PRAGUE MEETING ON HISTORICAL PERSPECTIVES OF MASS SPECTROMETRY

Opening of the Czech Mass Spectrometry Museum

October 13-14, 2021

Book of Abstracts

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Prague Meeting on Historical Perspectives of Mass Spectrometry

October 13-14, 2021

Organizing institutions

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PROGRAM

13th October 2021 (Wednesday)

7:30 - 8:20 **REGISTRATION**

8:20 - 8:40 **OPENING OF THE CONFERENCE**

SESSION I (NLT) ***Chair: František Tureček (University of Washington, United States)***

8:40 - 9:30 **Computational and Gas-phase Studies in Service of Catalysis Research: Identification of the "Aristocratic Atoms"**
Helmut Schwarz (Technische Universität Berlin, Germany)

9:30 - 10:00 **Mass Spectrometry at the J. Heyrovský Institute of Physical Chemistry**
Miroslav Polášek (Heyrovský Institute of Physical Chemistry of the CAS, Czechia)

10:00- 10:30 **Gas Phase Ion-molecule Reactions in Czechia over 60 Years**
Patrik Španěl (Heyrovský Institute of Physical Chemistry of the CAS, Czechia)

COFFEE BREAK

SESSION II (NLT) ***Chair: Michal Holčapek (University of Pardubice, Czechia)***

11:00 - 11:30 **Past, Present and Future of Orbitrap Mass Spectrometry** (virtual presentation)
Alexander Makarov (Thermo Fisher Scientific, Germany)

11:30 - 12:00 **Development of Novel Ion-less Matrices for Profiling and Imaging Mass Spectrometry**
Aleš Svatoš (Max Planck Institute for Chemical Ecology, Germany)

12:00 - 12:30 **Mass Spectrometry at the Institute of Organic Chemistry and Biochemistry & Museum Opening**
Josef Cvačka (Institute of Organic Chemistry and Biochemistry of the CAS, Czechia)

LUNCH BREAK

SESSION III (NLT) ***Chair: Miroslav Polášek (Heyrovský Inst. of Phys. Chem. of the CAS, Czechia)***

14:00 - 14:50 **High-resolution Native Mass Spectrometry**
Albert J. R. Heck (Utrecht University, The Netherlands)

14:50 - 15:20 **Noncanonical Ionized DNA Nucleobases and Nucleosides. A Dark Matter of DNA Ionization?**
František Tureček (University of Washington, United States)

15:20 - 15:50 **Mass Spectrometry at the University of Pardubice: Focus on Lipidomics**
Michal Holčapek (University of Pardubice, Czechia)

15:50 - 16:20 **Forty Year Evolution of High Throughput Mass Spectrometry: A Perspective** (virtual presentation)
Tom Covey (SCIEX, Canada)

BREAK

POSTER SESSION & DINNER (OWTP)

17:30 - 19:00 **POSTER SESSION**

19:00 - 23:00 **SOCIAL EVENT** - *Conference dinner, concert performance, guided underground tours*

PROGRAM

14th October 2021 (Thursday)

SESSION IV (NLT) ***Chair: Jan Preisler (Masaryk University, Czechia)***

8:40 - 9:30 **FT ICR Mass Spectrometry. Is There Room for Further Improvements?** (virtual presentation)
Evgeny Nikolaev (Skolkovo Institute of Science and Technology, Russia)

9:30 - 10:00 **Mass Spectrometry: from Metabolites to Art**
Karel Lemr (Palacký University Olomouc, Czechia)

10:00 - 10:30 **Historical Perspectives on Bruker's Involvement in Mass Spectrometry and the Development of Modern Q-TOF Mass Spectrometry**
Gary H. Kruppa (Bruker, Czechia)

COFFEE BREAK

SESSION V – YOUNG SCIENTISTS (NLT) ***Chair: Karel Lemr (Palacký University Olomouc, Czechia)***

11:00 - 11:15 **Identification of Novel Fatty Acids in Vernix Caseosa using UV Photodissociation Mass Spectrometry in Combination with Fixed-charge Photo-labile Derivatives**
Lukáš Cudlman (Institute of Organic Chemistry and Biochemistry of the CAS, Czechia)

11:15 - 11:30 **Mass Spectrometry-based Chemical Proteomics in Protein Post-translational Modifications Analysis**
Pavel Kielkowski (Ludwig-Maximilians-Universität München, Germany)

11:30 - 11:45 **Mapping of Regional and Subregional Unbound Drug Transport Across Blood-Brain Barrier Enabled by Quantitative Mass Spectrometry Imaging**
Dominika Luptáková (Uppsala University, Sweden)

11:45 - 12:00 **Visualization of Polyamines and Amino Acids Alterations in Neonatal Brain Hypoxic-Ischemic Injury in Rats by Mass Spectrometry Imaging**
Hynek Mácha (Institute of Microbiology of the CAS, Czechia)

12:00 - 12:15 **Temperature-Controlled Electrospray: A Window into Solution Thermochemistry of Non-Canonical Nucleic Acid Complexes**
Adam Pruška (ETH Zurich, Switzerland)

12:15 - 12:30 **Freeing *Aspergillus fumigatus* of Polymycovirus Infection renders it More Resistant to Competition with *Pseudomonas aeruginosa* due to Altered Iron-acquiring Tactics**
Rutuja Patil (Institute of Microbiology of the CAS, Czechia)

LUNCH BREAK

SESSION VI (NLT) ***Chair: Aleš Svatoš (Max Planck Institute for Chemical Ecology, Germany)***

14:00 - 14:50 **Native Ambient Mass Spectrometry: Mass Spectrometry Imaging of Intact Proteins and Protein Complexes** (virtual presentation)
Helen J. Cooper (University of Birmingham, United Kingdom)

14:50 - 15:20 **A (Time of) Flight from Ames to Brno: Laser Desorption Mass Spectrometry at Masaryk University**
Jan Preisler (Masaryk University, Czechia)

15:20 - 15:50 **The History of Time-of-Flight Mass Spectrometry at: VG-Micromass-Waters** (virtual presentation)
Mike Morris (Waters Corporation, Manchester, United Kingdom)

15:50 - 16:20 **Mass Spectrometry Innovation at Agilent Technologies** (virtual presentation)
George Stafford (Agilent Technologies, United States)

16:20 - 16:30 **CONFERENCE CLOSING**

PROGRAM

14th October 2021 (Thursday)

IOCB ChemBioDrug Workshop (NLT) ***Chair: Josef Cvačka (IOCB, Czechia)***

17:00 - 17:05	**OPENING**
17:05 - 17:25	**Orbitrap Fusion Lumos: A Versatile Mass Spectrometer for Biomolecules** *Martin Hubálek (Institute of Organic Chemistry and Biochemistry of the CAS, Czechia)*
17:25 - 17:45	**Protein Structure and Interactions Studied by Cross-Linking Mass Spectrometry** *Petra Junková (Institute of Organic Chemistry and Biochemistry of the CAS, Czechia)*
17:45 - 18:05	**Protein Structure and Interactions Studied by Hydrogen Deuterium Exchange Mass Spectrometry** *Jakub Sýs (Institute of Organic Chemistry and Biochemistry of the CAS, Czechia)*
18:05 - 18:30	**HPLC/ESI-MS² – Based Lipidomic Analysis and MALDI Imaging of Lipids** *Vladimír Vrkoslav, Štěpán Strnad (Institute of Organic Chemistry and Biochemistry of the CAS, Czechia)*
18:30 - 19:00	**Discussion / User Meeting**
	WORKSHOP CLOSING

Lectures

Computational and Gas-phase Studies in Service of Catalysis Research: Identification of the "Aristocratic Atoms"

Helmut Schwarz

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Gas-phase studies on isolated, mass-selected reactants – be these atoms or clusters – provide an ideal arena for probing experimentally the energetics and kinetics of a chemical process in an unperturbed environment at a strictly molecular level without being obscured by difficult-to-control or poorly understand solvation, aggregation, counter ions and other effects. Thus, an opportunity is provided to explore *inter alia* the concept of single-atom catalysis or, more generally, to help identify the active part(s) of single-site catalysts directly. As will be shown, pairing cryonegic ion trap vibrational spectroscopy with advanced quantum chemistry represents a generally applicable approach to address these problems and to identify the elementary steps of genuine catalytic cycles at ambient condition.

Topics to be discussed include:
1) The low-temperature redox-reaction in the N_2O/CO couple as catalyzed by $[AlVO_x]^+$ (x = 3, 4).
2) The selective $C_2H_4 \rightarrow CH_3CHO$ conversion mediated by $[PVO_4]^+$.
3) The room-temperature cleavage of N_2 by pristine $[Ta_2N]^+$: A perfect catalytic cycle.

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[3] Geng, C.; Li J., Weiske, T.; Schwarz, H. *PNAS* **2019**, *116*, 21416–21420
[4] Li, Y.-K. et al. *Angew. Chem. Int. Ed*. **2019**, *58*, 18868–18872

Mass Spectrometry at the J. Heyrovský Institute of Physical Chemistry

Miroslav Polášek

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The story of mass spectrometry[1] at the J. Heyrovský Institute began in 1949 when four graduates of the Charles University, V. Čermák, V. Hanuš, J. Cabicar and Č. Jech joined the postgraduate studies at the Institute of physical chemistry of the Charles University with professors J. Heyrovský and R. Brdička, and together they started working on the construction of a Nier-type mass spectrometer in 1950. This spectrometer was commissioned in 1953, already in the newly established laboratory of physical chemistry of the Czechoslovak Academy of Sciences. In 1955 this laboratory expanded and became the Institute of Physical Chemistry of the Czechoslovak Academy of Sciences. At that time, J. Cabicar and Č. Jech left the group; however, the team of V. Čermák and V. Hanuš soon grew by two other extraordinary personalities - electrical engineer M. Pacák and mechanician J. Protiva. At about the same time, three younger colleagues, Z. Herman, Z. Dolejšek and L. Hládek joined the group. Initially, they all worked together on solving analytical problems coming from external partners, but soon their own entirely mass-spectrometric research program arose. In the late 1950s, the group began to specialize. V. Hanuš and Z. Dolejšek began to concentrate more on the mass spectra of organic compounds and unimolecular reactions of ions, while V. Čermák and Z. Herman began to work on ion/molecule collision processes. Since the early 1960s, V. Hanuš intensively studied the relations between the structure of organic molecules and their mass spectra, a field in which he gained international recognition. He worked mainly on the structural analysis of alkaloids (in collaboration with L. Dolejš from IOCB) and products of organic syntheses and heterogeneous catalysis. In 1962 Hanuš's laboratory was equipped with the Soviet mass spectrometer MCh 1303, which improved the laboratory's experimental capabilities. Further improvement came in 1973 when the Jeol JMS-D100 double-focusing spectrometer was purchased and when an extremely fruitful collaboration with the young organic chemistry student F. Tureček began. This lasted until his emigration in 1987. V. Hanuš's adherence to the use of classical mass spectrometric techniques (i.e. EI HRMS) resulted in the J. Heyrovský Institute's organic mass spectrometry laboratory being gradually equipped with two donated sector-type instruments, which have determined the direction of its research for many years. After V. Hanuš's retirement, his followers began to gradually reconnect with the ion/molecule chemistry group and to work on various aspects of both high- and low-energy ion/molecule phenomena.

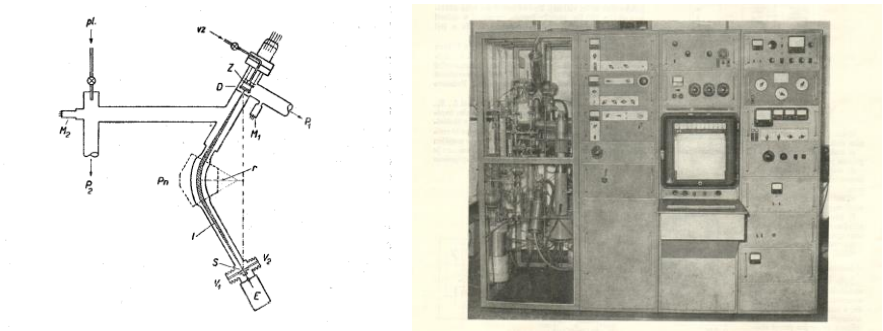

Figure 1. The first Czechoslovak mass spectrometer[2]

References

[1] Herman, Z. In *Počátky a historie Československé hmotnostní spektrometrie*; Pól, J.; Volný, M.; Eds.; Česká společnost pro hmotnostní spektrometrii, Praha, Czech Republic, 2010

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Gas Phase Ion-Molecule Reactions in Czechia Over 60 Years

Patrik Španěl

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The history of research in the area of ion-molecule reactions in Czechoslovakia has its beginnings in the late nineteen fifties and is closely related to the construction of the first mass spectrometer in this country in 1954. An active international community was formed in the subject of dynamics and kinetics of gas phase reactions of ions with molecules, and two Praha centres were its active members: The institute of Physical Chemistry (Čermák, Herman and colleagues) and the Mathematics and Physics Faculty of Charles University (Glosik, Tichý and colleagues). The experimental research carried out over the last six decades in these centres, included many original studies of fundamental importance and also projects motivated by the urge to understand the ion chemistry of natural gaseous media such as the terrestrial ionosphere and the interstellar medium and also technological plasmas and even fusion reactors. The insights and understanding gained of the kinetics of atmospherically important ion-molecule reactions eventually resulted in the "homecoming" of analytical mass spectrometry, especially the techniques for detection and quantification of trace gases and vapours of volatiles in polluted air, exhaled breath and the headspace of biological samples like bacterial cultures and foods and food products.

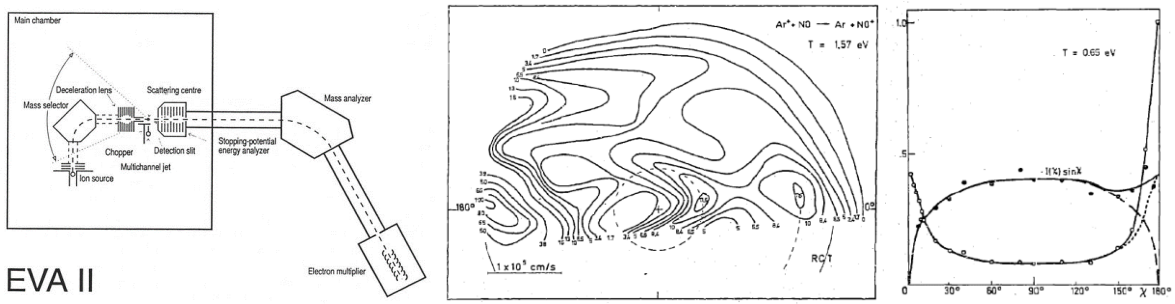

Figure 1. Schematics of the beam scattering apparatus EVA II and illustrative results of angular product ion distributions from Herman, Z. et al. *Chem. Phys. Lett.* **1976**, *37*, 329

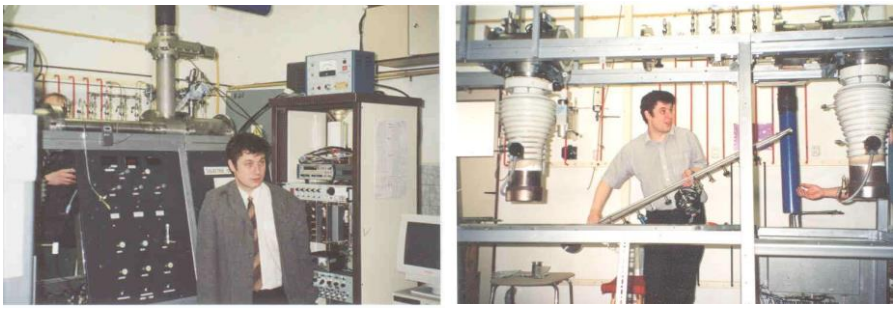

Figure 2. Commissioing of the first Selected Ion Flow Tube (SIFT) instrument in Praha, 1998. (Photo Z. Dolejšek)

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[1] Herman, Z. *Chem. Listy* **2010**, *104*, 955–990
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Past, Present and Future of Orbitrap Mass Spectrometry

Alexander Makarov [a,b]

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This talk will provide a first-hand overview of the short but eventful history of Orbitrap mass spectrometry, from laying down the first principles of the technology to its current status in mainstream mass spectrometry as the leading technique for high-resolution, high mass accuracy quantitative analysis.

Originating from an ideal (and therefore never realized) Kingdon trap, this analyzer can provide high performance analytical characteristics only when it is highly integrated with the ion injection process. The advent of pulsed injection from an external ion storage device has allowed the Orbitrap analyzer to enter mainstream mass spectrometry as a part of a hybrid instrument. Since its introduction, the utility of Orbitrap mass spectrometry has been extended by coupling with additional capabilities such as higher-energy dissociation (HCD), ETD, UVPD and their combinations as well as multiple ion sources. These capabilities were implemented in four major families of instruments with relentlessly expanding performance envelope.

While describing new possibilities arising from the recent extensions of two latest families of instruments, Orbitrap Fusion and Orbitrap Exploris, a special emphasis will be placed on technical solutions that enhance quantitative, structural and high-mass analysis in these instruments.

In conclusion, future trends and perspectives of Orbitrap mass spectrometry are discussed. It is shown that Orbitrap-based mass spectrometers possess compelling potential as an (ultra-) high resolution platform not only for high-end proteomic applications but also for routine, translational and clinical analysis.

Development of Novel Ion-Less Matrices for Profiling and Imaging Mass Spectrometry

Aleš Svatoš [a,b]

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Twenty years ago, the MALDI methodology was fully developed for the analysis of peptides or proteins thanks to the extensive efforts of Karas, Hillenkam, Tanaka and co-workers in the 1980s and 1990s. However, most or the developed organic matrices were not suitable for the analysis of small metabolites, due to the presence of intense matrix-related ion signals in the low mass range. My group started in 2003 to develop new matrices (**1**) for small molecules[1] in order to use our chemical knowledge and contacts to design suitable matrices. We have found that a sufficient type of ionic matrices for the analysis of acids and similar substances has been proposed by sufficiently increasing the basicity of the matrix (**2**,**3**,**4**) and eliminating slightly ionized functional groups in the matrix molecule.[2-5] Later, we used artificial or natural nanostructures (**5**) for the direct desorption of the metabolite without the use of organic matrices and thus significantly prevented the influence of matrix ions on the analysis result.[6] The applicability of these matrices will be illustrated in our application work.

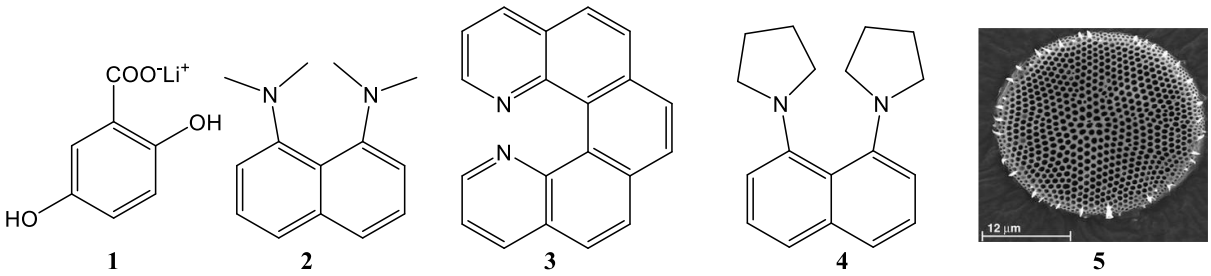

Figure 1. Developed matrices or supports.

References

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Mass Spectrometry at the Institute of Organic Chemistry and Biochemistry & Museum Opening

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The history of mass spectrometry at the IOCB began in the early 1960s when the director František Šorm sent Ladislav Dolejš to the Institute of Physical Chemistry, Prague, for a long-term internship. L. Dolejš worked together with Vladimír Hanuš, mainly on alkaloids. Later, he founded the Laboratory of Mass Spectrometry at the IOCB and headed it for many years. The first mass spectrometer was the Soviet magnetic sector MCH1303, and it served from 1967 to 1972. An incomparably better instrument, double-focusing MS902 (AEI Scientific Apparatus; Fig. 1a), was installed in 1969 and used to elucidate the structure of many organic compounds. Interesting natural products like insect pheromones or defense compounds were discovered. The growing need for studying high molecular weight substances resulted in acquiring a new double-focusing spectrometer with FAB ionization in 1987. ZAB EQ (VG Analytical) was used to study numerous compounds, including the nucleotide analogs from the laboratory of Antonín Holý that dramatically impacted HIV treatment in the world. At that time, Karel Ubik headed the Mass Spectroscopy Group. The declining interest in FAB and problematic maintenance of the aging ZAB EQ led to the purchase of the LTQ Orbitrap XL (Thermo Scientific) in 2007.

a b c

Figure 1. Jitka Kohoutová is operating MS902 (a), an experimental ion source for imaging non-planar objects (b), and a view of the display case with the Czech Mass Spectrometry Museum exhibits (c).

Mass spectrometry continued to gain importance at the IOCB in chemistry and also in biologically oriented disciplines. The Mass Spectrometry Group, headed by Josef Cvačka since 2005, gradually expanded. Now it is one of the research-service groups, which serve as core facilities and, at the same time, conduct independent research. The MS group is unique in its scope of expertise, which covers the structural and quantitative analysis of small molecules, lipidomics, classical, quantitative, and structural proteomics, mass spectrometry imaging, technical development (Fig. 1b), and other areas. Mass spectrometry also developed outside the MS group, mainly in the former Department of Natural Products. The IOCB is proud of the work of Detlef Schröder, an exceptionally talented scientist in the field of physical organic chemistry and mass spectrometry, who worked at the IOCB in 2006 - 2012.

The idea to establish an MS museum was around since the late 2000s, when a collection of ion sources, optics, and detectors from the disassembled ZAB EQ decorated the office of the group leader. The first steps to establish the museum were taken in 2013. Almost another eight years were spent negotiating the exhibition's location, searching for and cataloging exhibits, and creating the museum website `www.ms-museum.org` (Fig. 1c).

High-resolution Native Mass Spectrometry

Albert J.R. Heck

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Native mass spectrometry (MS) involves the analysis and characterization of macromolecules, predominantly intact proteins and protein complexes, whereby as much as possible the native structural features of the analytes are retained. As such, native MS enables the study of secondary, tertiary, and even quaternary structure of proteins and other biomolecules. Native MS represents a relatively recent addition to the analytical toolbox of mass spectrometry and has over the last decades experienced an immense growth, especially in enhancing sensitivity and resolving power, but also in ease-of-use. In my lecture I will provide a personal perspective on the early development of native mass spectrometry, but also highlight some recent developments, in particular in high-resolution native MS, describing applications in the structural analysis of protein assemblies, proteoform profiling of – among others – biopharmaceuticals, plasma proteins and viruses.

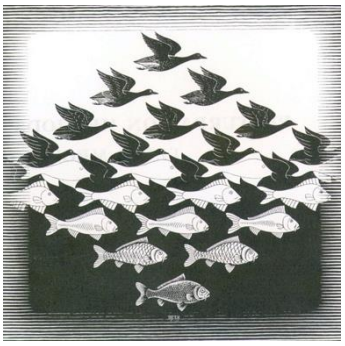

Figure 1. Native Mass Spectrometry; from solution to the gas phase. Sky and Water, M.C. Esher (1938)

References

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Noncanonical Ionized DNA Nucleobases and Nucleosides. A Dark Matter of DNA Ionization?

František Tureček,[a] Shu R. Huang,[a] Gabriela Nováková,[b] Aleš Marek [b]

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DNA ionization is a process of a considerable interest due to chemical reactions of cation and anion radicals that can lead to mutations or life-threatening strand breaks. The vast majority of experimental and computational studies of DNA ionization have implemented the paradigm of ionized DNA nucleobases retaining the chemical structure of their neutral counterparts, adenine (A), guanine (G), cytosine (C), and thymine (T). We have reported the generation of stable non-canonical cation-radical isomers of thymine,[1] 1-methylcytosine,[2] and 9-methyladenine.[3] These ions have been generated in the gas phase, selected by mass, and characterized by tandem mass spectrometry and UV-Vis photodissociation action spectroscopy in combination with CCSD(T)/CBS ab initio calculations of energies and time-dependent density functional theory calculations of vibronic spectra. The lecture will present an example of the targeted synthesis of gas-phase 9-methylene-(^1H)adenine cation radical and its spectroscopic characterization by UVPD-MS^3 and structure analysis of its major dissociation product using UVPD-MS^4 measurements. UVPD action spectroscopy has been shown to be a powerful tool for structure elucidation of gas phase ions,[4] as illustrated in Figure 1 by comparing the mass-resolved action spectra of the methyladenine isomers. Non-canonical isomers of ionized 2'-deoxyadenosine, guanosine, cytidine, and thymidine have been calculated by CCSD(T)/CBS to be thermodynamically more stable than their canonical forms in both the gas phase and when solvated by water.

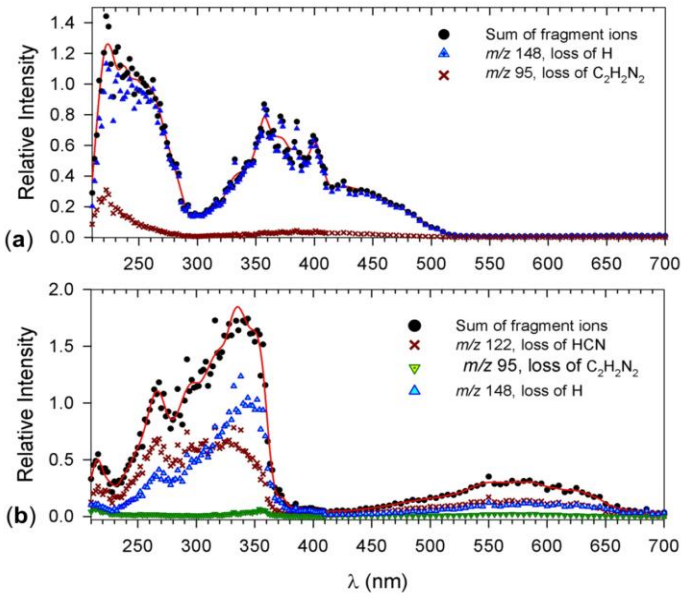

Figure 1. UVPD action spectra of (a) noncanonical 9-methylene-(^1H)adenine and (b) canonical 9-methyladenine cation radicals.

References

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Mass Spectrometry at the University of Pardubice: Focus on Lipidomics

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The history of mass spectrometry research at the University of Pardubice started in 1995 with the installation of a single quadrupole mass analyzer for LC/MS in the group of Prof. Pavel Jandera, who was a world class leader in the fundamentals of liquid chromatography. This was the first LC/MS system in the Czech Republic used routinely for LC/MS coupling, with the initial paper[1] on the retention behavior of ethoxylated alcohol surfactants published in 1998. In this starting period, various organic compounds typical for the chemical industry in the Pardubice region were analyzed, such as organic dyes.[2] A fruitful cooperation with research groups at the Department of Inorganic Chemistry focused on the synthesis of organometallic compounds began.[3] Later, we established cooperation with the Faculty of Pharmacy in Hradec Králové, where our task was the characterization of the metabolism of various drugs.[4] The first article related to lipid analysis was published in 1999 on the monitoring of biodiesel production by LC/MS.[5] The analysis of lipids as biomolecules involved in numerous metabolic pathways had attracted my attention from the beginning and continued with further work on the detailed characterization of triacylglycerols. We developed various LC/MS methods for the analysis of all existing types of triacylglycerol isomers, including regioisomers, double bond (DB) positional isomers,[6] DB geometrical isomers (cis / trans)[7] up to enantiomers.[8] Finally, we moved toward the comprehensive lipidomic analysis of many classes of phospholipids, sphingolipids, glycerolipids, fatty acyls, and sterols with the emphasis on validated quantitative workflows applicable in clinical analysis.[9] The development of LipidQuant 1.0 tool for automated processing of lipidomic data[10] was essential for high-throughput analysis of large clinical cohorts. The method for early detection of pancreatic cancer from human blood was patented,[11] and the revised version of the manuscript is now being considered.[12] A similar pattern of lipid dysregulation is also observed for other types of cancer.[13] My group has actively contributed to the MS community in the Czech Republic with the organization of several MS (https://holcapek.upce.cz/skoly-ms-archiv.php) and participated in the establishment of the Lipidomics section within the Czech Society for Biochemistry and Molecular Biology (http://lipidomics.uochb.cas.cz). I am also a founding member of the International Lipidomics Society (https://lipidomicssociety.org) with the position of vicepresident for conferences.

Acknowledgement: I greatly appreciate the skillful work of numerous current and former group members and other cooperators who contributed to individual papers and grant projects.

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Forty Year Evolution of High Throughput Mass Spectrometry: A Perspective

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The field of drug discovery has been the primary driver behind the development of quantitative high throughput mass spectrometry (HTMS) over the past several decades. Only in the past few years has the throughput of mass spectrometry-based assays risen to a point to match the daily throughputs of optical plate reader technology by achieving sampling rates of 1 Hz. It is the purpose of this presentation to attempt to explain how this came about, by defining what the trends in the field of HTMS were over the past 40 years using specific examples to illustrate its evolution. This perspective attests to the importance of addressing as many of the bottlenecks in the overall HTMS workflow as possible so a wide variety of technologies are shown to be contributors to the solution, in particular, the development of liquid introduction interfaces to atmospheric pressure ionization (API) that exhibits broad compound coverage, can be adapted to high inlet fluid flow rates, and is reliable and robust under high sample loads. High speed and parallelized chromatographic systems have played a major role and ion mobility has entered the HTMS scene as a faster alternative to some of the functions high performance liquid chromatography (HPLC) provides. Improvements to the sensitivity of mass spectrometers over time have been key to enabling HTMS systems by reducing and sometimes eliminating sample preparation requirements as a consequence of the reduction in sample volumes required to achieve biologically relevant quantitation limits. And finally, the emergence of low volume, contact free dispensers and the development of interfaces to transport these samples to the ion source indicate that throughputs an order of magnitude faster than one sample per second are within reach in the not too distant future. The historical relationship and interdependence of these various technologies as components of HTMS systems are described.

FT ICR Mass Spectrometry. Is There Room for Further Improvements?

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Fourier transform ion cyclotron resonance (FT ICR) mass spectrometer offers highest resolving power and mass accuracy among all types of mass spectrometers. Its unique analytical characteristics made FT ICR important tool for proteomics, metabolomics, petroleomics, imaging and investigation of complex mixtures. This technique is now almost fifty years old. Many laboratories and very talented scientists participated in its development. The main elements of FT ICR instruments - the FT ICR ion trap (called a measuring cell) and magnets, which create a strong magnetic field (necessary for the occurrence of cyclotron resonance), have undergone significant changes during this time. The main manufacturer of FT ICR mass spectrometers offers an instrument with a routine mass resolution of about ten million, equipped with almost all major ion sources and fragmentation methods for MS/MS.

Two unique 21 Tesla devices were developed, which allow measuring mass spectra of very complex chemical mixtures in a dynamic range close to 10^5. Possibility of equipping the device by the manufacturer with all known ion fragmentation methods (CID, SID, ECD, ETD, IRMPD, UVPD) has been demonstrated by many laboratories. A mass measurement accuracy of about 10 ppt has also been demonstrated. Modern superconducting magnets no longer consume liquid nitrogen and liquid helium in large amounts. The consumption of this expensive cryogenic liquid has been a major limiting factor in the widespread adoption of FT ICR mass spectrometry in previous years.

In recent years, many new ideas have been proposed to further improve the resolution and accuracy of the FT ICR mass spectrometer. These include an open dynamically harmonized FT ICR cell and a cell built into a vacuum system, making the mass spectrometer much more reliable for research. The European and Korean FT ICR consortia plan to build 24 Tesla devices over the next 3-4 years. Fourier-transform ICR MS continues to be the slowest MS method among other modern mass spectrometry methods. Many efforts have been made to shorten the measurement and signal processing times. Searches in this direction continue.

If successful, LC-MS and GC-MS will be taken to the next level in mass resolution and mass accuracy. All of these innovations can help bring FT ICR mass spectrometry back from the niche of unique instruments to the niche of conventional laboratory instruments.

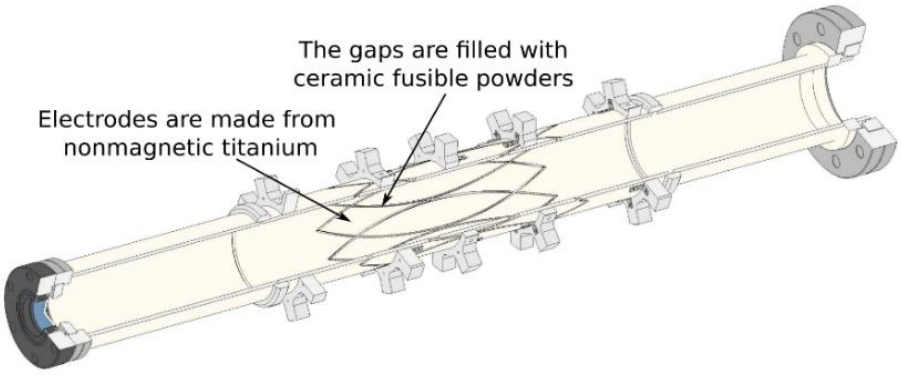

Figure 1. Open dynamically harmonized FT ICR cell

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Mass Spectrometry: From Metabolites to Art

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The first mass spectrometer (an ion trap) was installed at the Department of Analytical Chemistry (Faculty of Science, Palacký University) in 1997. Since this time, the department has been equipped with different systems coupling separation techniques (gas, liquid, supercritical chromatography, and capillary electrophoresis) with mass spectrometry (quadrupoles, ion traps, and Q-TOFs). In December 2019, a cyclic ion mobility-mass spectrometry system was installed (the third installation in the world). Each system offers new possibilities beneficial to our research and the development of analytical applications. A few selected examples are presented to document almost 25 years of mass spectrometry at our department. Besides mass spectrometry applications, they show ion source development and the interesting behavior of analytes.

The identification of metabolites and products of electrochemical reactions[1,2] belonged to the first solved tasks. The ionization and fragmentation of purines and pyrimidines as markers of inherited metabolic disorders were studied.[3,4] In 2007, we described desorption nanoelectrospray reaching lateral resolution in tens of micrometers.[5,6,7] Ion mobility-mass spectrometry allowed us to discriminate isomeric oligosaccharides derived from hyaluronic acid[8,9] and to contribute to the investigation of adrenaline binding to a peptide.[10] Gunshot residue analysis on latent fingerprints was performed using laser ablation.[11] The identification of historical pigments,[12] the detection of plant gum binders in watercolors,[13] and mass spectrometry imaging of egg yolk and oil binders represent artwork analysis and cultural heritage studies. Many other applications could be mentioned, e.g., the analysis of pharmaceuticals, abused drugs, precursors of chemical warfare agents, beverages, and food dyes. Mass spectrometry has become an important tool in our research and allows us to focus on new topics that would hardly be solved without its contribution.

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Historical Perspectives on Bruker's Involvement in Mass Spectrometry and the Development of Modern Q-TOF Mass Spectrometry

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Bruker's involvement with mass spectrometry began with its acquisition of Franzen Analysentechnik GmbH & Co. KG in 1980. Franzen Analysentechnik was founded by Dr. Jochen Franzen in April 1977, in Bremen, which itself has a rich history in mass spectrometry. Franzen Analysentechnik initially focused on the successful development of mobile mass spectrometers based on novel quadrupole and later ion trap analyzer mass spectrometers. The target market for these devices were military and environmental, the detection of chemical and later biological warfare agents, and the in-field detection of toxic environmental contaminants. In 1980 Bruker acquired Franzen Anlysentechnik, which then became in Bruker-Franzen Analytik GmbH. In the 1980's Bruker-Franzen Analytik also began a research program to develop a time-of-flight (TOF) mass analyzer with multi-photon ionization using a laser focused on gas pulsed into the source region. One goal of the research program was to develop a high-speed mass spectrometer that could acquire spectra at a rate sufficient to study the composition of internal combustion engine exhaust as a function of engine cycle, fuel composition etc. The resulting product, called the turboTOF was used in many interesting applications. In 1988 Bruker-Franzen Analytik again changed its name to Bruker Daltonik GmbH, and Dr. Franzen continued to serve as a managing director and head of the patent department.

One of the most interesting aspects of the development of the turboTOF was that when Tanaka and Karas and Hillenkamp began publishing on the use of MALDI-TOF for the intact ionization and mass analysis of large biomolecules, including proteins, in the late 1980's Bruker Daltonik already had a commercial TOF-MS product that used laser ionization. It was thus relatively quick and easy for Bruker to adapt the turboTOF for use as a MALDI-TOF, which was started in the late 1980's and Bruker's Reflex MALDI-TOF system was launched in the early 90's, among the first commercial MALDI-TOF systems available on the market. There have and continue to be many interesting applications of MALDI-TOF systems, but after the development of electrospray ionization (ESI) for large biomolecules by Fenn and co-workers also in the late 1980's, interest in the mass spectrometry community shifted to coupling ESI to mass analyzers due to the ease of coupling liquid chromatography (LC) separations to an ESI type source. Bruker also coupled ESI source technology to its high resolution FTMS systems as well developing ESI-TOF and ESI-Q-TOF systems, the first products being the BioTOF and BioTOF-Q developed in the mid '90s.

This presentation will be a tribute to the career of Prof. Dr. Jochen Franzen and focus on a historical perspective of Bruker's involvement in mass spectrometry and specifically on the development of modern Q-TOF mass spectrometers for biological applications.

Identification of novel Fatty Acids in Vernix Caseosa Using UV Photodissociation Mass Spectrometry in Combination With Fixed-Charged Photo-Labile Derivatives

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Vernix caseosa (VC) is a white biofilm protecting a human fetus during the late stages of in-utero development. It contains a complex mixture of lipids, including free and esterified fatty acids (FA) many of which are unique to VC. These include FA with positions of methyl-branching and carbon-carbon double bonds that are unlike any structures reported in human biology.[1] Besides traditional GC-MS methods, LC-MS approaches are increasingly favoured for FA analysis due to enhanced sensitivity, particularly for ultra-long chain species. To complement these workflows, ozone-induced dissociation[2] and ultraviolet photodissociation (UVPD)[3] can be applied to localize double bond(s) and methyl-branching positions, respectively. In the case of UVPD, FA have been derivatized to incorporate a fixed-charge pyridinium moiety that incorporates a photo-labile functional group that is activated by a UV laser to promote radical-directed dissociation.[3]

In this work, FA standards and FA isolated from VC were derivatized with 1-(3-(aminomethyl)-4-iodophenyl)pyridin-1-ium (4-I-AMPP$^+$) and ionized by electrospray source. MS and MS/MS spectra were acquired using Orbitrap Fusion Lumos Tribrid mass spectrometer (Thermo Fischer Scientific) equipped with a 213 nm laser (Figure 1). Reversed-phase Acclaim C30 HPLC column (2.1 x 150 mm, 3 µm; Thermo Fischer Scientific) and a mobile phase containing water, acetonitrile, 2-propanol, and ammonium formate were used for the separation. UVPD spectra showed structurally diagnostic signals arising from radical-directed dissociation of carbon-carbon bonds. While product ions from saturated FAs were separated by 14 Da, a double bond manifested itself as a difference of 12 Da. The presence of double bonds also led to increased abundance of product ions separated by 54, 94, or 120 Da, depending on the number of double bonds within the chain. In the spectra of methyl-branched FAs, a branching site created a 28 Da gap in the product ion series, thus enabling straightforward assignment of methyl-branching positions position. Using this LC-UVPD workflow it was possible to identify a wide range of unsaturated and methyl-branched FA in VC, including previously unreported species such as the triply branched species; 8,10,13-trimethyltetradecanoic acid. These methods will contribute to a better understanding of the biological functions of VC as well as insights into the biochemical origins of this fascinating substance.

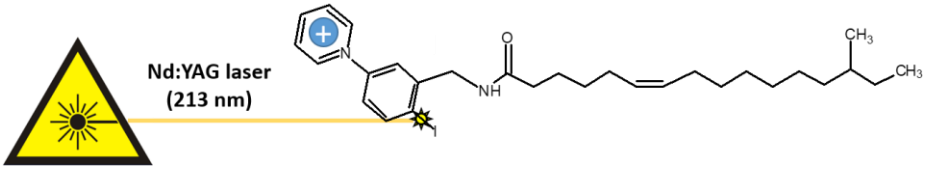

Figure 1. UVPD MS/MS of FA 4-IAMPP$^+$ derivatives

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Mass Spectrometry-based Chemical Proteomics in Protein Post-translational Modifications Analysis

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An increasing speed and sensitivity of nowadays mass spectrometers in combination with small compound probes, which are attached onto proteins in parallel to the endogenous post-translational modifications (PTMs) enable to discover the previously unknown scope of protein modification in living cells.[1,2] Here, I focus on a chemical proteomic analysis of protein AMPylation in differentiating neurons.[3,4] Protein AMPylation is characterized by attachment of an adenosine 5'-*O*-monophosphate onto protein's serine, threonine or tyrosine and it is catalyzed by thus far two known AMP-transferases in human cells.[5-7] The low abundance of the AMPylated peptides in a tryptic digest of a full proteome together with a decreased ionization efficiency due to the phosphate group necessitate for an enrichment of the modified peptides before the mass spectrometry analysis. However, the enrichment strategies tend to be tedious and require rather large amounts of the starting total protein, which restrict to carry out inevitable big scale studies mapping the protein AMPylation patter in many different cell types and conditions with high temporal resolution. Our laboratory has therefore focused on development of a novel high-throughput strategy for enrichment of PTM proteins, subsequent mass spectrometry analysis and essential bioinformatics of resulting data. The strategy promises to provide biologists with an automated analysis of protein PTMs to disentangle AMPylation and other protein PTMs complex regulatory networks.

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Mapping of Regional and Subregional Unbound Drug Transport Across Blood-Brain Barrier Enabled by Quantitative Mass Spectrometry Imaging

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Comprehensive determination of the extent of drug transport across the region-specific blood-brain barrier (BBB) is a major challenge in preclinical studies. To understand the biological mechanisms of low-molecular weight drug transport, it is essential to recognize that only unbound (free) molecule can cross the membrane to interact with targets in the brain and initiate a pharmacological response.[1] To study, spatially visualize and quantify unbound BBB drug transport, we determined the partition coefficient (K_p) of unbound drug (uu) in the brain ($K_{p,uu,brain}$). We combined *in vivo* brain neuropharmacokinetics (neuroPK) and *in vitro* brain slice drug distribution studies with MALDI Fourier transform ion cyclotron resonance quantitative mass spectrometry imaging (qMSI), and established a novel approach the qMSI for unbound drug determination (qMSI-uD). Using this method, we studied antipsychotic drugs risperidone, clozapine, and olanzapine, displaying different neuroPK properties. qMSI-uD enabled to visualize the extent of unbound drug regional and subregional BBB transport characteristics at 20 µm lateral resolution, as well as the post-BBB cerebral drug distribution. We showed significant differences in all three drugs between total and regional $K_{p,uu,brain}$, higher unbound drug BBB transport in gray matter compared to white matter, as well as differences in between brain cortex subregions. qMSI-uD method allows investigation of heterogeneity in BBB transport and presents new possibilities for molecular psychiatrists and neuroscientists by facilitating interpretation of regional target-site exposure results and decision making.

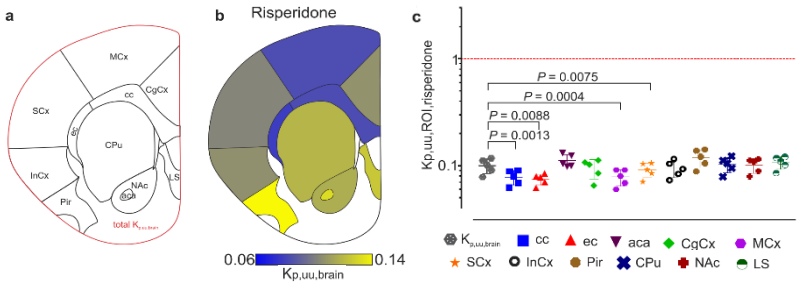

Figure 1. Mapping the extent of risperidone unbound drug transport across the BBB on regional and subregional levels using $K_{p,uu}$ in the imaged brain section and annotated brain regions (**a**), and heat map (**b**) and scatter dot plot (**c**) of regional $K_{p,uu,ROI}$ values. $K_{p,uu,ROI}$ under unity indicates predominantly passive BBB transport. cc, corpus callosum; ec, external capsule; aca, anterior commissure; Cg, cingulate; M, motor; S, somatosensory; In, insular; Pir, piriform; Cx, cortex; CPu, caudate putamen; NAc, nucleus accumbens; LS, lateral septum.

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Acknowledgments

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Visualization of Polyamines and Amino Acids Alterations in Neonatal Brain Hypoxic-Ischemic Injury in Rats by Mass Spectrometry Imaging

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Neonatal brain hypoxic-ischemic (HI) injury represents a major cause of mortality and morbidity in newborns characterized by two distinct types of brain energy metabolism failure[1]. Primary energy metabolism failure occurs due to lack of oxygen under hypoxic phase; delayed in time, secondary energy metabolism failure occurs under reoxygenation due to hypoxia-induced membrane depolarization and resulting intracellular calcium overload. Excitotoxicity and redox imbalance (oxidative stress) may subsequently be involved. Importantly, occurring within 6-24 hours after the birth, the degree of the secondary failure underlies the severity of the HI insult that per se determines the neonate's outcome. In the present study, using the Rice-Vannucci model in 7-day old rats, we focused on early metabolic changes in the brain. Metabolic profiling was spatially visualized and relatively quantified by high-resolution MALDI Fourier transform ion cyclotron resonance mass spectrometry imaging in brain tissue samples collected 12, 24, and 36 hours after the insult. At a 12-hour time point, a significant increase in polyamines – putrescine, cadaverine, and 3-aminopropanal, and amino acids – histidine, glycine, and valine were found in HI-insulted brain structures, particularly in the cortex, thalamus, and hippocampus. Taurine decreased in all three-time points, particularly in the cortex and hippocampus. Disruption of polyamine metabolism may reflect ischemia[2] and electron transport chain malfunction, and antioxidant taurine decrease and glycine increase may indicate oxidative stress and excitotoxicity, respectively. In conclusion, the observed metabolic alterations may be prospective as early biomarkers of neonatal brain HI injury in human newborns.

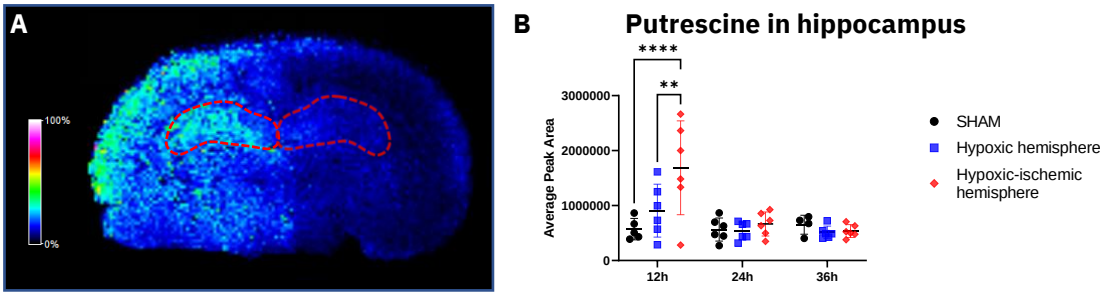

Figure 1. Altered distribution of putrescine ($[M+FMP10-CH_3]^+$ = 356.2126 *m/z*) in the HI-insulted rat brain tissue at 12 hours after the insult showing increased intensity, particularly in HI hemisphere (A) with representative relative quantification from the hippocampus (B). **, $p<0.01$; ****, $p<0.0001$; FMP10, 4-(Anthracen-9-yl)-2-fluoro-1-methylpyridin-1-ium iodide, reactive derivatization matrix

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Temperature-Controlled Electrospray: A Window into Solution Thermochemistry of Non-Canonical Nucleic Acid Complexes

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Repeated nucleic acid (NA) sequences readily form non-canonical structures such as G-quadruplexes (GQ), i-motifs, and multi-way junctions. Despite the importance of these structures in cellular processes, *in situ* characterization is limited in part because suitable detection methods have been lacking. Multi-way junctions and hybrid GQ-duplex oligonucleotide assemblies composed of multiple domains (Figure 1A) are particularly challenging to study with conventional biophysical methods due to their structural complexity.[1] Detailed characterization of NA thermodynamics and ligand binding aids for developing targeted genetic disease therapeutics. Here we demonstrate our development of temperature-controlled electrospray ionization (TC-ESI). The source design includes a nanoESI emitter placed between two copper blocks guaranteeing uniformly distributed heat (Figure 1B). It is coupled to a Synapt G2S or cyclic ion mobility mass spectrometer (cIMS MS).[2] MS thermal denaturation experiments were designed to acquire mass spectra with increasing source temperature, to observe unfolding steps of individual domains of multi-stranded oligonucleotide constructs (Figure 1C). We observed changes in thermodynamic parameters of individual DNA domains depending on the number of domains, their position, and order. Thermal denaturation of multi-domain complexes showed a significant difference in the melting temperature (T_m) compared to individual domains. Moreover, both three-way and Holliday junction unfolding pathways have been discovered and characterized. Specific binding of newly synthesized junction-specific ligands, TrisPOB and 2,7-TrisNP, has been investigated and shown destabilizing effects using van't Hoff analysis.[3] In conclusion, our methodology is a powerful tool to identify binding events and quantify unfolding intermediates if they significantly differ in thermal stability as demonstrated on simple D-G two-domain complex (Figure 1D).

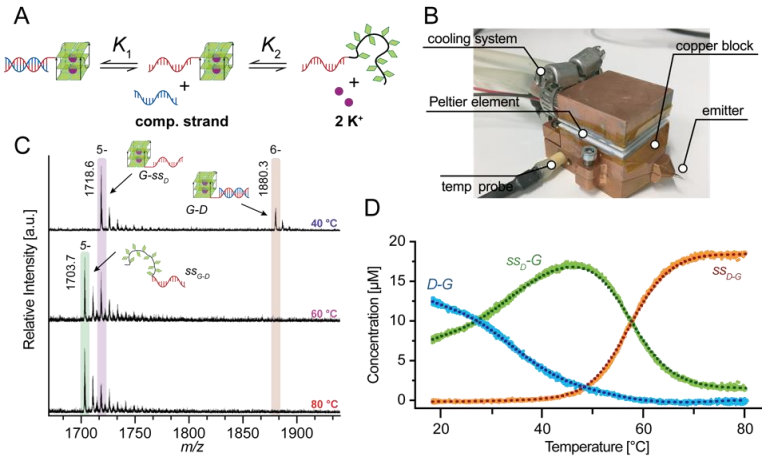

Figure 1. (A) Duplex-G-quadruplex (D-G) unfolding pathway, suggesting subsequent unfolding of individual domains. (B) Picture of TC-ESI showing the essential parts. (C) Mass spectra required at various temperature demonstrating change in signal intensities representing various complex forms. (D) Thermal denaturation melting curves of D-G complex showing clear evidence of half-folded intermediate.

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Freeing *Aspergillus fumigatus* of Polymycovirus Infection Renders it More Resistant to Competition with *Pseudomonas aeruginosa* due to Altered Iron-Acquiring Tactics

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Background: Mycovirus families are diverse and can affect their fungal hosts, including inducing hypovirulence, hypervirulence, or a killer phenotype via toxin production.[1] However, the mechanisms controlling mycovirus-induced phenotypic alterations or host-virus protein-protein interactions are yet unclear. Previous studies showed, virus-free *A. fumigatus* better-withstood competition with *P. aeruginosa*, i.e., the mycovirus infection weakened *A. fumigatus* by a mechanism somehow linked to iron metabolism.[2]

Methods: One of 2 polymycovirus (AfuPmV-1)-infected *A. fumigatus* isolates was cured of virus using cycloheximide, generating an isogenic *A. fumigatus* virus-free strain (VF). We re-infected VF strain with purified virus and concurrently studied VF (18-42) and 4 virus-infected (VI) lines: two infected parents (10-53, 18-95) and two re-infected strains (19-40, 19-47). The siderophore biosynthetic machinery efficiency was compared using three biological and three technical replicates/strains. The extracellular profiles of all five strains were sampled at 24, 31, 48, 54, and 72 hours and quantitatively examined by liquid chromatography and mass spectrometry. Intracellular profiles were quantitatively accessed at the stationary phase.

Results: When grown in an iron-limited medium, VF demonstrated the best fitness represented by the fastest onset of its exponential growth. The exponential phase and transitional production phase of the extracellular triacetylfusarinine C (TAFC) were achieved at 24 and 31 hours, respectively, contrary to VI strains. The VF strain had significantly higher intracellular ferricrocin (FC) concentration monitored in the stationary phase of growth. All these differences were statistically significant; Kruskal–Wallis, $p < 0.01$.

Conclusions: Our data show that, siderophore reservoir was consumed sooner in VF strain. Virus gene expression in the infected fungal cell represents a more substantial metabolic burden illustrated by a slower rate and lower dry cell weight achieved in the stationary phase (an effect similar to recombinant bacterial cells with high copy number plasmid DNA). The viral proteins or RNA may interact with fungal siderophore synthesis and iron metabolism. Fungal virulence attenuation through transfection of *Aspergillus* with an AfuPmV-1 may represent a future antifungal therapy analogous to antibacterial phage therapy.

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Native Ambient Mass Spectrometry: Mass Spectrometry Imaging of Intact Proteins and Protein Complexes

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Mass spectrometry imaging (MSI) provides information on the spatial distribution of molecules within a biological substrate, such as a thin tissue section, without the requirement for labelling. Ambient mass spectrometry, in which biological substrates are sampled at ambient temperature and pressure, and which requires little or no sample preparation, is ideally suited to *in situ* analysis of biomolecules. A suite of ambient mass spectrometry techniques exist including liquid extraction surface analysis (LESA), desorption electrospray ionisation (DESI) and nanoelectrospray desorption electrospray ionisation (nano-DESI), all of which have found applications in MSI.

A separate branch of mass spectrometry, native mass spectrometry, provides information relating to protein structure, including stoichiometry of protein assemblies and protein-ligand complexes, through retention of solution-phase interactions in the gas-phase. When native mass spectrometry is combined with ion mobility spectrometry, it is possible to determine rotationally-averaged collision cross sections.

Our goal is to combine native mass spectrometry with ambient mass spectrometry imaging to enable simultaneous acquisition of spatial and structural information on intact proteins directly from their physiological environment. Latest developments with LESA and nano-DESI, and their integration with ion mobility spectrometry, for the identification, structural characterisation, and imaging of monomeric proteins, protein assemblies, and protein-ligand complexes directly from a range of tissue types and pathologies will be presented.

A (Time of) Flight from Ames to Brno: Laser Desorption Mass Spectrometry at Masaryk University

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In the Laboratory of bioanalytical instrumentation at Masaryk University in Brno, we focus on the development of mass spectrometry (MS) instrumentation and methods for faster and more sensitive bioanalysis. The object of our interest ranges from small organic molecules to metal nanoparticles. This contribution will attempt to give a historical overview of the projects we were involved in.

A prerequisite for the instrumental development was mastering the time of flight (TOF) mass analyzer construction; the first apparatus was built for fundamental studies of matrix-assisted laser desorption/ionization (MALDI) using 193 nm laser photodissociation.[1]

An important research direction was the coupling of microcolumn separations to the TOF mass analyzer; a variety of interfaces were built for in-line and off-line capillary electrophoresis (CE)-MALDI MS and MS/MS analyses of peptide mixtures. Unmatched speed of the TOF mass analyzer along with a kHz laser and a fast galvanometer optical scanner allowed simultaneous detection of multiple separation eluents with a single mass spectrometer.[2] A similar idea helped to achieve acquisition rates above 100 pixels per second in MALDI MS imaging experiments recently.[3]

Another research area was the development of two original techniques of sample introduction for atomic mass spectrometry; combined bioanalysis and elemental analysis hold promising potential in the field of metallomics. Examples involve simultaneous MALDI, electrospray (ESI), and inductively coupled plasma (ICP) MS detection of separated metallothioneins and a thin layer (TLC)-ICP MS of cobalamins or selenium species. One of the sample introduction techniques was employed for the characterization of nanoparticles; it allows the introduction of intact gold nanoparticles into inductively coupled plasma operated in single-particle mode.[4]

Current research includes multimodal imaging of 3D cell aggregates,[5] MS imaging of double bond positional isomers,[6] nanoparticle tags for sensitive MS imaging, and a new arrangement for ionization of volatile organic compounds.[7]

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The History of Time-of-Flight Mass Spectrometry at VG-Micromass-Waters

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Time-of-flight mass spectrometry was first described in the 1940s. The rapid pulsing of ions into the analyser (milliseconds) and rapid flight times (microseconds) make it an inherently fast detector that is very dependent on the detection and control electronics.

In the late 1980s, Guilhaus[1] described the use of orthogonal-acceleration time-of-flight and this approach was adapted by Bateman[2] at VG as the second analyser in a tandem sector-ToF configuration. This approach was developed further in 1996[3] with the introduction of the quadrupole - time-of-flight configuration. Coupled with electrospray ionization, the Q-ToF became an accepted configuration in a wide range of applications.

ToF analysers have evolved significantly over the last 25 years, and have seen improvements in mass resolution and mass accuracy. The latest generation of ToF instruments now employs multi-reflecting ToF analysers[4] and is capable of delivering mass resolution of over 200,000 coupled with mass accuracy of better than 500 ppb.

The evolution of ToF instruments will be reviewed.

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Mass Spectrometry Innovation at Agilent Technologies

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The story of Mass Spectrometry at Agilent Technologies starts with the founding of Hewlett-Packard in 1938 by Bill Hewlett and David Packard. HP entered the analytical instrumentation field in 1965 which would lead to the development of the first mass spectrometer and introduction in 1971 of the 5930A GC/MS System. This system included an integrated oscilloscope for tuning and a strip chart recorder and featured a dodecapole mass filter and offered a mass range up to *m/z* 650.

Innovation and technology developments at Agilent would then lead to the introduction in 1974 of the computer-controlled GC/MS 5980 series and in 1976 the first benchtop GC/MS 5992A.

With the commercial success of chemical ionization, the switchable EI/CI ion source was introduced in 1977 and in 1990 the 5971 GC/MS was introduced with the glass structure hyperbolic quadrupole. Continued mass filter technology developments would produce the gold-plated fused silica quad and introduction of the 5973 MSD in 1996.

With the strong commercial interest in LC/MS and the development of ESI technology, the benchtop LC/Iontrap was commercialized in 2000 and in 2006 the LC/QTOF and the LC/triple quad were introduced.

More recent developments include the incorporation of the ion funnel to improve ion transmission and sensitivity (collaboration with Dick Smith, PNNL, Richland, WA, USA) and ECD ion fragmentation for improved MS/MS of large biomolecules (collaboration with e-MSion Inc., Corvallis, OR, USA).

Agilent has also added the new dimension of ion mobility separation combined with QTOF MS/MS to provide enhanced separation and identification of analytes. Ion mobility separation has been developed using both uniform field drift tube and by SLIM technology (collaborations with Dick Smith, PNNL, Richland, WA, USA and MOBILion Systems Inc, Chadds Ford, PA, USA).

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Orbitrap Fusion Lumos: A Versatile Mass Spectrometer for Biomolecules

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Mass Spectrometry innovation made tremendous progress during last 20 years. The impact of improved sensitivity together with ion source versatility and array of fragmentation techniques influenced heavily the analytics of difficult biological systems. The term OMICS would not exist without such improvements. Orbitrap Fusion Lumos may be considered as an example of such development. The instrument combines three different mass analyzers, could be operated in four different fragmentation regimes and can be coupled to at least three different ion sources. The possibilities of application to the biomolecular research and service are immense.

I will introduce the overall geometry of the instrument, introduce the basic protein and proteomic application and explain the advances of the instrument to other applications.

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Protein Structure and Interactions Studied by Cross-Linking Mass Spectrometry

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Protein cross-linking coupled with mass spectrometry analysis (XL-MS) allow the description of the structure of individual proteins or protein complexes. It is based on application of the cross-linking reagents (cross-linkers) that consist of at least two reactive groups connected by spacer arm of defined length that enable the identification of proximal structural regions of proteins on amino acid level. Although the recent development in MS instrumentation made XL-MS a powerful tool, it has also some limitations and individual steps of the XL-MS procedure need to be carefully designed.

Since proteins fulfill a huge number of very different functions, their structures significantly differ. As follows from the principle of XL-MS analysis, which is highly dependent on individual protein structure, there can be no uniform guidance to perform the procedure on every protein. Therefore, the crucial steps in the XL-MS procedure reside in the choice of suitable cross-linker, its amount and also the choice of suitable protease. Further, it is needed to secure the required amount of desired cross-link products in the sample for their successful identification by MS/MS analysis. This can be achieved by the sufficient quantity and purity of prepared protein sample or by the enrichment of cross-linking products at the peptide level. Also, the usage of highly sensitive MS/MS system with high resolution is useful to overcome this step. The application of MS/MS analysis providing the efficient fragmentation of cross-linked peptides as well as the measurement in high resolution also significantly increase the possibility of reliable identification of desired cross-linked peptides.

Despite all the difficulties mentioned, XL-MS is often the method of the first choice, mainly in the case of the clarification of protein complex's structure. The main reason for this is its straightforward approach which is based on standard and well available proteomic procedures.

The work was supported from European Regional Development Fund; OP RDE; Project "ChemBioDrug" (№ CZ.02.1.01/0.0/0.0/16_019/0000729).

Protein Structure and Interactions Studied by Hydrogen Deuterium Exchange Mass Spectrometry

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Hydrogen-deuterium exchange coupled with mass spectrometric detection (HDX-MS) is the proteomic approach widely used for structural analysis of proteins and protein-protein, protein-ligand or protein-membrane interaction studies. In respect to the protein structure, the investigation of each protein can require different experimental conditions, especially within the protein labelling step such as different time points, various of protein:deuterium solvent ratio or labelling under different temperature. The highly structured proteins or protein regions, are well-protected against deuterium incorporation and they have a suppressed rate of deuteration compared with unstructured proteins or loops. In contrast to highly structured proteins, the intrinsically disordered ones can be studied mainly by using shorter time points in combination with incubation under low temperatures to slow down the process of exchange. It enables us to see also the process of deuteration in case of generally fast deuterated regions and notice the changes triggered by protein structure formation or interaction partner binding.

As an example of differential analysis with biological context, we have successfully mapped the structural differences of myristoylated and nonmyristoylated Mason-Pfizer Monkey Virus matrix protein caused by the presence or absence of myristate at the N-terminus of protein, respectively. We claim that the exposure of myristate from matrix protein structure, which occurs naturally after interaction with plasma membrane, reveals the cleavage site for viral protease, which could be the essential factor of triggering the maturation of viral particle.

The work was supported from European Regional Development Fund; OP RDE; Project "ChemBioDrug" (№ CZ.02.1.01/0.0/0.0/16_019/0000729).

HPLC/ESI-MS² – Based Lipidomic Analysis and MALDI Imaging of Lipids

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Lipids are structurally diverse chemical compounds, that performs different functions in living organisms such as energy storage, endocrine actions, cell signaling, acting as structural components of cell membranes, and so on. Lipids play important roles in the pathogenesis of many diseases such as obesity, stroke, atherosclerosis, hypertension, neurodegenerative disorders or diabetes. Using a lipidomics approach, it has become easier to study the lipids species in an organism. Lipidomics is a relatively young field of science that is developing in the context of rapid advances in medicine, information technology, and analytical chemistry. The analysis of lipidome composition means the identification and quantification of hundreds of molecular lipid species. Information from these studies plays important role in explaining diseases pathogenesis at the molecular level.

Mass spectrometry imaging (MSI) is an analytical technique capable of the direct analysis of molecules within tissues. It allows label-free measurement of the distribution and abundance of molecules, including lipids. The spatial localization of the lipid species in the tissue gives additional important information for the understanding of various mentioned diseases and suitably complements the findings obtained by lipidomic analysis.

In workshop, we will present optimized workflow suitable for lipidomic analysis of tissues, body fluids, and cells. The procedure includes lipid extraction, lipidomic data acquisition, evaluation of the obtained data and basic statistical analysis. Lipidomic analysis is based on HPLC/ESI-MS with data-dependent setting of MS2 approach employing Orbitrap Lumos (Thermo Scientific) hybrid mass spectrometer. The instrument allows us to determine the elemental composition of lipids and their fragments. The data are evaluated in the LipidSearch (Thermo Scientific).

MSI studies were measured on MALDI-TOF instrument UltrafleXtreme (Bruker Daltonic). Developed workflow includes tissue preparation, matrix deposition, MALDI-TOF MSI data acquisition and data evaluation. Finally, examples of selected results will be shown.

The work was supported from European Regional Development Fund; OP RDE; Project "ChemBioDrug" (№ CZ.02.1.01/0.0/0.0/16_019/0000729).

Posters

P-01
Use of MS-Cleavable Cross-linkers for Improved Identification of Cross-linked Peptides

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Protein cross-linking mass-spectrometry (XL-MS) is a popular method of determining protein-protein interactions and protein structures. Various crosslinkers have already been developed for XL-MS studies, out of which MS-non-cleavable crosslinkers are being used extensively. A significant step was the introduction of MS-Cleavable crosslinkers in crosslinking experiments, where a urea group is introduced into the crosslinker spacer arm which can be cleaved in gas phase by CID/HCD fragmentation during the MS/MS analysis. This leads to the generation of two pairs of signature ions with a definite mass difference, which ultimately improves the identification of the crosslinked peptides by lowering false positive identifications. In our study, we have prepared a new non-commercial, water-soluble and MS-cleavable crosslinker based on the structure of disuccinimidyl dibutyric urea (DSBU). We propose to call the new crosslinker disulfosuccinimidyl dibutyric urea (DSSBU). Taking bovine serum albumin as our model system, we have performed the comparative study of the efficiency of commercial and non-commercial MS-cleavable (DSBU and DSSBU) with MS-non-cleavable (DSS and BS3) cross-linkers. Our results show that both MS-cleavable crosslinkers (DSBU and DSSBU) provide more crosslinks to those non-cleavable (DSS and BS3), which apparently relates to the more efficient identification of cross-linked peptides.

P-02
Laser Desorption Mass Spectrometry at the Chemistry Department of Masaryk University

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The development of novel instrumentation and methodology is an essential part of analytical chemistry driven by demanding edge-cutting applications. This contribution will cover mass spectrometry instrumentation and method development projects our group accomplished during the last 20 years.

The first discussed project aimed at coupling column separation techniques with desorption ionization mass spectrometry. We developed two original techniques of sample introduction for inductively coupled plasma (ICP) MS, surface assisted laser desorption (SALD) and diode laser thermal vaporization (DLTV). SALD found application in interfacing capillary electrophoresis to ICP MS for chromium speciation[1] and in detailed characterization of metallothioneins by simultaneous matrix-assisted laser desorption/ionization (MALDI), electrospray (ESI), and ICP MS detection after liquid chromatography[2] and characterization of nanoparticles.[3,4] DLTV was employed for determination of lead and cadmium in whole blood[5] or coupling to thin-layer chromatography for speciation of cobalamins[6] and selenium-containing compounds.[7]

The second project was the assembly of a high-throughput axial time-of-flight mass spectrometer and its application in MALDI mass spectrometry imaging (MSI). The spectrometer reaches acquisition rates up to 150 pixels per second in MSI due to a fast scanning mirror redirecting the laser beam across the sample.[8,9]

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P-03
Unprecedent Stereospecific Control of Gas-Phase Peptide Backbone Dissociations with Cyclo-Ornithine Residues

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Gas-phase ions produced from stereoisomers may display a completely different reactivity in unimolecular decompositions or ion-molecule reactions, thus showing stereospecific behavior.[1] Here, we report a rare example of rationally designed peptide stereoisomers with the 1,4-diaminocyclohexane-1-carboxylic acid (cyclo-ornithine) moiety which display pronounced gas-phase ion stereochemistry of different charge states.[2]

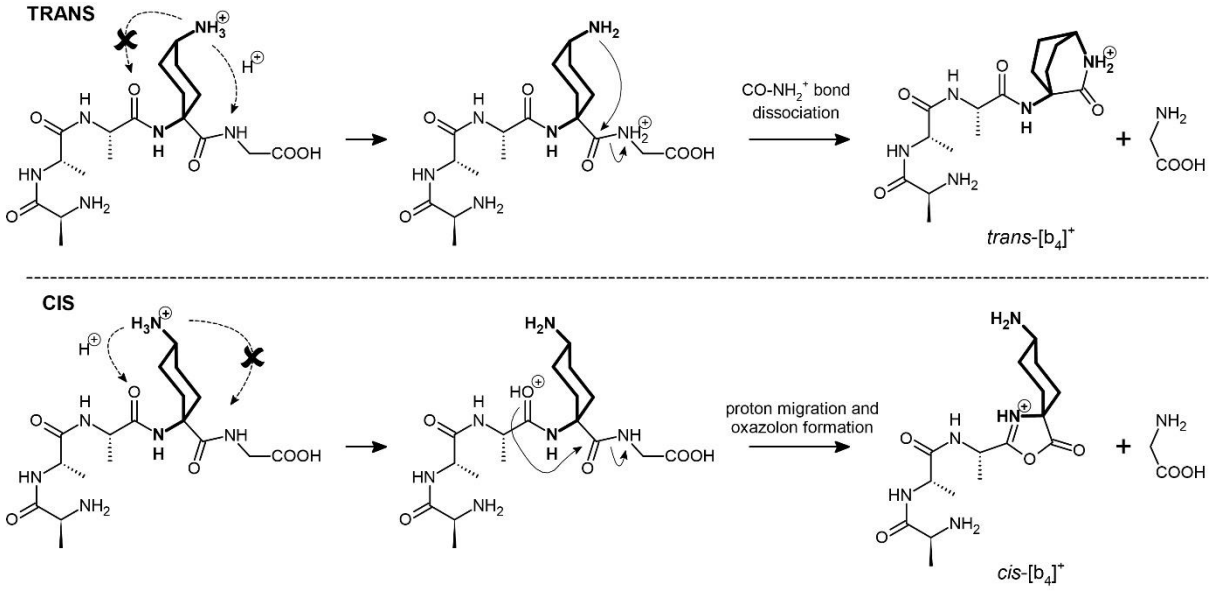

Figure 1. A plausible mechanism for the amide bond dissociation in the *cis*/*trans* single-charged form

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P-04
CE-UV and CE-MS Separation and Identification of Standard Oligonucleotides

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Capillary electrophoresis (CE) is a valuable tool in the analysis and characterization of (bio)molecules and their interaction.[1,2] CE is particularly useful for analysis of polar and charged molecules, which makes it a complementary technique to other methods, such as RP-HPLC. As inherently miniaturized technique, its advantage is the very low consumption of sample, which is in the order of nanoliter volumes for a solution of analyte in the range of micromolar concentrations per one analysis. Apart from the standard CE with the UV absorption detection, the CE is also compatible with a mass spectrometer, allowing for highly sensitive and information-rich detection. The online coupling of CE and MS is established through an appropriate ESI based interface, such as nanoflow sheath-liquid interface.[3,4]

The analysis of a mixture of eight standard oligonucleotides was conducted on both CE-UV and CE-MS. At first, the separation conditions were optimized by CE-UV and after that transferred to CE-MS. Volatile ammonium acetate and formate background electrolytes (BGE) were in focus for the suitability with the MS. Different pH values were tested in order to achieve the best results. Remarkable variation in separation selectivity was observed in the 2.5 – 3.5 pH region thanks to partial deprotonation of nucleobases with different pK_a. Furthermore, small amounts of EDTA in the buffer proved to be helpful for the improvement of the peak shape. The developed CE method was transferred to CE-MS. The oligonucleotides were separated as anions in acidic BGEs and analyzed by a TOF-based MS in positive mode. To avoid the contamination of the MS with the EDTA from the BGE, a partial filling setup was used. So, instead of having the EDTA containing BGE in the CE capillary, only a plug of a small concentrated EDTA solution of desired length was injected in the separation capillary before the sample, reducing the amount sprayed in the MS and in the same time keeping the peak shape improvement.

A good separation was achieved by both CE-UV and CE-MS, proving the good compatibility between the CE separation and the MS detection.

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P-05
Hyperbilirubinemia Is Associated with Changes in Cholesterol Metabolism and Fat Breakdown

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Elevated concentration of bilirubin in human plasma is associated with lower BMI. Animal studies also showed connection between high levels of unconjugated bilirubin and reduced adiposity. Physiologic function of bilirubin could also include protection against cardiovascular diseases where its role as an antioxidant agent has been proposed. Recent report showed that hyperbilirubinemia is associated with reduced fat mass and increased hepatic mitochondrial biogenesis, specifically in female animals, suggesting a dual role of elevated bilirubin and reduced UGT1A1 function on adiposity and body composition.[1]

Our aim was to investigate the impact of unconjugated hyperbilirubinemia on metabolite and lipid composition in Gunn rats. Rat plasma of 14 weeks old animals of both genders was examined in metabolomics and lipidomics screening. Extracted plasma samples were measured on Orbitrap ID-X Tribrid using ZORBAX Eclipse Plus C18 column and on 6546 LC/Q-TOF using Accucore C30 column.

The most prominent differences in plasma metabolite composition were found in female cohort of Gunn rats. 5 subclasses of metabolites were found: bile acids, flavonoids, fatty acids, phenylalanine and, as expected, bilirubin and its metabolites. As opposed to male Gunn rats, lipidomic data analysis in female Gunn rats revealed substantial decrease of all measured lipid classes in comparison with control, with the main difference in compounds containing long unsaturated fatty acids.

This is the first study to comprehensively assess metabolomics and lipidomics in hyperbilirubinemic rats. Our findings show that hyperbilirubinemia, specifically in female animals, is associated with changes in cholesterol metabolism and breakdown of fat.

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P-06
Sensitivity of SESI-MS to a Range of Volatile Organic Compounds: Ligand Switching Ion Chemistry and Influence of ZSpray™ Guiding E-fields

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Secondary electrospray ionization (SESI) is currently only semi-quantitative. To make SESI-MS more quantitative, the mechanisms and the kinetics of the reaction processes, especially ligand switching reactions of the hydrated hydronium reagent ions, $H_3O^+(H_2O)_n$, with trace volatile organic compounds (VOCs) need to be understood. 25 compounds from various classes including 4 aliphatic primary alcohols, 3 monoketones, 4 diones, 3 saturated and 3 unsaturated aldehydes, 3 carboxylic acids, 3 hydrocarbons and 2 terpenoids were involved in the present study to investigate the sensitivity of SESI-MS to various classes of VOCs and to understand the ion chemistry occurring between the reagent cluster ions and molecules, M.

A Mass Spectrometer with a ZSpray™ ESI source was adapted for SESI-MS operating at sub-atmospheric pressure with analyte sample gas introduced via an inlet coaxial with the spray. The electrospray was operated in the positive ion mode using ultrapure water containing a trace of formic acid (0.1%). The ions formed in the spray react with the molecules in the gaseous sample and the resulting ion swarm was analyzed by a quadrupole mass spectrometer.

SESI ion chemistry inevitably results in hydrated ions of the kind $MH^+(H_2O)$,[1,2,3] and also minor fractions of proton-bound dimer ions MH^+M. SESI-MS sensitivity variation for the various classes of organic compounds is very large, being about two orders-of magnitude greater for monoketones, unsaturated aldehydes and menthone to the lowest values for the primary alcohols, diones and saturated aldehydes. The saturated and unsaturated aldehydes have greatly different reactivity. This can be explained by significantly greater values of both dipole moment and proton affinity for these two classes of VOCs. Thus, it is the combination of chemical and physical properties of the molecules that determine SESI-MS sensitivity.

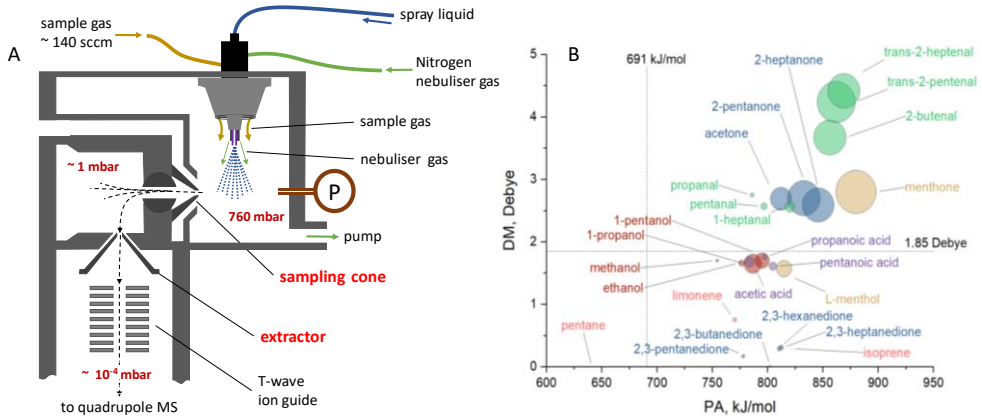

Figure 1. (A) a schematic of the Waters Micromass Quattro Premier ZSpray™ ion source adapted for SESI-MS. (B) a bubble plot showing the combined effect of dipole moment, DM, and proton affinity, PA, to the value of the measured SESI-MS sensitivity.

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P-07
Systematic Data Processing in Untargeted LC-MS Metabolomics: Application towards Common Variable Immunodeficiency Disease

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Exploratory mass spectrometry metabolomics generates thousands or tens of thousands of features. However, depending on the data processing pipeline, >85% of detected features may consist of false positives as an artifact of chemical noise or a relative mass spectrometry signal. Data processing is considered a significant bottleneck to unravel the translational potential in metabolomics. Metabolomics studies inadequately explaining technical details of data processing strategy are challenging to reproduce. The rationale of the inherent properties of the analytical method is often underestimated in metabolomics data processing. We describe a systematic, robust, and facile workflow to refine metabolomics data and mitigate false positives. We demonstrate the application of the feature filtering workflow towards a case/control study on biomarkers of common variable immunodeficiency diseases (CVID). In the first stage, features were detected using conventional XCMS processing and refined using blank subtraction and reproducibility measures. Then, features were mapped to metabolomics repositories to produce a list of tentatively identified metabolites. Next, the agreement between predicted and experimental reversed-phase LC retention time of a tentative identification was examined using a prediction model calibrated on 42 retention indices (cLogP range -6 to 11). We applied the retention time-based analytical feature filtering to datasets from the Metabolomics Workbench (www.metabolomicsworkbench.org), demonstrating the broad applicability. The data refinement, considering the inherent properties of the analytical method, reduced the number of tentatively identified metabolites from 6940 to 839 further submitted to statistical analysis. A subset of tentatively identified metabolites that were significantly different between CVID and control groups was validated by MS/MS acquisition to gain potential biomarkers of CVID. We found eight statistically significant metabolites which demonstrate metabolic dysregulation in CVID patients. Our proposed metabolomics data processing pipeline effectively removes the interference from the chemical background and chimeric signals inherent to the analytical technique. The feature filtering workflow verifies the physicochemical properties of tentatively identified metabolites, improving the reliability of identifications.

P-08
High Analytical Power of Mass Spectrometry Based on the Pesticide Modified QuEChERS

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Pesticides, a persistent environmental problem from forties of 20. century (1939, DDT), are, nowadays, likely behind the increased decline of bee populations, especially in Europe. The crucial aim of this work is to establish the maximal effective, comprehensive analytical system for monitoring these substances and their residues in a specific ecosystem of a given field in cooperation with our colleagues of the Czech University of Agriculture in Praha and the Faculty of Military Health Sciences, University of Defence in Hradec Kralove (dedicat.). We use our developed homemade modifications of the original QuEChERS method to process primary analytical samples of various agricultural forages, crops, individual parts of the plant body, pieces of soils, and bee bodies. The samples are extracted with acetonitrile–water or plus acetic acid–sodium acetate buffer solution eventually and salted out by anhydrous MgSO4 and NaCl. Then the extracts were cleaned up with N-propyl ethylene diamine (PSA), octadecylsilane (C18), and anhydrous MgSO4 again. Finally, the cleaned extracts were detected by multi- instrumental system consisted in UHPLC–MS/MS (TSQ Access Max, Thermo Fisher Scientific) in multi-reaction monitoring (MRM) mode, UHPLC-HRMS in full scan and PRM mode (Q Exactive Focus, Thermo Fisher Scientific) and UHPLC-LIT (LTQ XL system by Thermo) for additional confirm MS^n respectively. Quantitative determination of individual analytes is solved by the method of standard additions and construction of a calibration curve for the calculation of the γ-intercept. Pesticides, mainly of the groups: organophosphates, carbamates, sulphonylureas, that are detected and quantified. Our used multi - mass spectrometry system is currently able to capture more than 500 substances in total number.

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P-09
Monitoring of the Posaconazole Therapy Response in Rats Infected with *Aspergillus fumigatus*

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Invasive pulmonary aspergillosis (IPA) is a severe fungal infection arising after inhalation of the airborne conidia of *Aspergillus fumigatus*.[1] *This pathogen* produces virulence factors siderophores which steal iron in a host environment. Annually, with the mortality rate up to 95 %, IPA affects more than 300 000 people worldwide.[2] Current diagnostic procedures lack adequate specificity and sensitivity and are time-consuming and invasive.[3] We infected eight rats with *A. fumigatus* intratracheally and confirmed the onset of infection with micro positron emission tomography. We started a posaconazole (POS) therapy in four rats three days later. We collected urine twice daily, and for next-generation IPA diagnosis, we performed an *infection metallomics* approach combining high-performance liquid chromatography and Fourier-Transform Ion Cyclotron Resonance mass spectrometry of extracellular siderophore triacetylfusarinine C (TAFC), its degradation product triacetylfusarinine B (TAFB), and intracellular ferricrocin (FC). We monitored the kinetics of infection by detection of these siderophores between (un)treated rats. We detected and quantified at least one of the analytes one day after inoculation already. The severity of the IPA was reflected by a steep increase of monitored siderophores. With the beginning of the POS treatment, both siderophore production and thus infection itself slowed down (**Fig. 1**). Furthermore, we detected POS metabolites in the urine too. To conclude, we can non-invasively monitor the IPA in the early stages from the urine, correlate the severity of infection by simultaneous detection of both siderophores and applied antimycotics, and detect potential reinfection.

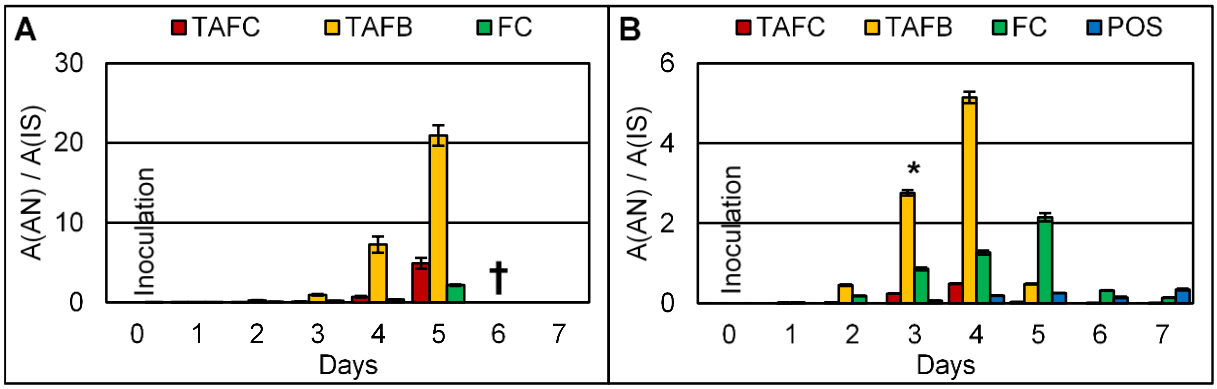

Figure 1. A representative infection diagram showing the distinct progression of aspergillosis in one untreated (left, A) and one treated (right, B) rat. The untreated rat died (†) five days post-inoculation as mirrored by sharp increase of all virulence factors. In opposite, POS treatment applied since day 3 (*) ensured surviving the rat. The severity of infection slowed down as showed either by reducing the amount of FC or disappearance of TAFC and TAFB signal. Data are presented as a ratio between peak area of analyte [A(AN)] and internal standard [A(IS)].

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P-10
Characterization of Gangliosides in Cerebral Organoids: A New Avenue to Study Aging and Alzheimer's Disease-Related Cerebral Changes

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Current development in three-dimensional cerebral organoids (COs) generation has revolutionized our understanding by recapitulating the critical aspects of aging and the underlying mechanisms of neurodegenerative diseases such as Alzheimer's disease (AD). Analysis of COs provides us the unique opportunity to examine different stages in brain development. The brain is a lipid-rich organ with membrane lipids playing a crucial role in maintaining the structure of the neural cells. Our study focused on gangliosides (GSs), an essential class of sialic acid-containing glycosphingolipids abundant in the plasma membrane with a known role in AD pathophysiology. We profiled eight GS sub-classes with corresponding molecular species based on different ceramide chain lengths using selected reaction monitoring (SRM) and ultrahigh performance liquid chromatography (UHPLC-ESI-MS/MS) in a single CO. The current study profiled longitudinal changes in GSs levels in wild-type (WT) COs harvested at five time-points – 50, 85, 110, 130, and 160 days, to monitor their levels throughout the CO development. We also profiled the GSs in WT and AD COs with different Apolipoprotein E (ApoE) isoforms, namely ApoE3/3 and ApoE3/4, at a similar time since ApoE is a significant risk factor for AD. Profiling of the GS levels revealed an abundance of simple GSs such as GD3 and GM3 compared to the complex GSs in the COs. We observed a subsequent increase in all neuron-specific GS – GM1, GD1a, GD1b, and GT1b levels till D110, followed by a decline at later time points. GSs such as GM3 and GD3 did not undergo massive changes with time. GSs such as GD2 and GM2 kept increasing till D160, resulting from the degradation of complex neuronal GSs. GSs from the same sub-class with different ceramide chains portrayed similar trends, and ceramides with shorter fatty acyl chains – 34:1 and 36:1 was the most abundant in COs. Upon comparing GSs in WT with ApoE3/3 and ApoE3/4 phenotype, we found higher levels of GSs in ApoE3/4 WT COs. In the ApoE3/3 line, there was a higher level of GSs in AD COs than WT COs. This result contrasted with the ApoE3/4 line, where the AD COs had lower levels of GSs compared to WT. Our study using induced pluripotent stem cell-derived (iPSC) COs recapitulates GSs changes with time as reflected in brain tissues and suggests ApoE related changes in GSs which can contribute to AD pathogenesis.

P-11
How Can Mass Spectrometry Uncover Retrovirus-Host Interplay?

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Recent development in mass spectrometry (MS) instrumentation enabled to employ this technique is various scientific disciplines. In last two decades MS became very supportive in proteomic research. Nowadays, MS enables the analyses of complex protein samples, as well as the targeted proteomic analyses enabling the elucidation of the protein function or structure.

This study shows the significant role of MS in the clarification of the mechanisms of retroviral proliferation. As was already discovered in the case of human immunodeficiency virus (HIV), the retroviral virions are not composed just from retroviral proteins, but also host-cell proteins promoting the retroviral proliferation are specifically packed into virions.[1] Therefore, we used shotgun proteomic approach to identify host cell proteins in Mason-Pfizer monkey virus (MPMV). As a result, 39 of host cell proteins were identified to be specifically packed into isolated wild type MPMV virions. Interestingly, human helicase DHX15 was also identified among these proteins suggesting that this helicase is used during the viral reverse transcription in the host cells. Subsequently, the relative quantification of proteins between wild type virions and virions with deleted so called G-patch domain confirmed the connection between DHX15 and the retroviral G-patch domain. Finally, the direct interaction between DHX15 and retroviral G-patch domain was confirmed and further examined by targeted structural proteomic approaches based on protein cross-linking and H/D exchange.

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P-12
Laboratory of Atomic Spectrochemistry - Research and Development in Plasma Spectrometry

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The Laboratory of Atomic Spectrochemistry was established in 1997 based on a project from the program of the Ministry of Education, Youth and Sports "Strengthening Research at Universities". This year it is 25th anniversary since the laboratory started its scientific research activities in the field of atomic spectrometry with plasma excitation sources. Inductively coupled plasma atomic emission spectrometry was investigated as a powerful detector for laser-based sampling of solid materials. Laser ablation has been connected since 2005 with Inductively coupled plasma mass spectrometry. Laser induced breakdown spectrometry has been investigated using laboratory-constructed instrumental setup from the very beginning of the existence of the laboratory, whose name was originally Laboratory of Plasma Sources for Chemical Analysis. Besides inductively coupled plasma, microwave excitation sources have been studied for atomic emission spectrometry. Main research tasks of the laboratory comprised in the past and encompass study of laser-matter interaction form the viewpoint of direct solid analysis, study of laser-produced aerosol with mass and aerosol spectrometers, analysis of structured materials, depth profiling, elemental mapping, imaging. Geological, archaeological, and biological materials are being investigated and methods of quantitative determination are being developed. Nanoparticles and surface roughness are currently systematically studied as means of enhancement of laser ablation inductively coupled plasma mass spectrometry signal and laser induced breakdown spectrometry signal. Rocks and minerals are investigated in the framework of project focused on searching of suitable geological locality for spent nuclear fuel repository. Nanoparticles as tags for identification of molecules by laser ablation inductively coupled plasma mass spectrometry are studied. Advanced chemometric tools are used for data treatment and evaluation. Future development will follow basic research of laser – material interaction, geology, archaeology, and biology. Examples of research tasks are presented.

P-13
Analysis of Alachlor, Acetochlor and Metolachlor by Liquid Chromatography Coupled with Tandem Mass Spectrometry

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Alachlor, acetochlor and metolachlor are herbicides used in the agricultural industry for control of broadleaf weeds. These substances are toxic to aquatic organisms. Analysis of these substances by liquid chromatography in reversed phase (RPLC) mode is difficult, because the compounds have very similar structure with the same retention on classical C18 columns. An alternative approach thus involves coupling of RPLC with MS / MS (tandem mass spectrometry) detection, which allows discrimination of these three herbicides in one sample. Analysis of these herbicides was performed by liquid chromatography in combination with electrospray ionization and triple quadrupole detection. A Kinetex C18 column (150×3 mm, 2.6 µm) was used for separation and mixture of methanol and water with ammonium acetate was used for gradient elution. The MS / MS parameters were optimized, the obtained fragmentation transitions (alachlor (270/239), acetochlor (270/225), metolachlor (284/253)) were used for MS / MS detection in the positive ion mode.

P-14
Huperzine A Interacts with Gold Nanoparticles and Forms $Au_m(Hup\ A)_n$ Clusters. Mass Spectrometric Study

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Huperzine A (Hup A) is a naturally occurring sesquiterpene alkaloid found in *Huperzia serrata*. It has been used in traditional Chinese medicine for centuries for the treatment of swelling, fever and blood disorders. Recently, Hup A was considered as a candidate drug for Alzheimer disease as it shows inhibitory effects on the acetylcholinesterase enzyme similarly to galantamine[1] or donepezil[2] already used to treat symptoms of Alzheimer's disease. Recently, we have developed a sensitive and fast methods of CE and CE LIF for Hup A determination[3]. Here we show that Hup A interact with gold nanoparticles (GNP). Gold nanoparticles have interesting properties improving detection and analysis of biomolecules[4] and also serving as drug carriers *in vivo*.

In this study we have investigated formation of Hup A and GNPs structures. We demonstrated that various $Au_m(Hup\ A)_n$ clusters are formed by laser ablation synthesis from mixtures of Hup A and gold nanoparticles prepared by reduction of auric acid with gallic acid in aqueous medium. In the mass spectra, we detected the following $Au_m(Hup\ A)_n$ clusters: $Au_m(Hup\ A)$ (m = 1-14), $Au_n(Hup\ A)_2$ (n = 5-8) (Fig. 1), example of proposed structure GNP $(Hup\ A)_4$ is shown in Fig. 2.

This report shows pilot results demonstrating the laser ablation synthesis of $Au_m(Hup\ A)_n$ nanoparticles, paving the avenue for preparations in larger scale or applications in vivo.

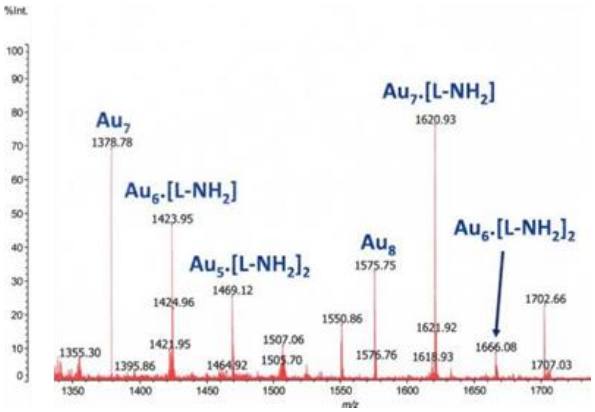

Figure 1. Example of mass spectrum

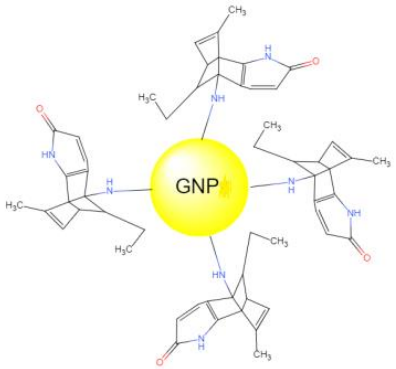

Figure 2. Example of $Au_m(Hup\ A)_n$ structure

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P-15
History of the Secondary Ion Mass Spectrometry in the Czech Republic

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One of the first, if not the very first, Secondary Ion mass Spectrometer (SIMS) instrument in the Czech Republic (CR) or rather in former Czechoslovakia was built in 1973 by Zdeněk Šroubek at the Institute of Radio Engineering and Electronics – the predecessor of the Institute of Photonics and Electronics (IPE) of the Czech Academy of Sciences (CAS). It was based on a commercial quadrupole mass spectrometer (QMS) with innovative ion velocity filtering setup[1], which enabled to study some fundamental aspects of ion formation in SIMS. At around the same time a new mass spectrometry group was established at TESLA - Vacuum Technology (TVT) company with a mission to design a QMS and then a quadrupole SIMS (QSIMS). As a result, a QSIMS instrument Tesla VT-250 equipped with a high performance 1 - 300 a.m.u. QMS have been designed and manufactured.[2,3] The performance of the instrument was comparable to the state of the art "macro-probe" type of SIMS. Only one prototype of Tesla VT-250 has been made before the mass spectrometry program at TVT was cancelled. That instrument was eventually installed at the J. Heyrovsky Institute of Physical Chemistry of CAS, where it was used for fundamental research of ion formation in SIMS till the end of 90's. In 1998 a simple design of linear time-of-flight (TOF) SIMS for fundamental studies has been presented.[4] There have been other in-house built QSIMS instruments in the CR and applied for analyses of thin films and surfaces or for education.[5,6]

The first commercial SIMS in the CR has been installed at the Faculty of Science of J.E. Purkinje University in Ústí and Labem in 2005. It was an upgraded QSIMS ATOMICA 3000 that was then used for depth profiling analyses of thin films, diffusion studies, and measurement of light elements in metals until its decommissioning in 2020. A notable event was the introduction of an orthogonal TOF-SIMS[7] integrated into a Focused Ion Beam Scanning Electron Microscope (FIB-SEM) model LYRA3 from TESCAN (Czech Republic) to the world market in 2012 with the first production piece being installed at the IPE of CAS in the CR. The most recent milestone of the SIMS technique in CR was the installation of two state of the art instruments in 2015: a double focusing magnetic SIMS IMS-7f from CAMECA (France) at Research Centre Řež (Centrum výzkumu Řež s.r.o.) and TOF SIMS model TOF.SIMS5 from IONTOF (Germany) at Central European Institute of Technology (CEITEC) in Brno. Since then, the R&D community in the CR could benefit from the analytical support from the inland laboratories equipped with high-performance SIMS instruments.

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P-16
Charge-Tagged Nucleosides in the Gas Phase. UV-Vis Action Spectroscopy and Structures of Cytidine Cations, Dications, and Cation Radicals

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Charge-tagged nucleoside conjugates provide a common molecular platform for acquiring photodissociation action spectra of neutral, protonated, and radical nucleobase chromophores in the gas phase. Combinations of these units occur in cation-radicals derived from more complex DNA oligonucleotides, and thus the absorption bands obtained from the action spectra of simpler, well-defined, nucleoside conjugates can serve as standards for interpreting action spectra of larger ions.

The cytidine radicals reported here did not show strong absorption bands in the visible region of the spectrum, which was consistent with previous studies of deoxycytidine dinucleotide cation radicals.[1] Cytidine radicals were found to be intrinsically stable against spontaneous unimolecular dissociation by loss of cytosine and hydrogen atom. Their stability exceeds that of purine nucleoside radicals where spontaneous loss of hydrogen atom from the nucleobase has been found to complicate experimental studies.[2,3] The present results for cytidine,[4] as well as spectra interpretation supported by theory, will be utilized in upcoming studies of larger DNA cation radical oligomers, where theoretical analysis by DFT and TD-DFT calculations exceeds current computer technology limits.

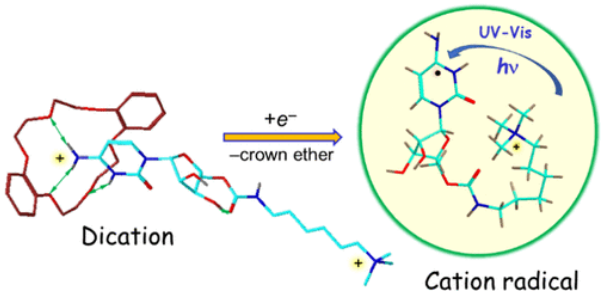

Figure 1. The Generation of Charge-Tagged Cytidine Cation Radical

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P-17
Practical Aspects of High-Resolution Mass Spectrometry for Characterization of Pyrolysis Products

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Pyrolysis, a thermal decomposition process in the absence of oxygen, is one of the attractive and effective ways of reducing the amount and utilizes different types of waste. Pyrolysis converts waste, such as plastics and biomass, into various products which are considered as alternative resources in the petroleum and chemical industry**.** The conditions of pyrolysis, technology unit set-up and feedstock pretreatment influence the distribution of the final products into three main groups, i.e., gas, liquid (oil), and solid (pyrolysis wax and char). Research in the field of pyrolysis is one of the main topics of Consortium 3U, which was recently established between the Jan Evangelista Purkyně University in Ústí nad Labem (UJEP), ORLEN UniCRE and Unipetrol.

Pyrolysis products are complex mixtures of hundreds of components and chemical compounds. A combination of different analytical methods with the necessary sample pre-treatment techniques are needed for the characterization and identification of these products. Mass spectrometry detection in combination with either gas or liquid chromatography provides selectivity crucial for characterization of such complex mixtures. It is widely used for identification of pyrolysis products in the laboratories of Department of Environmental Chemistry and Technology, Faculty of Environment, UJEP. The research focuses on target quantification by using triple quadrupole mass analyzers applied for polycyclic aromatic hydrocarbons, singlequad for identification and quantitation of volatile organic compounds, organic acids, etc. High resolution qTOF analyzers in combination with 1D or 2D chromatography separation and various ionization techniques are utilized for detailed and robust non-targeted identification of pyrolysis products followed by targeted quantitation of several compound classes. Detailed insight into pyrolysis products helps to understand the mechanism of the process, identify valuable components and optimize the technology. It also contributes to better control, prevention or reduces the risk of environmental contamination and to minimize the negative impact on human health as toxic and carcinogenic compounds are known to be formed during pyrolysis.

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P-18
Mass Spectrometry in Clinical Laboratory Research: The Fat-Soluble Vitamins Determination

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During the last few years, the interest in a fat-soluble vitamins (A, D, E, K) has dramatically increased in clinical medicine. The research is focused on complete clarification of their role in prevention, pathophysiology and therapy of diseases.[1] Nowadays, vitamins are extensively studied in the context of COVID-19.[2] Regarding to this findings, the reliable and precise laboratory methods are necessary. Immunoassays and HPLC are the most common methods in clinical laboratories. Immunoassays are commercially available, easy to use, fast and automated, however they are unable to distinguish between different forms of vitamin D and K_2.[1] Liquid chromatography tandem mass spectrometry techniques enable more selective and sensitive identification and quantification of individual forms then immunoassay kits. Nevertheless, there are some limitations as solubility, protein binding, unavailability of blank matrices or control material, and high matrix effect due to complex matrices. These limitations can be solved by development of new UHPLC-MS/MS methods using of modern sample pre-treatments procedures and stationary phases.

In the Research Laboratory at University Hospital Hradec Králové several liquid chromatography methods for vitamins analysis were developed, validated and used for clinical studies. UHPLC-MS/MS technique was applied for the determination of fat-soluble vitamins in case of natural low levels occurrence in various human biofluids (namely different forms of vitamin K, 25-hydroxyvitamin D_3 and 25-hydroxyvitamin D_2, and retinol in urine). This approach has enabled sensitive, accurate, precise and high-throughput analyses in complex biofluids. These methods will be presented and discussed.[3,4]

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P-19
Combination of Comparative Proteomics and Metabolomics for Complex Analysis of Wheat Response to Drought

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Arid climates with continually reduced precipitation cause water deficit in soil leading to imbalances between plant water uptake and water release resulting in reduced crop yield represents the most widespread environmental stress globally. Due to complex impacts of drought on plants, a complex view is needed to understand plant responses to drought.[1] During the last two decades, many alone standing proteomic and metabolomics studies dealing with drought responses in model plants *Arabidopsis thaliana* and rice but also, more recently, in *Triticeae* species.[1] However, complex physiological and molecular studies using combined "omics" approaches to describe crop drought stress responses are still very scarce. Our study is one of the first studies interconnecting areas of proteomics and metabolomics, thus the worlds of two different fields of mass spectrometry (GC-MS, MALDI-TOF MS). In addition, the study aims to compare the stress response of tolerant and sensitive winter wheat varieties at different organ levels.

In addition to the classic markers of drought stress (LEA proteins, heat shock proteins, osmoprotectants and ROS scavenging proteins and metabolites),[2] the obtained results provided interesting connections of metabolic pathways with proteins, specifically changed only in tolerant variety, and these findings have also been confirmed in other proteomic or metabolomic studies. These results include noteworthy biological processes like cell wall remodeling,[3] changes in endocytosis,[4] and cell signaling.[5-7] Surprisingly, changes of metabolism of oxalate and oxalate oxidase were found, especially in tolerant variety. Oxalate, or oxalate crystals, is degraded by oxalate oxidase to form two molecules of CO_2. In correlation with some studies,[9] we can assume, that thanks the "oxalate CO_2", photosynthesis may continue even with closed stomata, and the homeostasis during the drought stress can be effectively maintained. This hypothesis correlates very well with our measured data of physiological parameters and show how important the interdisciplinary connection is for obtaining a comprehensive idea of the biological world.

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P-20
Mass Spectrometry-Based Targeted Assay for Profiling Cell-Specific, Developmental and Neurodegeneration Protein Markers in a Single Cerebral Organoid

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Immune-based assays are mostly used for protein analysis.[1] However, these methods suffer from limited throughput and multiplexing capacity. Moreover, the development of a new immunoaffinity assay is time-consuming. Recent developments in analytical mass spectrometry[2] offer a unique opportunity to investigate diseases with unknown causes, such as neurodegenerative disorders. Three-dimensional cell cultures, cerebral organoids (COs), represent an emerging model system to study biological processes leading to brain diseases. The COs recapitulate the 3D cytoarchitecture of the brain tissue mimicking the *in vivo* brain development (Figure 1).[3] However, this technology has limitations, i.e., reproducibility and homogeneity of the generated COs tissue. Suitable tools are required to characterize cerebral organoids in cellular composition and neuronal maturation adequately. Our study aims to profile a panel of protein markers (Figure 1) in a single cerebral organoid using selected reaction monitoring (SRM) quantitative proteomics.

We developed an SRM assay for quantification of 50 protein targets per analysis in a single cerebral organoid. The total protein content in single cerebral organoids ranged between 7-141 ug. We used a minimum of 1 ug of the total protein equivalent per analysis. We quantified 27 out of 37 selected cell markers for the organoids cytoarchitecture characterization (neural stem cells, radial glial cells, neurons, astrocytes) and neurodevelopmental stages (markers indicating cell maturity) in all collected COs. We found COs a suitable *in vitro* model system that recapitulate *in vivo* developmental features. The remaining 13 targeted proteins were quantified in each cerebral organoid and used to monitor neurodevelopment and neurodegeneration. We discovered age-dependent variation in cytoarchitecture and levels of protein markers in cerebral organoids.

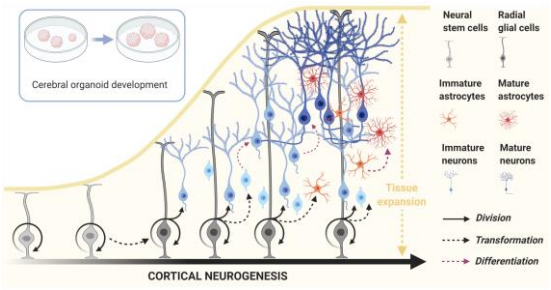

Figure 1. Cerebral organoid development. Cell types during neurogenesis.

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P-21
The Elusive Non-Canonical Isomers of Ionized 9-Methyladenine and 2´-Deoxyadenosine

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The DNA nucleobases represent chemically stable heteroaromatic molecules of essential importance for life on the Earth. Ionization by high-energy particles or photons disrupts the nucleobase π-electronic system, rendering nucleobase cation radicals susceptible to fast electron and proton transfer reactions within DNA or with surrounding molecules. Previous investigations of nucleobase cation radicals have largely considered these reactive species as having canonical structures produced by electron removal from neutral nucleobases.[1] However, this has been challenged recently by the generation of stable non-canonical isomers of nucleobases cation radicals in the gas phase.[2,3] Non-canonical nucleobases and nucleosides represent newly discovered species of relevance for DNA ionization.

This contribution4 reports a targeted synthesis of gas-phase 9-methylene(1*H*)adenine cation radical (**A**$^{+\cdot}$) as a low-energy isomer of ionized 9-methyladenine. The ion displayed unique collision-induced dissociation and UV-Vis photodissociation action spectra that distinguished it from other cation-radical isomers. We also report a mechanism for the major dissociation of **A**$^{+\cdot}$ by a H loss, which was elucidated by deuterium labeling at positions N-1, NH_2 and C-8 combined with RRKM kinetic analysis.

The noncanonical 9-methylene(1*H*)adenine cation radical was generated for the first time using targeted gas-phase ion chemistry. The discovery of stable non-canonical nucleobase and nucleoside cation radicals presents a challenge for future experimental and computational studies to search for the formation and role of such non-canonical intermediates in DNA ionization.

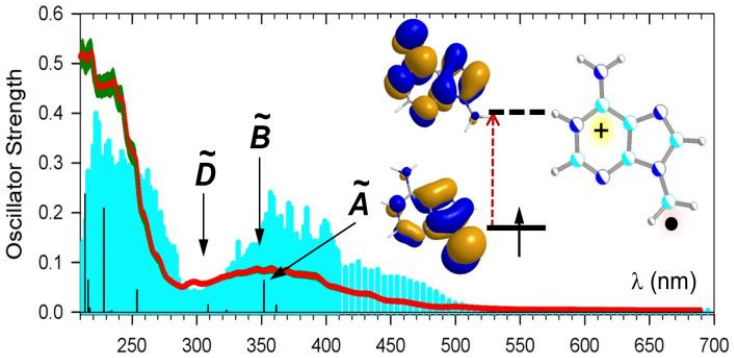

Figure 1. UV-vis photodissociation action spectrum of 9-methyladenine cation radical (**A**$^{+\cdot}$).

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P-22
Multipass Separation of Synthetic Cathinones by Cyclic Ion Mobility: Collision Cross Section Determination

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Synthetic cathinones belong to the class of new psychoactive substances (NPS) causing serious social and health problems. They show isomerism that significantly complicates their analysis. Electrospray ionization mass spectrometry (ESI-MS) coupled to cyclic ion mobility (cIM) was applied to discriminate three such isomeric pairs: a) 3-methylcathinone (3-MMC) and buphedrone; b) 3-fluoromethylcathinone and flephedrone; c) 1,3-benzodioxolylbutanamine (BDB) and methedrone (concentration 100 ng·ml^{-1}). A mass spectrometer with a novel Q-cIM-TOF geometry featuring a cyclic ion mobility device enables multipass experiments to be performed increasing the resolving power of ion mobility.[1] A single pass experiment was compared to 5 and 10 pass experiments for each pair of isomers. CCS calibration was carried out using mixture of small molecule compounds.[2] In our work, we developed a novel method for CCS determination in multipass measurements based on calculations of the individual cycle time. Five and ten pass experiments revealed that isomers exist in many conformations or protomers. Their partial separation caused complex arrival time distributions for some of the isomeric pairs studied, which complicated their separation. For example, the mixture of 3-MMC and buphedrone was not separated after the ten-pass experiment due to complete overlap of ATDs of isomers. To improve the separation, sodium and lithium adducts were generated (**Fig.1**). CCS values from multi-pass measurements agree well with those observed (**Table 1**). Similar agreement was also observed for sodium adducts. A novel approach determining collision cross section from multipass experiments is advantageous for the identification of compounds with small differences in CCS values where high IMS resolving power is needed.

Table 1: CCS values for lithium adducts and corresponding relative standard deviations

	CCS-single pass (Å²), RSD (%)	CCS-ten passes (Å²), RSD (%)
3-MMC	153.0, 0.03	152.1, 0.05
Buphedrone	150.4, 0.02	149.6, 0.04

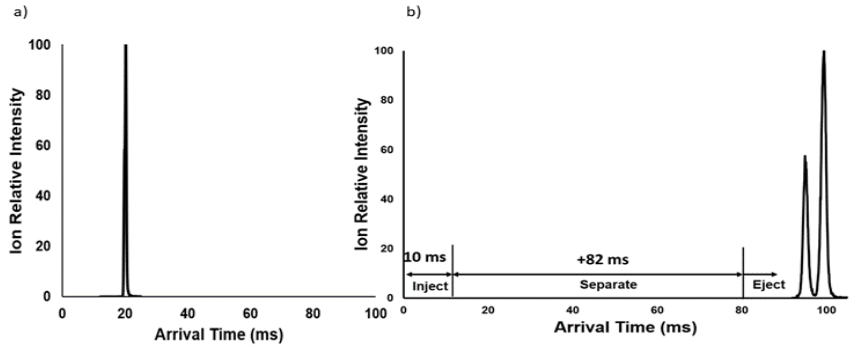

Figure 1. ATD of a mixture of 3-MMC and buphedrone: a) 1 pass; b) 10 pass separation of $[M+Li]^+$

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P-23
Assesment of Soft Ionization Techniques Coupled to GC-qTOF for Non-targeted Characterization of Pyrolysis Products and Natural Organic Compounds

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Traditional hard electron ionization provides highly fragmented spectra referenced to well developed „fingerprint“ knowledge base. With the developement of high-resolution mass spectrometers, this approach became infufficient for some applications as soft ionization techniques provides more information about the target molecules. Molecular ion yield and adduct/fragment formation during ionization are essential parameters for succesfull determination of monoisotopic mass and further structure elucidation by fragmentation patterns. Yield of M+H mostly depends on ionization principle, functional groups in target compounds, its polarity, and conditions of ion source. Except widely spread hard electron ionization by 70 eV, many soft techniques can be utilized with GC for highly accurate identification with more evidence (exact mass + isotopic pattern = sum formula; framentation at various collision energies = structure). Our main aim is to assess suitability of 3 soft ionization techniques: low-energy electron ionization (EI at 12 eV), atmospheric pressure chemical ionization (APCI), and soft ionization by reaction in transfer (SICRIT) for M+H yield on standards and samples in positive mode of the instrument.

Evaluated compound classes includes: phenols, chlorinated alka(e,y)nes, bacterial acid methyl esters, PAHs, C18 – C40, BTEX, dinitrophenyhydrazine derivatives of aldehydes and ketones, headspace of *Pinus sylvestris* and *Cupressus sempervirens* leaves, pyrolysis oils of *Picea abies* biomass, and mixture of 5 plastics. These small molecules with various polarity and functional groups were used to explore ionization mechanism and complexity of FullScan spectra for further non-targeted identifications.

Each of the ionization mechanism provide various benefits and drawbacks for each compound class. The highest positive identification rate is enabled by combination of these methods and implementation of retention indexes, even tough some isomers can be distinguished just by spectral information. Several compounds provide rich spectra full of quasi-molecular ions without M+H. Knowledge and deeper understanding of these ions combined with in-house databases contributes to more effective identifications.

P-24
Optimization of Lipoprotein Analysis by Liquid Chromatography and Mass Spectrometry

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Protein lipidation is one of the major post-translational modifications that play an important role in processes of cell differentiation, synaptic transmission and have an effect on the binding of proteins to cell membrane. Lipoproteins also play a key role in physiological processes and their defect or change in presence are associated with diseases such as Huntington's disease, schizophrenia or cancer.[1] For these reasons, the analysis of lipoproteins has become a needed procedure, however due to their specific properties it's very challenging. The most commonly used technique in proteomic analysis is liquid chromatography coupled with mass spectrometry because of its ability to handle difficulties occurred in analysis of proteins. Tandem mass spectrometry is key to sequence proteins and peptides and their modifications including phosphorylation, glycosylation, lipidation, etc.[2]

Our study was focused on lipoproteins which are modified on N-terminus by myristic acid - matrix protein of mouse mammary tumor virus (MMTV) and matrix protein of Mason-Pfizer monkey virus (MPMV). The aim of this study was to optimize LC-MS method for lipoprotein analysis following the enhanced filter-aided sample preparation (eFASP). After finding the optimal conditions of the RP-LC method, MS detection was optimized. Synthetic peptide modified by N-terminal myristoylation was fragmented using different fragmentation techniques (CID, HCD, EThcD, UVPD) under different settings in order to find optimal conditions for recording rich MS/MS spectra containing both structural information and information about the fatty acid modification.

The optimized LC-MS method was applied to a complex sample of human cell lysate and MMTV and MPMV lipoproteins. The final experiment confirmed that the optimized method is able to detect N-terminal lipomodification of proteins in complex samples.

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P-25
Nebulizer with a Virtual Nozzle for the Introduction of Liquid Samples into a Mass Spectrometer

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Atmospheric-pressure chemical ionization (APCI) and atmospheric-pressure photoionization (APPI) are valuable techniques for analyzing nonpolar and low polar compounds[1]. These techniques are alternative to electrospray ionization (ESI), which provides less efficient ionization of low-polar compounds such as some lipids and sterols.[2] Despite efforts to develop APCI and APPI sources for microliter and nanoliter flowrates,[3,4] no such ion sources have been commercialized yet.

In this work, a nebulizer with a virtual nozzle was assembled and tested for APCI and APPI at microliters per minute flow rates. It generates a narrow jet of neutral droplets by dynamic forces exerted on the liquid by co-flowing nitrogen.[5,6] The nebulizer is small, easy to fabricate, robust, and, in contrast to previous low-flow nebulizers,[7,8] essentially immune to clogging. Its properties make it a good candidate for nebulizing samples in low-flow APCI and APPI.

The nebulizer was assembled from an outer borosilicate glass tube, tapered-tip fused silica capillary for the liquid sample introduction, and PEEK Tee for 1/16 OD tubing. The tapered-tip capillary was centered with a custom-made gas-permeable spacer inside the outer borosilicate tube. A set of nebulizers with various outer orifice diameters and distances of the tapered-tip capillary from the orifice was tested to investigate the size and velocity of the droplets and the spray shape. Furthermore, the effect of liquid and gas flow rates on the droplet formation was studied. Finally, nebulizers were tested in APCI and APPI sources using compounds of different polarities sprayed from various solvents. A piece of resistively heated glass tube was used to make the solvent evaporation easier. All the compounds were detected with excellent sensitivities.

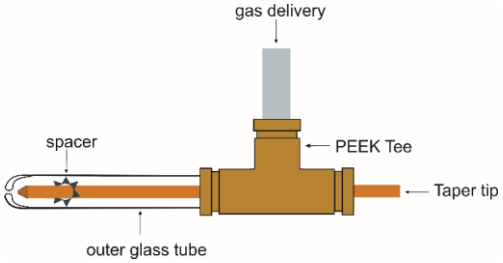

Figure 1. Scheme of a virtual nozzle nebulizer.

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P-26
Atmospheric-pressure Chemical Ionization and Photoionization as Alternative Ionization Sources in Proteomics

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In bottom-up approaches, complex protein mixtures are enzymatically cleaved, and the peptide products are analyzed by HPLC-ESI-MS/MS. This method is well established and has proven to be effective; however, several limitations exist.

This study aims to determine whether atmospheric pressure chemical ionization APCI and/or atmospheric pressure photoionization APPI could help improve the analysis of peptide mixtures, especially for peptides that are inefficiently ionized in electrospray. These alternative ionization sources already proved to be able to ionize peptides. While APCI is more effective for short peptide detection,[1,2] APPI is suitable for hydrophobic peptides.[3]

Synthesized peptide standards with various sequences (i) SLGK, VASLR, AWSVAR, VLASSAR, AEFVEVTK, QTALVELLK; (ii) LAE, SLGE, VASLE, AWSVAE, VLASSAE, AEFVEVTE, QTALVELLE; and (iii) LAF, SLGF, VASLF, AWSVAF, VLASSAF, AEFVEVTF, QTALVELLF represented peptides obtained by digestion with (i) trypsin, (ii) Glu-C and (iii) chymotrypsin. Fragmentation patterns in all ionization sources were investigated. Typical *b*, *y*, and less abundant *a* ions were determined in all ionization sources. Significant loss of water and ammonia were observed in MS^3 steps. Doubly charged ions were detected in ESI close to the detection limit. Limits of detection together with sensitivity were determined for all peptide standards in all ionization sources.

In APCI and APPI, optimization of the mobile phase pH and the ion source temperature were investigated. Both ionizations reached the lowest signal intensities in acidic conditions, which are typically used in ESI and often employed in APCI and APPI for peptide detection. Surprisingly, the optimal ion source temperatures were relatively high, and no degradation products were noticed.

Under optimized conditions, UHPLC-ESI/MS/MS, UHPLC-APCI/MS/MS, and UHPLC-APPI/MS/MS experiments were performed to analyze peptide mixtures resulting from trypsin, chymotrypsin, and Glu-C cleavage of bovine serum albumin (BSA). Chromatograms were obtained in a reversed-phase system with a C18 column kept at 40 °C with a 27-minutes gradient. The effects of the mobile phase composition and pH on detecting BSA peptides were investigated. In APPI, the highest sequence coverage was achieved with acetonitrile adjusted at a higher pH value, unlike APCI, where the best results were obtained in methanol with water.

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P-27
Predicting Electron Ionization Mass Spectra: Short History & Future Outlook

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Gas chromatography coupled with electron ionization-mass spectrometry (GC-EI-MS) is widely applied for the identification of small molecules in complex mixtures. Notable applications include clinical metabolomics (e.g. profiling steroid metabolism[1]), forensics (e.g. drug testing[2]) and environmental analysis (e.g. air pollutant analysis[3]).

Typically, GC-EI-MS screening relies on automated compound annotation procedures whereby the spectra (& retention characteristics) observed in samples are matched with those obtained from authentic compounds.[4] As a result, the identification of compounds that lack authentic spectra is limited, requiring further expertise and time to undertake interpretation.[5] The prediction of EI mass spectra is therefore valuable to extend automated annotation procedures to cover compounds without experimental spectra.

Various *in silico* approaches to predict EI mass spectra have been developed and can be largely categorized as those which rely on i) rules-based or combinatorial fragmentation,[6] ii) statistical and machine-learning[7,8] and/or iii) quantum chemistry.[9,10] Each approach has distinct limitations: briefly, rules-based methods have narrow applicability whilst combinatorial approaches show low accuracy. Statistical and machine-learning procedures achieve greater accuracy, yet are constrained by the availability of experimental data for training. Finally, quantum chemistry methods enable *ab initio* calculation but are complex and computationally intensive.

Past, present and future developments to improve the accuracy of *in silico* predicted EI mass spectra will be presented, focused upon enhancing GC-EI-MS screening of biospecimen.

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P-28
Mass Spectrometry Quantitative Proteomics and Exploratory Metabolomics for Biomarker Discovery

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

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

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

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

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Acknowledgment

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P-29

High Throughput Mass Spectrometry in Determination of Inhibitors for SARS-CoV2 Methyltransferase

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SARS-CoV2 pandemic already had a great impact on our lives, but is still far from over. There are attempts to develop effective strategies to control the pandemic. One such strategy is inhibition of enzymes involved in the viral replication. One of the targeted enzymes is viral nsp14 methyltransferase. In order to screen inhibitors of this enzyme, heterologously expressed nsp14 methyltransferase from SARS-CoV2 virus was used for methyltransferase assay.

We demonstrate ECHO-MS system in analysis of potential inhibitors and consider its advantages in comparison with the traditional HPLC-MS approach. The main advantage of ECHO-MS system is the speed of the analysis.

The IC_{50} concentration was in this case determined from measurement of *S*-Adenosyl-L-homocysteine (SAH), a product of the monitored enzymatic reaction. The MRM method for SAH was tuned on MRM transition 385.1 → 134.1 in positive mode. So far, six potential inhibitors were tested from which three showed a good potential of inhibiting nsp14 methyltransferase. They were even more effective than reference compound sinefungin (with IC_{50} concentration 1.31 µmol/L), namely T0738 (IC_{50} = 0.31 µmol/L), T0713 (IC_{50} = 0.15 µmol/L) and T0730 (IC_{50} = 0.02 µmol/L).

P-30

Mass SPECTROMETRY IMAGING of Free-Floating Sections for the Study of Neurodegenerative Disorders

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Alzheimer´s disease (AD) is a progressive and neurodegenerative disorder, which results in dementia. Currently, there is no drug available to cure neurodegenerative diseases. Instead, several compounds managing symptoms or slowing the progression of the disease are available only. However, some peptides have shown enhanced potential for the treatment, specifically, palm^{11}-PrRP31 (an anorexigenic and glucose-lowering analog of prolactin-releasing peptide) and liraglutide (a type 2 diabetes drug). In recent years, MALDI mass spectrometry imaging (MSI) has been repeatedly used for studying lipids in tissue sections and for clarification of their role in diseases such as AD. MSI is an analytical technique capable of direct distribution analysis of compounds of interest within the tissues.

In the present work, we investigated paraformaldehyde fixed free-floating sections and studied their applicability for the MSI analysis of lipids in the mouse brain. Moreover, we examined the neuroprotective properties of palm^{11}-PrRP31, using the lipid biomarkers found by the MSI method in the APP/PS1 mouse model of neurodegeneration.

From the free-floating sections we obtained lipid images without interferences or delocalization. In the APP/PS1 mouse model, we observed changed distribution of various lipids. For tracking of lipid changes in treated versus non-treated APP/PS model, we used visualization of GM2 36:1 because of its low concentration in the control model and the area surrounding plaques in APP/PS1 model. Two months of the treatment with palm11-PrRP31 reduced significantly area of lipids linked with senile plaques compared to controls treated with saline. The results obtained indicate that these drugs might be potentially useful in the treatment of neurodegenerative diseases.

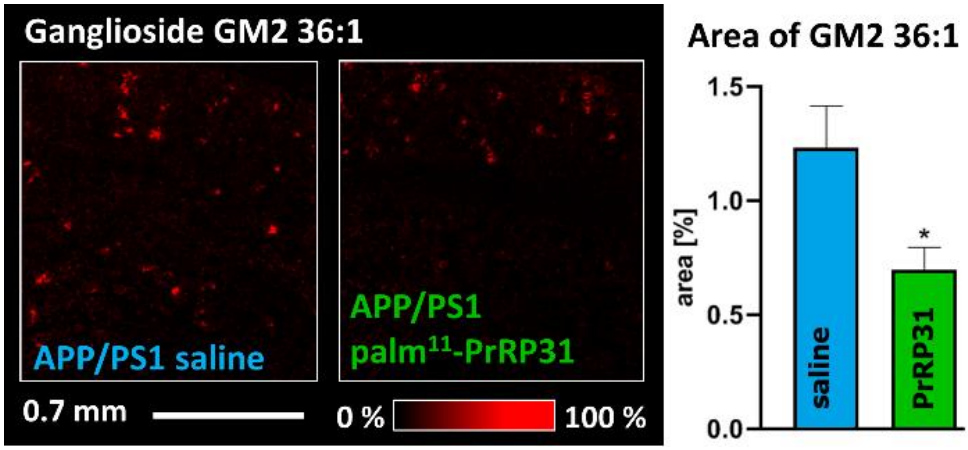

Figure 1. MALDI MSI analysis APP/PS1 mice treated with saline or palm^{11}-PrRP31. Ion images of ganglioside (GM2 36:1) were obtained in negative ion mode at a spatial resolution 15 µm. Data are mean ± SEM (4 mice in one group, *p < 0.05).

The work was supported from European Regional Development Fund; OP RDE; Project "ChemBioDrug" (№ CZ.02.1.01/0.0/0.0/16_019/0000729).

P-31
ESI MS Identification of Toxic Triton™ Derivatives Formed in E-shell 300 3D Printed Material After UV Photopolymerization

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The commercially available E-shell 300 Series material, declared as class IIa biocompatible,[1] used in the manufacture of hearing aids and other implants could be potentially exploited in microfluidic device.[2] Unfortunately we saw that the UV firmed material strongly influenced the cell proliferation and caused death of biological models *Danio rerio* embryos and B14 cells. We observed that post-printing treatment of 3D-printed material in 96% ethanol can reduce embryonic mortality in the FET test by 71%, and also completely eliminate negative effects on cell proliferation. Based on ESI MS data collected from LCQ Fleet Ion Trap of ethanol leachate, and in accordance with ^{1}H NMR, we conclude that Triton™ X-100 carboxylate, and especially Triton™ X-100 terminal alkene group modified surfactants could be leaching from the cured polymer objects and causing observed issues.[2] The Triton™ X-100 alcoholate is widely used non-ionic surfactant sensitive to UV irradiation[3,4] after several hours exposure. In summary, our results indicate the importance of biocompatibility testing of the 3D printing photopolymer material in direct contact with the given biological model. On the other hand, the possibility of eliminating toxic effects by an appropriate post-processing strategy opens the doors for broader applications of E-shell 300 photopolymers in the development of complex microfluidic devices for various biological applications.

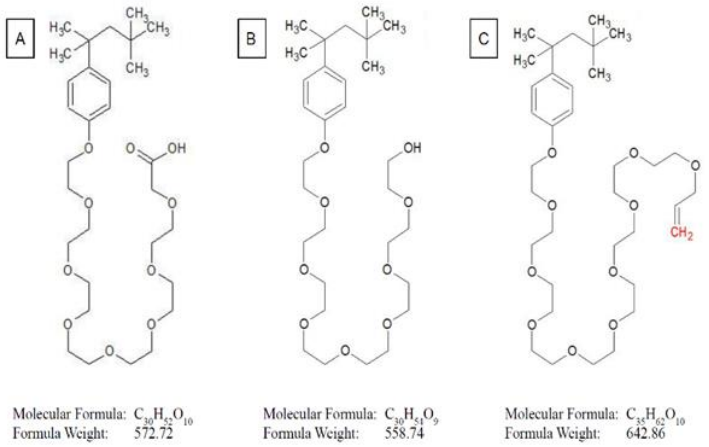

Figure 1. Structural formula of **A)** terminal carboxylic acid substitution derivative of Triton X-100 part 3,6,9,12,15,18,21-Heptaoxatricosan-1-carboxylic acid, 23-[4-(1,1,3,3-tetra-MeBu)phenoxy]-; **B)** Triton X-100 part 3,6,9,12,15,18,21-Heptaoxatricosan-1-ol, 23-[4-(1,1,3,3-tetra-MeBu)phenoxy]-; **C)** terminal alkene substitution derivative of Triton X-100 part 3,6,9,12,15,18,21,23,25-Nonaoxatricosan-1-en, 25-[4-(1,1,3,3-tetra-MeBu)phenoxy]-.

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P-32
A Robust LC-MS/MS Method for Quantification of Steroid Hormones of C_{18}-, C_{19}- and C_{21}-family

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Involvement of steroid hormones in various physiological and pathophysiological processes is well established phenomenon. Thus, accurate steroid metabolome analysis is crucial in the elucidation of detailed mechanisms of steroid-related diseases.

Aim of this study was to develop a robust and sensitive LC-MS/MS method for the quantification of steroid hormones of four major groups: estrogens, androgens, corticosteroids and progestins. As we focused on a wide range of analytes, it was necessary to consider their structural differences and resulting divergences in chromatographic, spectrometric and derivatization approaches.

The LC-MS/MS measurements were performed using Exion LC AD system connected to a mass spectrometer Sciex QTRAP 6500+. Both chromatographic and mass spectrometric conditions were optimized. The mass spectrometer was operated in the positive electrospray ionization mode using multiple-reaction monitoring transitions. Gradient chromatography was performed using a mixture of water and methanol with the addition of 0.1 mM formic acid on Kinetex C18 column (100 mm × 3.0 mm, 2.6 µm). Since we were not able to quantify non-derivatized estrogens and some of the androgens, a derivatization step had to be included.

After testing various approaches, modification of hydroxy-group with 2-fluoro-1-methylpyridinium *p*-toluenesulfonate (FMP-TS) was found to be the most suitable.

This method enables simultaneous analysis of 22 non-derivatized steroids and additional analysis of 10 steroids derivatized with FMP-TS in human plasma. Both analyses are carried out under the same spectrometric and chromatographic conditions and thus enable simultaneous profiling in a single analytical run. Validation of the method was performed according to the FDA Guidance for Industry.

This method will serve as a springboard for steroid analysis in other biological matrices, and will be a useful tool in exploring the mechanisms of a broad steroid-related diseases spectra.

P-33
Detection of *Pseudomonas Aeruginosa* Infection in Biological Specimens

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Pseudomonas aeruginosa is one of the leading causes of respiratory infections and is responsible for high mortality in clinical settings. It can cause severe pneumonia in patients with bronchiectasis and chronic obstructive pulmonary disease,[1] and the whole group of immunocompromised patients after chemotherapies, transplantations and with genetic disorders such as cystic fibrosis (CF).

Motivated by the importance of fast, noninvasive and sensitive detection of microbial infections, we have developed a method detecting bacterial secondary metabolites siderophores[2] or quorum sensing molecules.[3] In this novel approach, we are using liquid chromatography-mass spectrometry-based monitoring of microbial virulence factors in biological specimens such as urine, aspirate and even breath condensate.

First, we have established *in vivo* rat models, which were intramuscularly or intratracheally infected by *Pseudomonas aeruginosa.* Here we detected and quantified siderophores-pyoverdins and pyochelin. Later, we have tested the method on five patient samples from various Czech hospitals, where pyoverdines and 2-heptyl-4-quinolone quorum sensing molecule were found at ng/mL levels.

There is still plenty of work ahead (more robust method development, biomarker's threshold definition) to be able to transfer this basic research to clinical diagnostics, however direct and almost online monitoring of microbial virulence factors, may allow faster and more targeted treatment and increase the survivability of the patients at high risk.

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P-34
Ion Mobility Mass Spectrometry Characterization of Aβ42 and Its Oligomers

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Amyloid beta (Aβ) peptides fulfil a crucial role in the pathology of Alzheimer's disease (AD). One of the main characteristics of AD is the accumulation and aggregation of Aβ. Aβ42 tends to aggregate into neurotoxic oligomeric species and further forms protofibrils; it is a major component of senile plaques in the brain[1]. The oligomerization process is heavily dependent on the conformation of the peptide. β-sheet conformation of Aβ42 acts as a nucleation seed and also enhances the oligomerization of Aβ40[2]. Therefore, a detailed analysis of the conformations of Aβ42 and its oligomers is needed to understand the aggregation process. Conventional mass spectrometry methods are unsuitable for such analysis as the majority of the species are overlapping at the same m/z values. For this purpose, native mass spectrometry coupled with traveling wave ion mobility spectrometry (IMS) was utilized to separate and characterize Aβ42 oligomers formed in water. Experimental conditions were optimized to enhance the detection sensitivity of the oligomers. Lower molecular weight oligomers ranging from dimer to hexamer (9 kDa – 27 kDa) were detected alongside the monomer.

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This work was conducted in collaboration with Alzheon, Inc.

P-35
From Isotopes to Omics – Birth and Development of Mass Spectrometry

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Mass spectrometry (MS) has started with the discovery of catode and canal rays (**E. Goldstein** 1886[1]) that were proved as positively charged ions with trajectory bending in the electromagnetic field and the curvature dependent on the gas used in the discharge tube (E. Goldstein, W. Wien, E. Wiechert). This led to the construction of the first instruments: **Joseph John Thomson** – parabolic spectrograph (1897[2]), parabolic spectrometer (1912[3]), **Arthur Jeffrey Dempster** – mass spectrometer (1918[4]), **Francis William Aston** – mass spectrograph (1919[5]).

J.J. Thomson was the first (1910) who proved the existence of isotopes (^{20}Ne, ^{22}Ne)[6] with his parabolic spectrograph. The existence of natural isotopes of the majority of known elements was demonstrated by similar instruments. Moreover, such discoveries helped to clarify several "discrepancies" in Mendeleev periodic system, which confirmed its validity (Te – I, Co – Ni, As – K).

The utilization of MS in the petrochemical industry during World War II as a method of quality control of avgas (type analysis) contributed to the commercial production of mass spectrometers promoting their spread within analytical labs.

The study of fragmentation principles of molecules applying electron ionization allowed for the identification of a large number of organic compounds. A successful coupling of MS with separation techniques (GC, LC) expanded its applicabitlity to the field of complex mixture analysis with identification of individual components.

Nearly each innovative construction of an ion source or an analyzer opened up MS applications in new fields. Currently, analyses of complex natural extracts, trace contaminants in foods, drugs, and environmental samples, represent a common practice. New MS instruments take part in space research, forensic analysis, proteomics, and many other scientific areas.

Czechoslovak scientists have actively participated in the development of MS since the nineteen fifties. MS found its place at University of Chemistry and Technology, Prague in the late sixties, when the first GC-MS instrument in the former Czechoslovakia was put into operation.

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P-36
HILIC-Single MS Analysis of Nucleotide Pool in Bacterial Cells

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Small molecules as nucleotides, nucleosides and their derivatives are present in cells at various concentration levels. Exact knowledge of their concentrations in cells is of great importance, because nucleotides play crucial role in most of the metabolic pathway. For example, (p)ppGpp serves as an alarmone in bacterial stringent response, and its concentrations significantly influences the metabolic state of the bacterium.[1]

Previously published papers dealing with the analysis of nucleotides and other intracellular metabolites can be divided into two groups according to the analytical approach: a) metabolomic approach and b) targeted approach dealing with narrow group of target analytes. In the case a) most authors use the state-of-the-art LC-MS/MS technique, whereas in the case b) robust UV detection coupled mainly to IP-LC is widely used.

We took advantage from both approaches and developed new HILIC-single MS method for routine analysis of nucleotides and applied it on analysis of changes in nucleotide concentrations during stringent response in *E. coli*.[2]

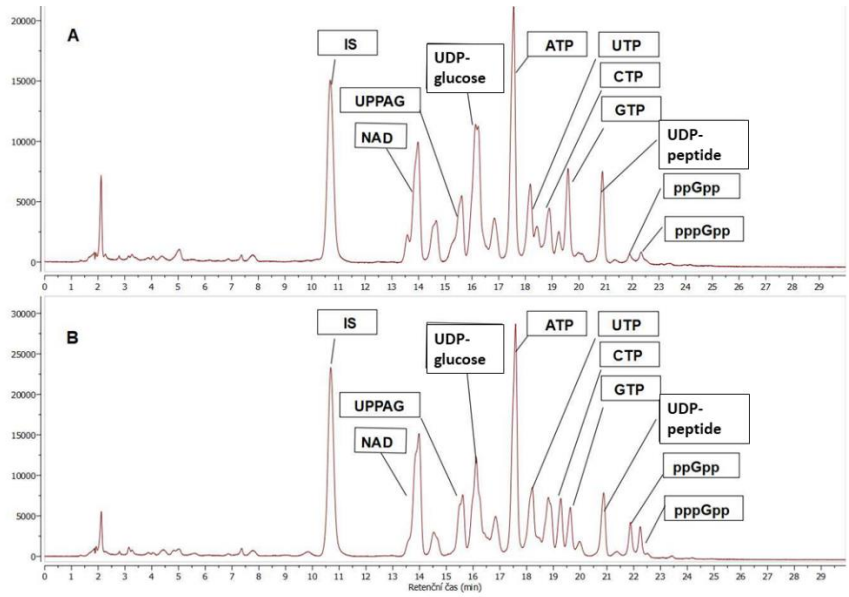

Figure 1. Comparison *Escherichia coli* K12 extracts at OD_{600} = 0.45. A – noninduced stringent response, B – addition of 150 μM mupirocine for stringent response induction. Column ZIC-cHILIC, 150 × 2.1 mm, 3 μm

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P-37
Ion Mobility - Mass Spectrometry Imaging: A Next-Generation Small Molecule Mapping Tool

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Mass spectrometry imaging (MSI), as a high throughput label-free molecular imaging technique, has been used for lipidomics and proteomics analysis of plant samples for many years. In our recent study, we visualized and compared phytohormone species in wounded *Arabidopsis* leaves by desorption electrospray ionization mass spectrometry imaging (DESI-MSI).[1] However, *in situ* mapping of isobaric or isomer species without the use of unique fragmentation ions in MS/MS remains elusive in most MSI analysis, yet isomers are very common in endogenous phytohormones and exogenous compounds. Ion mobility (IM) that separates all analytes based on molecular sizes and shapes is frequently coupled to MS instrument, which enables discrimination between isobaric/isomer ions in the drift time (df) prior to MS/MS analysis.[2] In this work, we established the IM-MSI method for the separation of auxin antagonist auxinole and its isomers. We then performed direct MSI analysis in 10 days old *Arabidopsis* plants co-treated with two isomeric compounds, auxinole and Me-PEO-IAA-OMe (MPM). We were not only able to identify the MPM peaks from auxinole and other isotopic interferences but also highlighted alternative distributions of those isomers in the co-treatment. The deprotonated MPM ions (*m/z* 320.16; df 94.34) are mainly detected in the root, whereas auxinole signals (*m/z* 320.16; df 102.01) can be observed across the plant. The MS/MS imaging further validated their unique spatial distributions and subsequent data segmentation uncovered their related metabolites independent from the parallel LC-MS analysis. With the development of IM-MSI methods, we believe direct mapping of endogenous phytohormones and synthetic plant growth regulators with similar structures will become possible in the MSI analysis, which will further extend the current application of MSI in plant research.

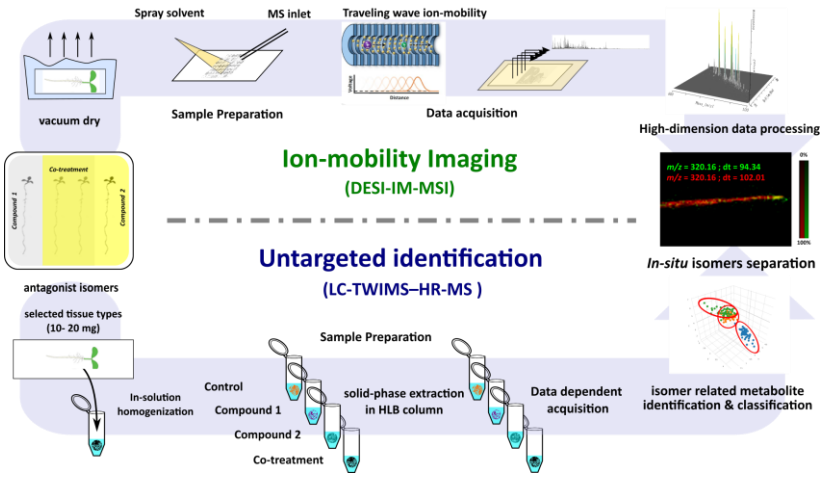

Figure 1. Procedures for IM-MSI based isomeric compounds *in situ* identification and visualization

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Laboratory Version of the Orbitrap Mass Analyzer with Several Types of Ion Sources, *HANKA* Space Instrument

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Application of mass spectrometry for asteroid exploration has recently become a hot topic. It is interesting both in orbit and on the asteroid. It can be used for the analysis of space dust, micrometeorites and particles from larger objects.

For the Czech ***SLAVIA*** satellite project was designed the ***HANKA*** (***H***motnostný ***AN***alyzér pre ***K***ozmické ***A***plikace) space instrument - a high-resolution Orbitrap-based electrostatic ion trap mass analyser. The instrument is based on a commercial mass analyser[1] established in biology and medicine research, the so-called Orbitrap™ and the space CosmOrbitrap prototype (developed by LPC2E Orleans[2]). ***HANKA*** will bring this new technology into space to combine a small CubeSat space version of this ion trap analyzer, with an innovative in-situ hypervelocity impact ionization source for micrometeoroids.

A laboratory version of this instrument (***CIARA***) is currently under construction, where ions can be generated by three different methods:

1. Photons with molecules in the liquid phase (coupled with experiment ***LILBID*** (Laser Induced Liquid Bead Ion Desorption)
2. Electrons with molecules in the gas phase (***EI*** source)
3. Photons with solid-phase molecules (**MALDI** or Laser Ablation)

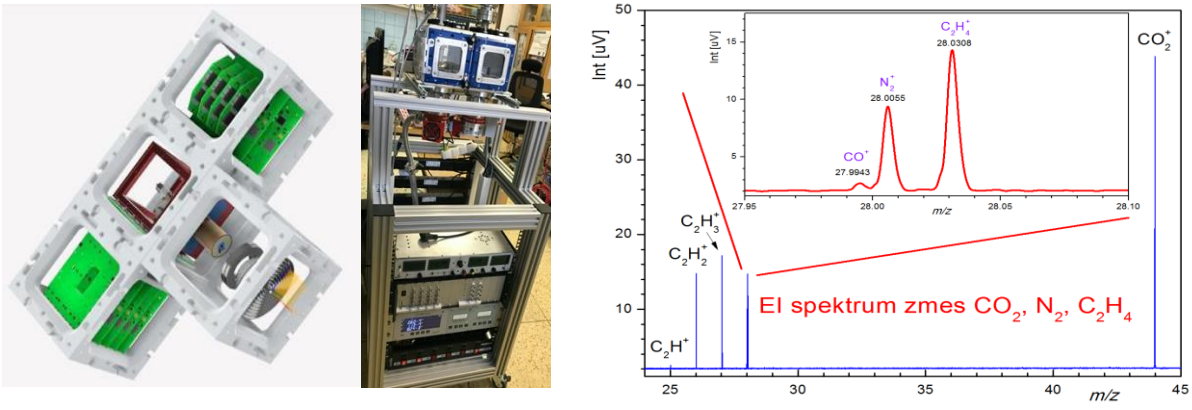

Figure 1. *HANKA* – proposal instrument, *CIARA* – laboratory prototype, preliminary data from EI source

Based on the results obtained on the laboratory prototype, a miniature version of the high-resolution space mass spectrometer – *HANKA* – will be constructed.

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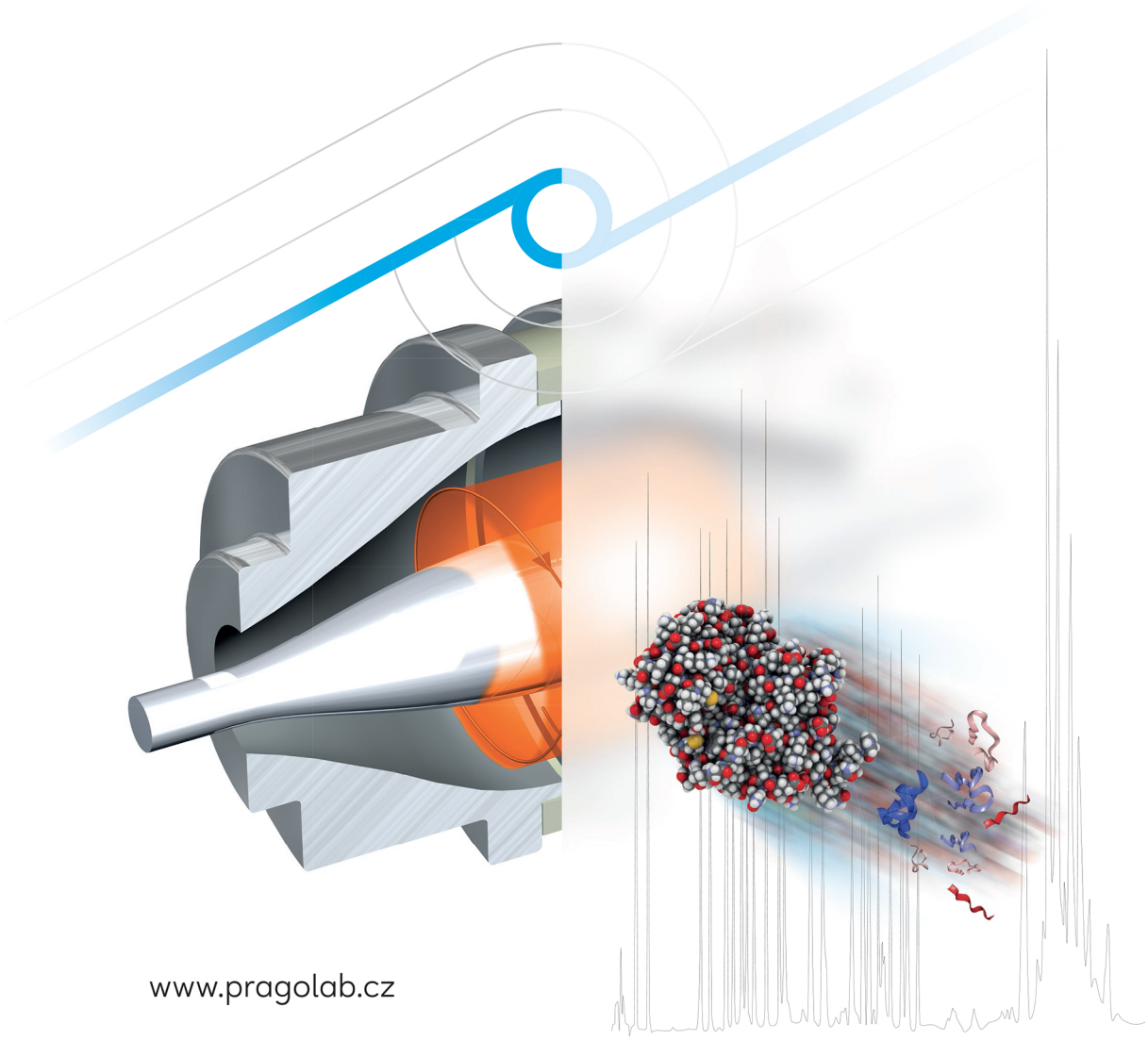

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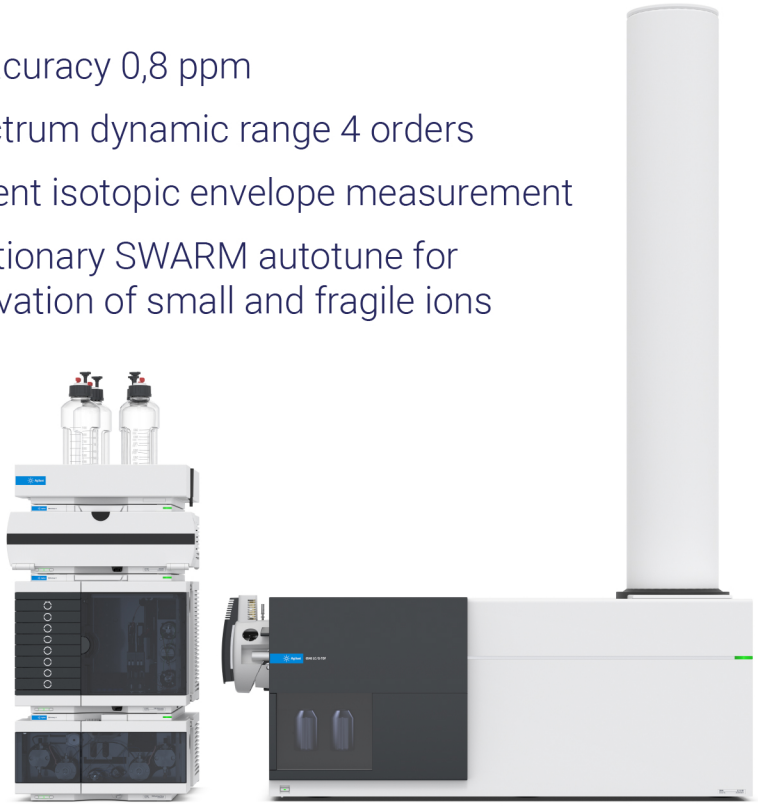

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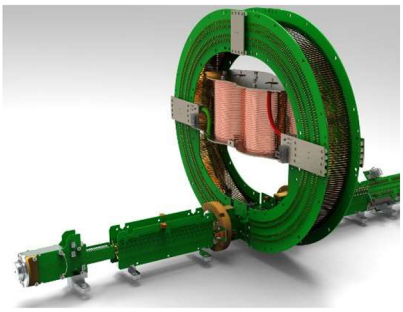

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