methods require blood sample collection via venipuncture, unsuitable for newborns and babies. On the other hand, collection of dried blood samples (DBS) is less invasive and requires less sample processing and handling.

In this study, we have applied our previously developed multiplex assay for absolute quantification of alpha-1-antitrypsin (A1AT), alpha-1-acid glycoprotein 1 (A1AG1), alpha-1-acid glycoprotein 2 (A1AG2), C-reactive protein (CRP), serum amyloid A1 (SAA1), serum amyloid A2 (SAA2), serum amyloid A4 (SAA4) and immunoglobulin A (IgA) [2] to 134 neonatal DBS from CELSPAC birth cohort study. Median of blood concentrations of A1AT, A1AG1 and A1AG2 were 189.8 mg/l; 105 mg/l and 28.5 mg/l respectively. Median of blood concentration of constitutive protein SAA4 was 8.7 mg/l. Circulating levels of SAA1, SAA2 a CRP are low in healthy individuals, but they increase between 10- to 1000-fold during acute phase of inflammation. Only a single neonatal DBS revealed CRP level above limit of quantification (7.2 mg/l), still within the normal range for CRP. However, SAA is arguably a more reliable marker of inflammation compared to CRP as SAA levels rise earlier, more rapidly and with higher amplitude [3]. In our study, higher than normal levels of SAA1 (> 10 mg/l) were observed in 13 newborns, presumably indicating an inflammatory condition.

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## ThP-026:
# Fast screening of fire accelerants by atmospheric solids analysis probe-mass spectrometry

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Ionization technique ASAP (Atmospheric Solids Analysis Probe) is a powerful tool for direct analysis of solid and liquid samples. It can be used for rapid analysis of a wide range of materials e.g. synthetic compounds, drugs, tissues, polymers, petroleum, nucleosides, etc. [1,2] ASAP allows crude oil analysis without any sample preparation. [3] In our work, ASAP ionization was tested for fast identification of fire accelerants from cloth before and after burning.

Experiments were performed using a Xevo TQD triple quadrupole mass spectrometer (Waters, Manchester, UK) equipped with ASAP. Analyses of the following samples were carried out: a) pure accelerants on a glass capillary, b) fabric soaked in accelerant and c) fabric soaked in accelerant and burned for defined time. The same materials without accelerant were used as blanks. In the case b) and c) the glass capillary was cut and cupreous wire was inserted inside to serve as a sample holder for fabric. Mass spectra were acquired using ASAP temperature ramp from 50°C to 250°C. For MS/MS experiments temperature was set at 200°C.

Mass spectra of fire accelerants were successfully acquired for all analysed samples and their typical features were observed for all used materials (glass capillary, unburned and burned fabric). Complex accelerants (gasoline, diesel, kerosene, thinner) were distinguished using their characteristic “fingerprints”. Simple accelerants (ethanol, methanol, toluene, etc.) were identified by their fragmentation. Signals of accelerants were observed at 50°C, but with higher temperature their intensity increased. Applicability of ASAP ionization for fast screening of fire accelerants with different structures and autoignition temperature was demonstrated.

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## ThP-027:
# Interaction between Mason-Pfizer monkey virus matrix protein and cytoplasmatic domain of polyprotein Env

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Incorporation of viral membrane Env glycoprotein into newly assembled viral particles is essential to maintain the infectivity of human imunodeficiency virus (HIV-1). The important role in this incorporation play homotrimers of matrix protein (MA) through their mutual interaction with the cytoplasmic tail (CT) of Env glycoprotein. Although, the similar relation was observed in the case of other retroviruses such as Mason-Pfizer monkey virus (M- PMV), the direct interaction between MA and CT has not been confirmed yet.

Our aim was to use the XL-MS for the study of the interaction between MA and CT in M-PMV. The interaction between MA and CT was initially studied by the pull-down experiment. Afterwards, the proteins were cross-linked in solution by bis(sulfosuccinimidyl)suberate (BS3) and the complexes of higher molecular weight were visualized. The mass-spectrometry data obtained from the analysis of mixture consisting of the cross-linked and noncross-linked peptides were evaluated by StavroX, Kojak and SIM-XL, the softwares designed to identify cross-linked peptides. As a result, high number of cross-linked peptides derived just from MA was identified. Nevertheless, also several cross-links derived from both studied proteins were identified suggesting that proteins interact through the domains which are located close to the phospholipid membrane. Cross-linked peptides derived only from MA were compared with the already-confirmed structure of MA homotrimers.

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## ThP-028:
# Photo-initiated chemical cross-linking & Electron Release: structure functional study of azurin

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Electron transport processes are an extremely important field of study in modern biochemistry and proteomics. Azurin of *P. aeruginosa* is one of the most utilized model proteins for study of redox and electron transport processes in proteins.

We have utilized photo-induced crosslinking (PIXL) to study oligomerization of azurin in solution by the effect of L-2-amino-5,5-azi-hexanoic acid (photo-Met) – a structural photoinducible analogue of canonical amino acid Met. We have also explored a novel technique – photo-induced electron release (PIER) – during same experiment on electron transport processes in azurin. We have also optimized expression and purification protocol for azurin recombinant production in auxotrophic *E. coli* B834 cells and its purification. The incorporation percentage of photo-Met was analyzed via MALDI-TOF MS. We were able to achieve 70% incorporation in cell lysate. Purified azurin was used in PIXL experiments. Samples of azurin with Met, photo-Met and "All-Phe" mutant (all Trp and Tyr were substituted by Phe) were exposed to intense light and the results were evaluated via UV-VIS spectrophotometry and SDS-PAGE. Dimers and intramolecularly linked azurin were observed and analyzed employing LC-MS.

Photo-Met incorporated into azurin sequence influences not only formation of dimers during PIXL experiment but also the redox state of copper ion at the active site (PIER).

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## ThP-029:
# Application of micromanipulation and Laser Desorption Ionization Mass Spectrometry in analysis of pea seed coat.

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

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

Significant decrease of normalized signals (NS) of fatty acids (FA) in peeled samples with respect to the untreated ones was observed. Among them decrease of NS of long chain FA, i.e. hexacosanoic and octacosanoic acids that are particular markers of L100 dormant genotype [2] by peeling off this genotype PSCs is of particular interest (decrease 9-10x). These measurements confirm effectivity of peeling and presence of FA particularly in cutin layers. Peeling of cutine layers exposed deeper PSC layers. NSs of sodiated fragments of sugar chains were higher in peeled PSCs compared to untreated ones (increase 3-28x). These pilot results suggest that combination of EC peeling with LDI-MS is efficient tool for study of seed dormancy mysteries.

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## ThP-030:
# Discovery proteomics proposes a tolerance mechanism maintaining the survival of *Coxiella burnetii* under doxycycline exposure

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*Coxiella burnetii* is an intracellular pathogen that causes Q fever. Due to similar clinical symptoms with commonly occurring infections, it's unambiguous diagnosis is difficult. For this reason, the disease can occasionally re-emerge with a considerable economic impact in farm industry and human health [1]. Because antibiotic resistance represents a global public health problem, understanding of the bacterial tolerance mechanisms is critical for effective therapy.

Doxycycline is an antibiotic with high cell membrane permeability used for the treatment of acute Q fever in a daily dose of 100 µg for 14 to 21 days or for several months in the case of chronic disease. To gain insight into the mechanisms involved in detoxification of doxycycline, we evaluated the adjustments in *C. burnetii* proteome at presence (0.5 and 4 µg/ml) and absence of doxycycline in axenic media [2]. The whole cell extracts were digested in-solution with trypsin and the peptides separated by LC system Ultimate 3000 (Dionex) using micro Pillar Array Column—µPAC. MS detection was performed using Orbitrap Q-Exactive plus and the spectra searched by Mascot Server against *Coxiella* database. The results were uploaded into Scaffold and additionally verified by X-Tandem. Due to the application of micro Pillar Array Column (µPAC) columns, 870 proteins were identified by spectral counting principle and 151 hits were found to be significantly adjusted as ANOVA $p \leq 0.019$ and the fold change ratio $\geq 2$.

We discovered several proteins affected by doxycycline exposure and related to i) primary metabolism, ii) homeostasis of the cell wall, iii) cell division, and iv) oxidative stress detoxification.

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## ThP-031:
# Purification of glutamyl endopeptidase from *Streptomyces griseus* and its preliminary testing for sample digestion in mass spectrometry-based proteomics

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Both protein extraction and digestion represent important steps, which critically influence the procedure of protein identification and quantification in proteomics. Bovine or porcine trypsin is typically used. Other endoproteases are rather rara but may become advantageous in specific experiments: e.g. when a limited amount of trypsin cleavage sites is present in the sample proteins or post-translational modifications are studied.

Pronase is a commercial mixture of extracellular proteases from the bacterium *Streptomyces griseus* with numerous applications in biochemistry and biology, which benefit from its strong digestion performance leading to a complete or nearly complete degradation of proteins into amino acids. It consists of various aminopeptidades and carboxypeptidases, trypsin, streptogrisins A to D, aqualysin, a subtilisin-like protease and others. There is also an endoprotease present, glutamyl endopeptidase II - streprogrisin E - SGPE, which cleaves its protein substrates at the carboxyl end of glutamic acid residues. This cleavage specificity resembles that of the staphylococcal Glu-C protease, which is used in proteomics, but SGPE has a substantially lower molecular mass value of 18 kDa.

We purified the enzyme using low-pressure ion-exchange chromatography and medium-pressure chromatography (the latter with a gel permeation medium, ion-exchangers or hydroxyapatite). During all steps, enzyme activity was monitored at 408 nm using N(alpha)-acetyl-L-glutamic acid alpha-4-nitroanilide as an artificial chromogenic substrate. We obtained a useful proteolytic reagent, which produces peptides with a length distribution of around 10 amino acids, although we could not fully separate SGPE from streptogrisin B with a similar molecular mass and isoelectric point.

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Bednář Petr.....ThO-004, WeP-010, ThP-022, ThP-029
Benada Oldřich.....WeP-003
Béres Tibor.....FrO-013, WeP-013
Bilkova Zuzana.....ThS-013
Bláhová Tereza.....WeP-002
Borek Ivo.....WeP-001, ThP-025
Borovcová Lucie.....ThP-026
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Coene Karlien.....FrO-012
Coufalíková Kateřina.....WeO-001, WeP-001, WeP-004
Cvačka Josef.....ThO-011, ThP-024
Černá Zuzana.....FrO-015
Damborský Jiří.....WeP-004
Danchenko Maksym.....WeP-007, ThP-030
Deininger Soeren-Oliver.....ThO-005
Dostálková Alžběta.....WeP-012
Dovrtělová Gabriela.....WeO-001, WeP-004
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Dvorak Vit.....WeP-008
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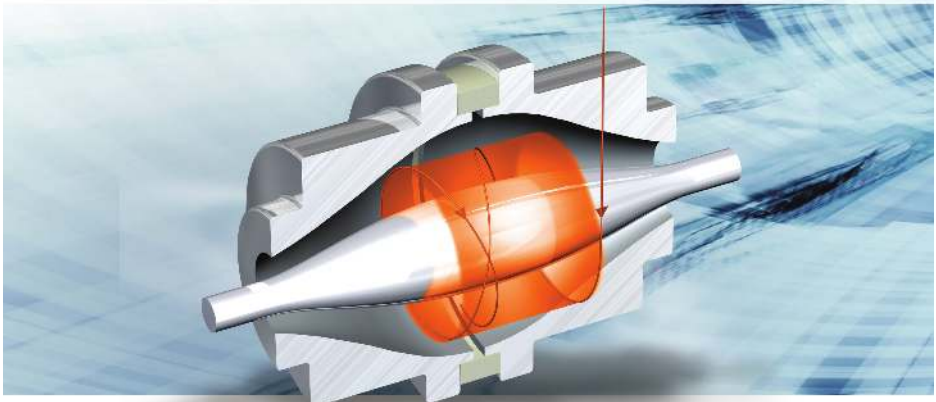

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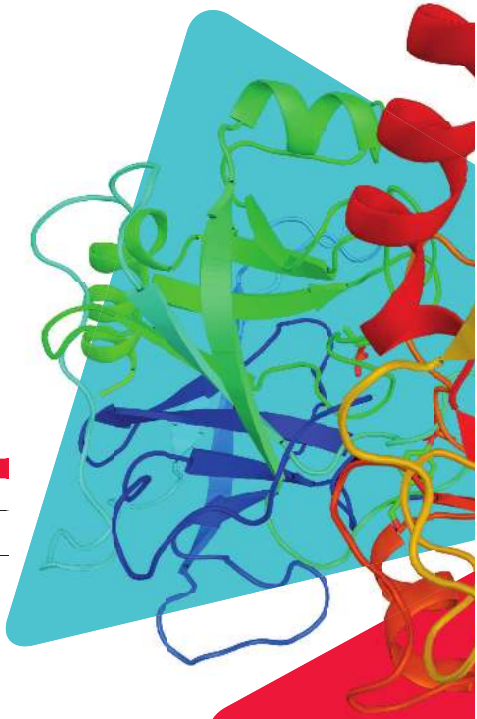

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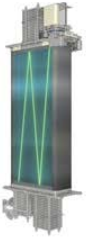

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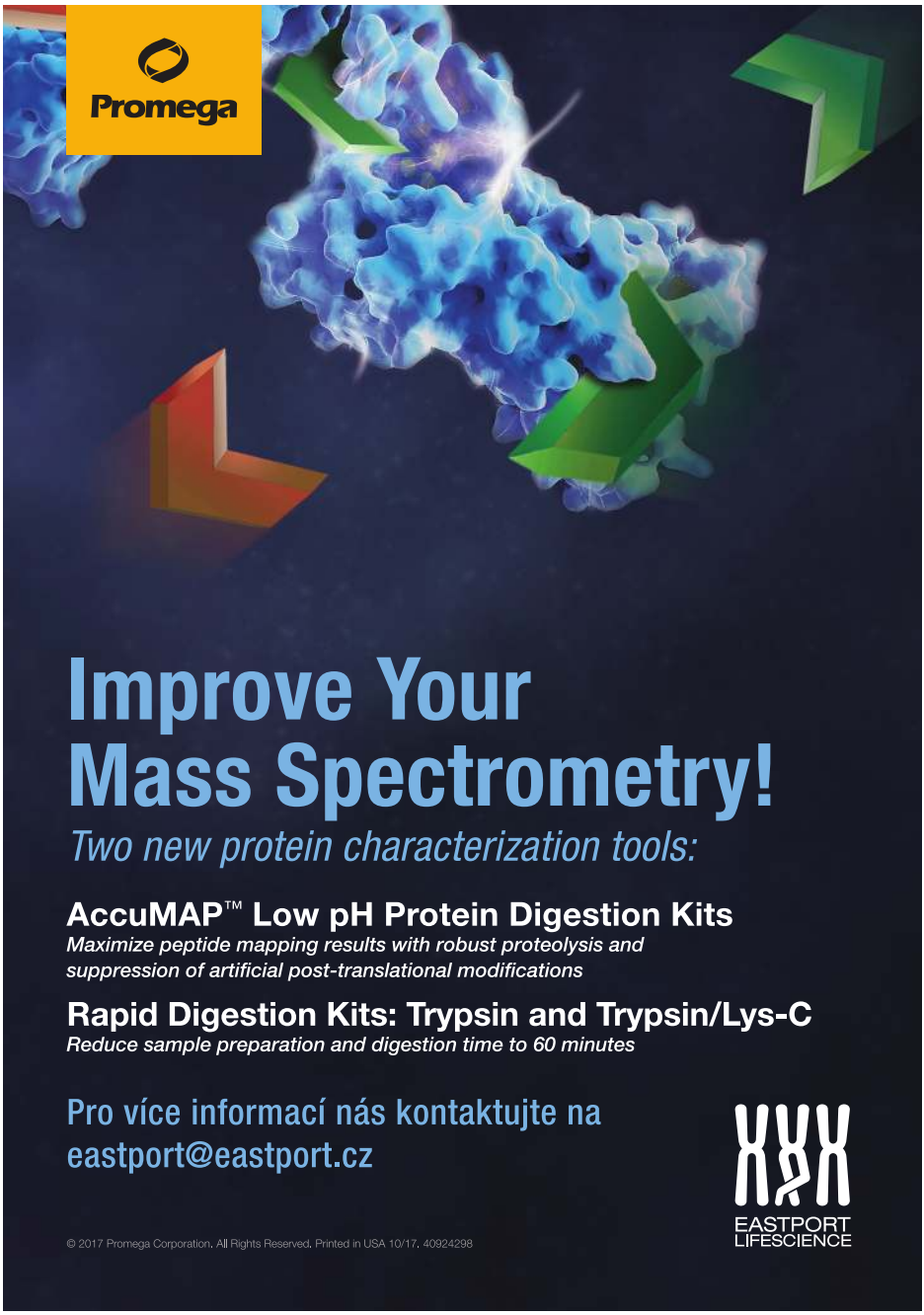
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