

TWELFTH ANNUAL CONFERENCE OF
THE CZECH SOCIETY FOR MASS
SPECTROMETRY

České Budějovice, October 30 – November 1, 2024

BOOK OF ABSTRACTS

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

Czech Society for Mass Spectrometry

České Budějovice 2024

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

Authors

Collective authorship

Published

October 2024, first edition

Publisher

Česká společnost pro hmotnostní spektrometrii

Přírodovědecká fakulta, Univerzita Palackého

17. listopadu 1192/12

771 46 Olomouc

Czech Republic

www.czechms.org

ISBN 978-80-907478-4-5



ISBN 978-80-907478-4-5



Financováno
Evropskou unií
NextGenerationEU



Národní
plán
obnovy

MS
MT
MINISTERSTVO ŠKOLSTVÍ,
MLÁDEŽE A TĚLOVÝCHOVY



Tento projekt je
podpořen z rozpočtu
Jihočeského kraje



Tento projekt je
spolufinancován
statutárním městem
České Budějovice

Important portion of the financial support needed to organize the conference was kindly provided by the government of the South Bohemian Region and by the city of České Budějovice



Přírodovědecká
fakulta
Faculty
of Science

Jihočeská univerzita
v Českých Budějovicích
University of South Bohemia
in České Budějovice

This year's conference is being organized in cooperation with Faculty of Science – University of South Bohemia and Biology Centre ASCR.

Main conference sponsors

*Czech Society for Mass Spectrometry thanks the following partners
for their kind and generous support*

GDa

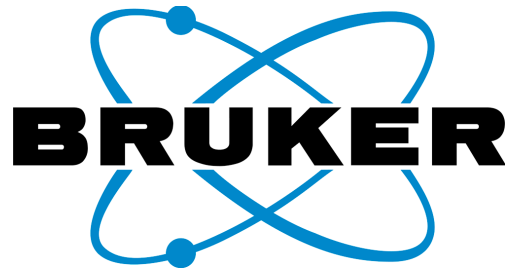


thermo
scientific

Authorized Distributor

*Pragolab s.r.o., Authorized Distributor of
Thermo Scientific*

MDa



Bruker s.r.o.

MERCK

Merck spol. s r. o.

Da



Altium International, s.r.o.



www.amedis.cz



Chromservis



*Shimadzu Handels GmbH,
branch office*

Twelfth Annual Conference of the Czech Society for Mass Spectrometry

Date

30th October – 1st November 2024

Venue

Jihočeská Univerzita

Branišovská 1760

České Budějovice

Czech Republic

Organizer

Czech Society for Mass Spectrometry, Olomouc

Institute of Microbiology of the Czech Academy of Sciences, Prague

Biology Centre, Czech Academy of Sciences, České Budějovice

University of South Bohemia in České Budějovice, Faculty of Science, České Budějovice

Faculty of Science, Charles University, Prague

Executive Board

Chair: Michael Volný (University of Chemistry and Technology, Prague)

Vice Chair: Volodymyr Pauk (Palacky University, Olomouc)

Members:

Zdeněk Kukačka (Institute of Microbiology of the CAS, Prague)

Martin Hubálek (Institute of Organic Chemistry and Biochemistry of the CAS, Prague)

Petr Novák (Institute of Microbiology of the CAS, Prague)

Adam Pruška (Swiss Federal Institute of Technology (ETH), Zurich, Switzerland)

Zdeněk Spáčil (ThermoFisher Scientific, Brno)

CONFERENCE PROGRAM

Wednesday 30th October 2024

10:00 – 17:30 Registration

13:00 – 13:20 Conference opening

13:20 – 14:10 Plenary lecture I: Prof. Wolfram Weckwerth (Chairperson: Martin Moos)

[PL-01](#) *Metabolomics, the Panome and AI-Based Metabolic Modelling – applications in Immunometabolism, Cancer and Natural Product Treatment*

14:10 – 15:40 **Session I**
(Chairperson: Zdeněk Kukačka)

14:10 – 14:30 Zdeněk Kameník

[WeO-01](#) *Functional Group Molecular Networking (FunMN) Applied to Mouse Gut Microbiota-Associated Polar Metabolites*

14:30 – 14:50 Kateřina Grabicová

[WeO-02](#) *Brown trout exposed to WWTP discharge - bioaccumulation and metabolome*

14:50 – 15:10 Petr Žáček

[WeO-03](#) *Comprehensive two-dimensional gas chromatography with mass spectrometric detection (GCxGC-TOF/MS) is a powerful tool for untargeted metabolomics*

15:10 – 15:40 Hamish Stewart

[WeO-04](#) *Orbitrap Astral and Stellar mass spectrometers, new stars for discovery and targeted analysis*

15:40 – 16:00 Coffee break

16:00 – 16:40 Company Workshop – Pragolab s. r. o.
(Dr. Hamish Stewart: The journey to the Astral analyzer)

16:40 – 17:30 Poster talks (Chairperson: Anton Škríba)

[WeS-01](#) *Tomáš Korba: Removing contaminating ions in Q0 region to increase robustness of MS analyzer*

[WeS-02](#) *Pavla Fialová: Mapping Chemical Space Sampled by Three Passive Samplers in Wastewater Effluent Using LC-HRMS and Suspect Screening*

[WeS-03](#) *Alina Sadchenko: Assessing Pesticide Levels in Soils of the Czech Republic: Methods and Findings*

[WeS-04](#) *Jana Schwarzerová: Transitioning from User-Based Applications to Cloud Computing: The Python-Based COVAIN v2.0 Toolbox for Metabolomics Data Analysis on Google Colab*

[WeS-05](#) *Michal Jágr: Comprehensive study of the effect of oat grain germination on the content of avenanthramides*

[WeS-06](#) *Marek Zákopčáník Dmitry Loginov: Peptide Identification framework for Fast Photochemical Oxidation of Proteins*

[WeS-07](#) *Michal Baka: Ion Exchange Trapping: A Solution for Excess Derivatization Reagent Removal*

17:40 – 23:00 Drinks and poster session

CONFERENCE PROGRAM

Thursday 31th October, 2024

9:00 – 9:50	Plenary lecture II: Dr. Robert Mistrík (Chairperson: Michael Volný) <u>PL-02</u> <i>mzCloud – 10 Years Striving for Perfection</i>
9:50 – 10:50	Session II (Chairperson: Petr Šítek)
9:50 – 10:10	Jan Fiala <u>ThO-05</u> <i>Protein-Centric Antibody Repertoire Profiling on timsTOF: Pioneering Insights into Sensitivity and Characterization</i>
10:10 – 10:30	Summra Ahmed <u>ThO-06</u> <i>Microbial Siderophores Intercalated into Lipocalin-1: Native Mass Spectrometry and Molecular Modelling</i>
10:30 – 10:50	Vítězslav Brinsa <u>ThO-07</u> <i>Utilizing the native MS to study protein-DNA binding modes</i>
10:50 – 11:10	Coffee break
11:10 – 13:00	Session III (Chairperson: Kristýna Gloc Pimková)
11:10 – 11:40	Goran Mitulovic <u>ThO-08</u> <i>No throughput/sensitivity compromises with 4-D proteomics at PASEF speed: Current and upcoming applications for deeper insights</i>
11:40 – 12:00	Peter Konik <u>ThO-09</u> <i>Profiling protein expression in Synechocystis using quantitative proteomics</i>
12:00 – 12:20	Tomáš Ječmen <u>ThO-10</u> <i>NKp46 receptor O-glycoprofiling by bottom-up proteomics using recently discovered O glycoprotease</i>
12:20 – 12:40	Tereza Kozelková <u>ThO-11</u> <i>Proteomic insight into the midgut of the hard tick Ixodes ricinus with the special focus on the lipolytic system</i>
12:40 – 13:00	Zuzana Kalaninová <u>ThO-12</u> <i>Post-proline Cleaving Enzymes in the Spotlight: Exploring the Hidden Specificity</i>
13:00 – 14:00	Lunch

14:00 – 15:00 Session IV

(Chairperson: Zdeněk Spáčil)

15:00 – 15:20 Roman Grabic

[ThO-13](#) *Can we find unknown unknowns? LC-HRMS DIA or DDA? That is the question.*

15:20 – 15:40 Alžběta Nemeškalová

[ThO-14](#) *Ambient ionization mass spectrometry provides screening of performance-enhancing drugs in clandestine products*

15:40 – 16:00 Filip Gregar

[ThO-15](#) *Ultraviolet LAESI/LAESCI: a novel approach for ambient MSI*

15:00 – 15:30 Zdeněk Herman Award presented by Resonance Foundation and presentation of the winning thesis

[ThO-16](#)

15:30 – 15:50 Coffee break

15:50 – 17:30 General assembly of the Society with drinks (in Czech/Slovak)

18:30 – 20:00 Guided Walk through historic center of České Budějovice – meet at the main square

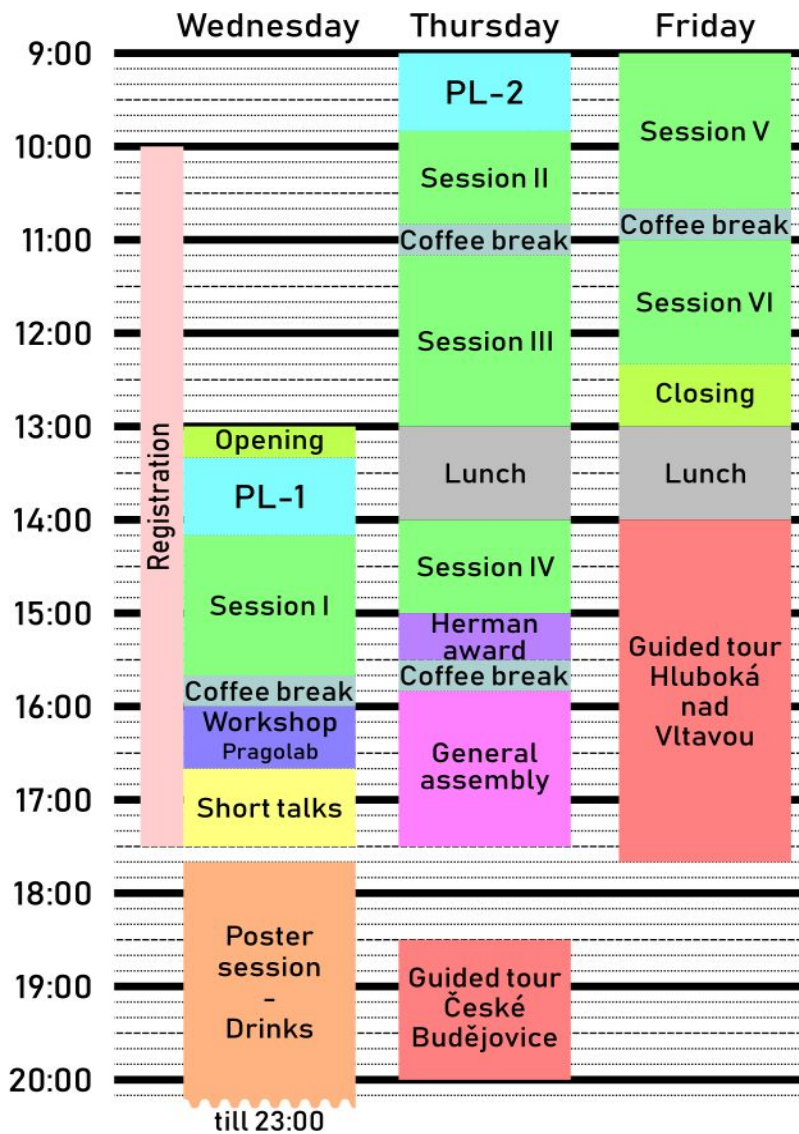
CONFERENCE PROGRAM

Friday 1st November, 2024

- 9:00 – 10:40 **Session V**
(Chairperson: Petr Novák)
- 9:00 – 9:20 Stanislav Opekar
[FrO-17](#) *The Longer, The Higher? ESI Responses of Model Oxo-Compounds labeled with Hydroxylamine Reagents Possessing Variable Alkyl Chain on a Quaternary Ammonium Moiety*
- 9:20 – 9:40 Markus Wierer
[FrO-18](#) *Non-Targeted Analysis of PFAS in Environmental Samples Using HPLC coupled with Drift Tube Ion Mobility and Quadrupole Time-of-Flight Mass Spectrometry*
- 9:40 – 10:00 Martina Poncarová
[FrO-19](#) *Products of photochemical degradation of the antidiabetic drug metformin*
- 10:00 – 10:20 Gabriela Lokočová
[FrO-20](#) *Bimodal imaging reveals a characteristic immune response to bacterial infection of the central nervous system*
- 10:20 – 10:40 Klára Švihovcová
[FrO-21](#) *Animal Behaviour Through the Lens of Mass Spectrometry*
- 10:40 – 11:00 Coffee break

- 11:00 – 12:20 **Session VI**
(Chairperson: Tomáš Pluháček)
- 11:00 – 11:20 Karel Hořejší
[FrO-22](#) *Glycosphingolipidomics: Mass spectrometry profiling of glycosphingolipids in biological samples*
- 11:20 – 11:40 Jan Šlauf
[FrO-23](#) *MALDI MSI Reveals Brain Region-Specific Changes in Lipids as Mediators of Inflammation in an Experimental Model of Bacterial Infection of the Central Nervous System*
- 11:40 – 12:00 Lucie Řimnáčová
[FrO-24](#) *Quantification of Lipid Fatty Acids by Transmethylation, Isotope-Coded Derivatization and GC-PICI-MS Analysis*
- 12:00 – 12:20 Joshua Smith
[FrO-25](#) *Feature-Based Molecular Networking Guided Discovery and Isolation of Novel Capsaicinoids from the chili pepper (*Capsicum* sp.) and their TRPV1 activation*
- 12:20 – 13:00 Poster prize, Final remarks
- 13:00 – 14:00 Lunch
- 14:00 – 17:40 Guided Tour to Hluboká nad Vltavou

CONFERENCE PROGRAM



PI-01: Metabolomics, the Panome and AI-Based Metabolic Modelling – applications in Immunometabolism, Cancer and Natural Product Treatment

Wolfram Weckwerth^{1*}

1. University of Vienna, Austria

The integration of metabolomics with AI-based metabolic modeling offers a powerful approach to understanding complex biological systems. This study builds on the application of metabolomics, proteomics and RNAseq in conjunction of AI-based metabolic modelling on macrophage metabolism, highlighting the role of mTOR signaling in various biological contexts. Recently, the Weckwerth lab demonstrated that PHGDH acts as a metabolic checkpoint regulated by mTORC1, influencing macrophage function and proliferation [1]. Based on this study PHGDH was proposed to be a metabolic checkpoint in tumor-associated macrophages (TAM). This could be confirmed in a follow-up study by deleting the PHGDH in a mouse model thereby reversing the immunosuppressive phenotype of TAMs through α -ketoglutarate and mTORC1 signaling and leading to reduced solid tumor growth [2]. Additionally, studies on natural products, such as hesperetin and norbergenin, reveal their potential to modulate mTORC1 signaling, mitophagy and apoptosis, thereby offering protective effects against inflammatory responses and lipotoxicity in NAFLD [3,4]. By leveraging high-throughput metabolomic and proteomic data and sophisticated AI algorithms, we aim to elucidate the metabolic pathways involved in immune responses, cancer progression, and the effects of natural product treatments. Our findings underscore the power of combining metabolomics and AI to uncover critical metabolic alterations and identify potential biomarkers for disease diagnosis and treatment efficacy. This interdisciplinary approach holds significant promise for advancing precision and natural medicine.

* Correspondence: wolfram.weckwerth@univie.ac.at

REFERENCES:

1. Wilson, J. L., Nägele, T., Linke, M., Demel, F., Fritsch, S. D., Mayr, H. K., Cai, Z., Katholnig, K., Sun, X. & Fragner, L. Inverse data-driven modeling and multiomics analysis reveals *phgdh* as a metabolic checkpoint of macrophage polarization and pro
2. Cai, Z., Li, W., Hager, S., Wilson, J. L., Afjehi-Sadat, L., Heiss, E. H., Weichhart, T., Heffeter, P. & Weckwerth, W. Targeting PHGDH reverses the immunosuppressive phenotype of tumor-associated macrophages through α -ketoglutarate and mTORC1 sign
3. Li, W., Cai, Z., Schindler, F., Bahriraii, S., Brenner, M., Heiss, E. H. & Weckwerth, W. Norbergenin prevents LPS-induced inflammatory responses in macrophages through inhibiting NF κ B, MAPK and STAT3 activation and blocking metabolic reprogramming.
4. Li, W., Cai, Z., Schindler, F., Afjehi-Sadat, L., Montsch, B., Heffeter, P., Heiss, E. H. & Weckwerth, W. Elevated PINK1/Parkin-Dependent Mitophagy and Boosted Mitochondrial Function Mediate Protection of HepG2 Cells from Excess Palmitic Acid by Hesperetin. *J Agric Food Chem* 72, 13039-13053, doi:10.1021/acs.jafc.3c09132 (2024).

PI-02: mzCloud – 10 Years Striving for Perfection

Robert Mistrík^{1*}

1. Bitmoderna, s.r.o.

It has been more than 10 years since we started developing the MSn spectra database called mzCloud. We didn't want to create yet another database and compete for the largest number of spectra or mindlessly collect citations, as some of our fellow mass spectrometrists still do today. Instead, we aimed to develop a completely new concept that would make the identification of small molecules a solvable problem. Have we succeeded? Partially. Although the number of users and citations is growing exponentially, small molecules are still a tougher challenge than proteins. Is the solution artificial intelligence, molecular mechanics, or another concept?

* Correspondence: rob.mistrik@gmail.com

WeO-01: Functional Group Molecular Networking (FunMN) Applied to Mouse Gut Microbiota-Associated Polar Metabolites

Zdenek Kamenik ^{1*}, Tommaso Stefani ¹, Anna Jelinkova ¹, Martin Schwarzer ¹

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

Polar metabolites, such as amino acids, organic acids, monoamines, nucleotides, thiols, and sugars, present challenges for LC-MS/MS analysis due to their high polarity and/or tendency to adhere to metal components of the instrumentation. Chemical derivatization can mitigate these issues by reducing the polarity of these metabolites, making them compatible with established reverse-phase chromatography. This presentation will explore the use of chemical derivatization for untargeted metabolomics, introducing a concept we call functional group molecular networking (FunMN). Specifically, we employed various derivatization agents, including 3-nitrophenyl hydrazine, dansyl chloride, 3-aminomethyl pyridine, and N-ethylmaleimide, targeting functional groups such as carboxyls, hydroxyls, amines, phosphates, and thiols. Their effectiveness was assessed using over 100 polar metabolite standards in both solvents and biological matrices. To demonstrate the method's relevance for real-world metabolomics, we analyzed metabolites linked to host-gut microbiota interactions, which are crucial for host physiology and disease regulation. Fecal samples were collected from three groups of five-week-old C57BL/6 male and female mice with distinct microbial colonization: germ-free (no microbiota), oligoMM12 (colonized with 12 bacterial strains), and conventional (common microbiota). We will demonstrate how each derivatization technique distinguishes between these groups, and how GNPS-based molecular networks can reflect the presence of a functional group rather than overall structural similarity, as in traditional molecular networking.

* Correspondence: zdenek.kamenik@email.cz

REFERENCES:

1. Wang M. et al.: *Nat Biotechnol* 34 (8), 828-837 (2016)

ACKNOWLEDGEMENT:

This work was supported by the Lumina Quaeruntur Program, No. LQ200202002, provided by the Czech Academy of Sciences.

WeO-02: Brown trout exposed to WWTP discharge - bioaccumulation and metabolome

Kateřina Grabicov^{1*}, Ning Zhang¹, Roman Grabic¹, Tomš Randk¹

1. Jihoesk univerzita v eskch Budjovicch, Fakulta rybřstv a ochrany vod

The emissions of micropollutants from wastewater treatment plants (WWTPs) into the receiving surface water is a recognised threat to the aquatic environment. The adverse effects of released chemicals on aquatic organisms belong to the most accented ones. In this study, brown trout (*Salmo trutta*) eggs were deployed to a natural stream, where treated wastewater contributed up to 25% of the total stream flow. The eggs placed upstream of the WWTP served as a reference locality. The bioaccumulation of selected pharmaceuticals and their metabolites in three developmental stages of brown trout (fish egg, yolk sac fry and young-of-the-year fish) was studied using liquid chromatography with high-resolution mass spectrometry. Besides the bioaccumulation, the changes in metabolome and exposome were evaluated applying two stage non-targeted workflow.

Fifteen pharmaceuticals were found to have clear bioaccumulation trends over time since hatching. The highest concentrations were observed for antidepressants and for cardiovascular drug. It was proven that individual developmental stages bioaccumulated different pharmaceuticals with different intensities. Based on the non-targeted screening, significantly changed signals (up- and downregulated) increased with exposure time.

* Correspondence: grabicova@frov.jcu.cz

ACKNOWLEDGEMENT:

This study was financially supported by the Czech Science Foundation, grant No. GACR 20-04676X (Holistic exposure and effect potential assessment of complex chemical mixtures in the aquatic environment).

WeO-03: Comprehensive two-dimensional gas chromatography with mass spectrometric detection (GCxGC-TOF/MS) is a powerful tool for untargeted metabolomics

Petr Žáček^{1*}

1. PřF UK BIOCEV

Biological matrices often pose significant challenges in analysis due to their complexity and potential interferences[1]. The number of detectable compounds can be in the thousands or more, making traditional one-dimensional separation techniques insufficient for handling such a large number of analytes. An approach using two-dimensional comprehensive gas chromatography coupled with mass spectrometry equipped with electron ionization (EI) and time-of-flight mass analyzer (TOF) (GCxGC-TOF/MS) offers a solution to analyze volatiles in complex matrices[2]. GCxGC-TOF/MS can be easily coupled with robotic devices enabling specific sampling methods such as headspace solid-phase microextraction (SPME) or dynamic headspace (DHS). Commercially available instrumentation was introduced more than twenty years ago. Since then the technique has become well-established due to its sensitivity, peak resolution, enhanced peak capacity, and reproducibility[3].

* Correspondence: Petr.Zacek@natur.cuni.cz

REFERENCES:

1. Keppler, EAH. et al.; *Trends Anal. Chem.* 109, 275-286 (2018).
2. de Souza, JRB. et al.; *Anal. Chim. Acta.* 1040, 1-18 (2018).
3. Zanella, D. et al.; *Anal. Sci. Adv.* 2(3-4), 213-224 (2021).

ThO-05: Protein-Centric Antibody Repertoire Profiling on timsTOF: Pioneering Insights into Sensitivity and Characterization

Jan Fiala^{1,2}, Dina Schuster^{1,2}, Simon Ollivier^{1,2}, Stuart Pengelley³, Markus Lubeck³, Florian Busch⁴, Andris Jankevics^{1,2}, Oliver Raether³, Jean-Francois Greisch⁴, Albert J. R. Heck^{1,2*}

1. *Biomolecular Mass Spectrometry & Proteomics, Padualaan 8, 3584 CH Utrecht, The Netherlands*
2. *Netherlands Proteomics Center, Padualaan 8, 3584 CH Utrecht, The Netherlands*
3. *Bruker Daltonics GmbH & Co. KG, Fahrenheitstrasse 4, 28359 Bremen, Germany*
4. *Bruker Switzerland AG, Fällanden, Zurich, Switzerland*

Antibodies or immunoglobulins (Igs) play a crucial role in immune defense, but their vast sequence variability and concentration levels pose analytical challenges. Recent advances in protein-centric analysis utilizing Orbitrap-based systems have facilitated the detailed profiling of human antibody repertoires by analyzing fragment antigen-binding (Fab) regions[1]. However, effective Fab profiling requires optimized separation techniques and rapid mass spectrometric analysis to differentiate hundreds of Fabs with nearly identical masses but potentially diverse biochemical properties.

In this work, we aimed to test the capability of the timsTOF system for Fab profiling, as this has been primarily performed using Orbitraps so far. To achieve this, first LC runs with 7 recombinant monoclonal Fab mixture were performed to compare the sensitivity of UHPLC system versus the nanoLC, whereby the nanoLC provided approx. 50-fold increase in intensity. Further, Fab fragments (injection amounts of ~56-110 ng) from serum samples, were profiled on Thermo Orbitrap and timsTOF platforms with identical nanoLC separation. By applying an in-house developed script simulating a sliding window algorithm, the timsTOF identified over 1.5 times more distinct IgG1 clones compared to the Orbitrap Eclipse, particularly excelling in the detection of low-abundance clones (<1%). Nevertheless, the top 100 most abundant clones exhibited high overlap between both platforms, underscoring the robustness of the proposed workflow.

The study introduces the first use of the Bruker timsTOF HT for profiling circulating antibodies in human serum. High sensitivity allows detailed Fab profiling from just 50 nL of serum, reducing sample consumption from 1-2 µL, with robust results across platforms[2].

* Correspondence: a.j.r.heck@uu.nl

REFERENCES:

1. Bondt A. et al.: *Cell Syst.* 12(12), 1131-1143.e5 (2021).
2. Fiala J. et al.: *J. Am. Soc. Mass Spectrom.* 35(6), 1292-1300 (2024).

ACKNOWLEDGEMENT:

We would like to thank our colleagues Linus Wollenweber, Danique van Rijswijck, Albert Bondt, and Maurits den Boer for their valuable feedback. We acknowledge funding through the Dutch Research Council (NWO) for The Netherlands Proteomics Centre through the X-omics Road Map program (project 184.034.019). A.J.R.H. acknowledges further support from The Netherlands Organization for Scientific Research (NWO) through the Spinoza Award SPI.2017.028.

ThO-06: Microbial Siderophores Intercalated into Lipocalin-1: Native Mass Spectrometry and Molecular Modelling

Summra Ahmed ^{1,2*}, Hynek Mácha ^{1,2}, Josef Chmelík ¹, Dominika Luptáková ¹, Alan Kádek ³, Milan Raška ⁴, Vladimír Havlíček ^{1,2}

1. *Institute of Microbiology of the Czech Academy of Sciences, Videnska 1083, Prague, Czech Republic*
2. *Department of Analytical Chemistry, Faculty of Science, Palacký University, 17. listopadu 12, Olomouc, Czech Republic*
3. *Institute of Microbiology of the Czech Academy of Sciences - BIOCEV, Průmyslová 595, Vestec, Czech Republic*
4. *Institute of Immunology, Faculty Hospital, Zdravotníků 248/7, Olomouc, Czech Republic*

The tug-of-war for nutrients between the host and the pathogen is crucial in the progression of an infection. Most microbial species produce siderophores for iron intake such as Enterobactin (Ent) Rhizoferrin (Rhf) and Triacetylfulsarinine C (TafC) [1-2]. The nutritional immunity against pathogens is partially driven by lipocalin-1 (Lcn1) secreted by some immune cells during microbial invasion [3]. The noncovalent lipocalin/siderophore complex was characterized using ESI-MS, FTICR and Q-TOF. The spectra of a 20 μM conc. of Lcn1 in 150 mM NH_4Ac were recorded in 1:1 stoichiometric ratio with Ent. The stability of the Lcn1-Ent complex was tested in the pH range of 5.4–9, Ent conc. 0.2–200 μM and with 50 to 120 $^\circ\text{C}$ ion block temperatures. The interaction was modeled using SwissDock, KVFinder, LigPlot, and Chimera tools. The native MS data indicated a decline in the capacity to form a noncovalent complex in the following order: Lcn1-(Ent/Rhf/TafC). The formation of the complex was found to be sensitive to pH and the ratio of siderophore to Lcn1. The formation of the Lcn1-Ent complex was at pH 7.4 and 20 μM conc. was approx. 30% compared to the native protein. The optimal pH was found to be 6.4–7.4 and Ent conc. was 200 μM with no thermal effect. Molecular modeling revealed a Lcn1-Ent interaction and Lcn1-dihydroxybenzoylserine dimer. The crucial interacting amino acid (AA) were Lys114, Asp25, and Leu33. Moreover, the AA of Fusarinine C were similar to Ent while Rhf binds to a different domain. In conclusion, Ent is the preferred transported siderophore over Rhf and TafC. Identifying novel protein binding partners of siderophores in the host and the characterization of new membrane receptors of Lcn-complexes on immune cells may lead to the discovery of new therapeutic targets.

* Correspondence: summra1.618@gmail.com

REFERENCES:

1. *Maret, W. (2024). Natural Product Communications, 19(8), 1934578X241271701.*
2. *Schalk, I. J. (2024). Nature Reviews Microbiology, 1-17.*
3. *Chandrasekaran, P. et al. (2024). International Journal of Molecular Sciences, 25(8), 4290.*

ACKNOWLEDGEMENT:

Czech Science Foundation (22-06771S).

ThO-07: Utilizing the native MS to study protein-DNA binding modes

Vítězslav Brinsa ^{1,2*}, Alan Kádek ³, Karolína Musilová ¹, Petr Novák ³, Aleš Hnízda ¹

1. *First Faculty of Medicine, Charles University in Prague*

2. *Department of Biochemistry, Faculty of Science, Charles University*

3. *Institute of Microbiology of the Czech Academy of Sciences*

Native mass spectrometry (native MS) represents a powerful tool for the studying of functional properties of biomolecules, including protein oligomerization, ligand binding, structural stability and many others. It provides precise readouts of molecular mass of the studied entities and complements well-established biophysical techniques.

In this talk, the benefit of using the nano-electrospray ionization mass spectrometry (nESI-MS) will be demonstrated in a project focused on human transcription factors (TFs) FOXK1 and FOXK2 bound to their DNA recognition motifs. Using fluorescence anisotropy and electrophoretic mobility shift assay, we found that FOXK proteins cooperatively bind to the short DNA fragments regardless of the presence of the consensus FOX binding motifs within their sequences. This implies that FOXK TFs bind to DNA in a multivalent binding mode combining specific as well as unspecific interactions. However, the applied biophysical techniques could not elucidate binding stoichiometry in the studied FOXK-DNA complexes. We therefore took advantage of nESI-MS, which allowed us to precisely determine composition of these complexes. Following these observations, comprehensive structural studies will be designed accordingly. This work exemplifies a power of the native MS for studying challenging biological systems with non-optimal behaviour.

* *Correspondence: vitezslavbrinsa@gmail.com*

ACKNOWLEDGEMENT:

This work was generously funded by the Cooperatio project.

ThO-09: Profiling protein expression in *Synechocystis* using quantitative proteomicsPeter Konik^{1*}, Vendula Krynická², Petra Skotnicová², Roman Sobotka^{2,1}

1. Jihočeská univerzita v Českých Budějovicích

2. Institute of Microbiology, CAS Centre Algatech, Třeboň

Control of protein expression is one of the key cellular mechanisms of response to changes in environment, nutrient supply, stress conditions and others. Quantitative proteomics, combined with robust statistical methods, is able to measure protein expression levels in cells grown under different conditions. In this study, we compared protein expression levels in the cyanobacterium *Synechocystis* PCC6803 in stationary phase of growth and after subsequent transition to the exponential phase induced by dilution and exposition to high light. We identified an excess of 2500 proteins, out of which 1000 have shown significant differences in expression levels between the tested conditions. These include ribosomal proteins and proteins generally associated with cell growth; proteins of photosystems I and II; proteins associated with various metabolic pathways and proteins with known regulatory functions. The expression profiles of well characterized proteins can be used as a template to point to proteins with unknown function and suggest their involvement in a metabolic or information pathway, involvement in a protein complex or indicate agonist/antagonist relationships between them.

* Correspondence: konik@prf.jcu.cz

ThO-10: NKp46 receptor O-glycoprofiling by bottom-up proteomics using recently discovered O glycoprotease

Tomáš Ječmen¹*, Shiva Nejadbrahim¹, Ondřej Vaněk¹

1. *Katedra biochemie, PřF UK*

The invasive candidiasis caused by *C. glabrata* is a feared complication for patients with hematological diseases. Epithelial adhesin Epa1 of this yeast is among ligands of NK cells' receptor NKp46, which is involved in triggering apoptosis in target cells. It was proposed that the NKp46:Epa1 interaction specifically depends on T225 O-glycosylation and N216 N-glycosylation in the NKp46 stalk region.

To investigate the effect of NKp46 glycosylation on binding Epa1, we expressed NKp46 wild type and T225A mutant, with complex and high-mannose type N-glycosylation. The affinity of Epa1 to the NKp46 glycovariants was measured by microscale thermophoresis. To characterize the proteins, we enzymatically removed N-glycans, cleaved the proteins sequentially by O-glycoprotease (Ogp) and trypsin, or by trypsin alone, and analyzed resulting (glyco)peptides by RP-ESI-qTOF. Ogp cleaving N-terminal to a glycosylated serine or threonine was used to obtain shorter peptides in the NKp46 stalk region, which is rich in serine, threonine and proline residues while lacking charged residues.

We found 5 novel O-glycosites (S218, T233, T237, T238, and T252), and 2 potential sites (T230, T240) in motives that are not cleaved by Ogp and thus cannot be unambiguously identified by the approach used. We also showed that multiple sites in the stalk region are O-glycosylated simultaneously, with type 1 or 2 cores that are truncated in the vicinity of N-glycans, or extended elsewhere. Our results confirm that O-glycans vary minimally between NKp46 from HEK293T and S cells.

Overall, we substantially broadened existing knowledge about NKp46 O-glycosylation and its crucial role in Epa1 binding.

* Correspondence: jecmen@natur.cuni.cz

ACKNOWLEDGEMENT:

This work was supported by the Czech Science Foundation (18-10687S), the Ministry of Education, Youth and Sports of the Czech Republic (LTC20078), and Charles University (GAUK 1436020). We acknowledge CMS BIOCEV ("Biophysical techniques") of CIISB, Instruct-CZ Centre, supported by the Ministry of Education, Youth and Sports of the Czech Republic (LM2018127 and CZ.02.1.01/0.0/0.0/18_046/0015974).

ThO-11: Proteomic insight into the midgut of the hard tick *Ixodes ricinus* with the special focus on the lipolytic system

Tereza Kozelková^{1,2*}, Matthias Schittmayer-Schantl³, Filip Dyčka², Stephen Lu⁴,
Veronika Urbanová¹, Helena Frantová¹, Daniel Sojka¹, Martin Horn⁵, Ruth Birner-Gruenberger³,
Petr Kopáček¹

1. *Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, Ceske Budejovice, Czech Republic*
2. *Faculty of Science, University of South Bohemia, Ceske Budejovice, Czech Republic*
3. *Institute of Chemical Technologies and Analytics, Vienna University of Technology, Vienna, Austria*
4. *Vector Biology Section, Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases: Bethesda, MD, US*
5. *Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic*

The tick midgut plays a crucial role as the main tissue for the storage and digestion of host blood, which is the sole source of energy and nutrients essential for the tick development and reproduction. During tick feeding, the midgut epithelium undergoes dynamic changes reflecting the changes in physiological processes in the tissue. This study provides a novel insight into the proteomics of the midgut during tick development, focusing on the protein-level changes that occur throughout feeding and development.

Label-free quantitative (LFQ) proteomics was used to elucidate changes during the blood meal and development of *Ixodes ricinus*. Midguts from different feeding stages of nymphs and adults were dissected, in-solution digestion using trypsin was followed by the peptide analysis carried out on the timsTOFPro(Bruker) mass spectrometer coupled to an Ultimate 3000 RSLnano System. Obtained raw data were submitted to the actual database available on UniProt for the *I. ricinus* and searched in MaxQuant software. Data were further analyzed using Perseus or in-house written scripts. To further access the lipolytic system, the affinity-based proteomics was performed. Midgut homogenates from different feeding time points were labeled with C6 ABP, allowing the selective isolation of active hydrolases using streptavidin-agarose beads.

In summary, while several transcriptomics studies of *I. ricinus* have been published, only few studies have investigated the tick proteomes. Potential targets for drug or vaccine treatment might be developed. Our pioneering activity-based proteomics study on lipid hydrolases contributes to the limited understanding of lipid metabolism in ticks and holds promise for finding susceptible targets for effective interventions against ticks.

* Correspondence: tereza.kozelkova@paru.cas.cz

ACKNOWLEDGEMENT:

Acknowledgement: Supported by GACR 21-08826S and ERDFunds (No.CZ.02.1.01/0.0/0.0/16_019/0000759).

ThO-12: Post-proline Cleaving Enzymes in the Spotlight: Exploring the Hidden Specificity

Zuzana Kalaninová^{1,2,*}, Jasmína Mária Portašiková^{1,2}, Barbora Jirečková^{1,2}, Marek Polák^{1,2}, Jana Nováková³, Daniel Kavan², Petr Novák^{1,2}, Petr Man²

1. Faculty of Science Charles University

2. BioCeV, Institute of Microbiology of the Czech Academy of Sciences

3. AffiPro s.r.o.

Classical as well as structural proteomic workflows rely heavily on protein digestion. In hydrogen/deuterium exchange (HDX) it provides spatial resolution, in fast photochemical oxidation or cross-linking it enables precise localization of modifications and in general proteomics it helps to overcome issues with ionization of entire proteins in complex mixtures. Although each workflow has its own “golden standard” protease, the search for new enzymes is a never-ending quest. Recently, the utility of post-proline cleaving enzymes (PPCEs), such as *Aspergillus niger* prolyl endopeptidase (*AnPEP*) and neprosin, has been highlighted as they complement proteolytic tools because proline is a stop site for many proteases.

In our study, while aiming at using *AnPEP* in online proteolysis, we found that this enzyme also displayed specificity to reduced cysteine. Two *AnPEP* sources, research grade ProAlanase and industrial grade alternative Clarity Ferm, were systematically evaluated by LC-MS/MS under various conditions possibly affecting cleavage preferences of this enzyme. Under specific conditions, we discovered previously overlooked cleavage dependencies and showed that the industrial grade *AnPEP* is a good source of enzyme, available in quantities suitable for immobilization and generation of protease columns. We also showed that post-cysteine cleavage is blocked by cysteine modifications such as disulfide bond formation, oxidation, or alkylation. In the same experimental design, neprosin mimicked this cleavage pattern. To facilitate cross-comparison of numerous digestion conditions and speed-up the extraction of cleavage specificities and other digestion-related metrics, we created a Java-based application DigDig.

* Correspondence: kalaninz@natur.cuni.cz

ACKNOWLEDGEMENT:

Financial support from NPO-NEURO-EXCELES (ID Project No. LX22NPO5107), the Technology Agency of the Czech Republic (Programme National Centre of Competence, ID Project No. TN02000132), Czech Science Foundation (22-27695S) and the MEYS/EU project OP JAK – Talking Microbes (CZ.02.01.01/00/22_008/0004597) is gratefully acknowledged. Access to the Centre of Molecular Structure (CMS), BioCeV, was provided by CIISB LM2023042 and ERDF “UP CIISB” (CZ.02.1.01/0.0/0.0/18_046/0015974).

ThO-13: Can we find unknown unknowns? LC-HRMS DIA or DDA? That is the question.

Roman Grabic ^{1*}, Kateřina Grabicová ¹, Andrea Vojs-Staňová ^{1,2}, Petra Mikušová ³, Zuzana Toušová ³, Klára Hilscherová ³

1. *University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters*

2. *Comenius University Bratislava, Faculty of Natural Sciences*

3. *Masaryk University, Faculty of Science, RECETOX*

The primary issue of recent pollution research is bridging the gap between toxicology (effect) and environmental chemistry (levels, fate etc). Because we cannot detect all chemicals present even in the extract, the battery of bioassays remains the only tool for biological effect evaluation [1]. However, there is a need to identify effect drivers, which can be forwarded to the next decision-making stage. Several approaches combine targeted, nontargeted analysis with bioassays to explain the effect of the mixtures. Still, the level of explicability of biological effect equivalent (BEQ) by chemical analysis varies from about 50% to < 1% [2].

The advantages and disadvantages of two different nontargeted workflows for identifying unknown chemicals with biological effects will be presented. Combining LC HRMS with fractionation can help lower the mixture's complexity. However, DIA can fail when nothing is known about the effect drivers or their concentration is lower than the identification threshold.

The workflow consisting of affinity purification and two-stage LC-HRSM workflow seems promising as the fraction's complexity level was lowered much more than in the case of fractionation only. DDA MS2 spectra quality allows easier identification or spectra interpretation. This approach yielded the successful identification of several classes of unknown TTR ligands and the explanation of up to 40% of the biological activity of the samples [3]. However, some limits of data interpretation are difficult to overcome at the recent knowledge level.

* *Correspondence: rgrabic@frov.jcu.cz*

REFERENCES:

1. *Escher B.I. et al.: Science, 367(6476), 388-392 (2020).*
2. *Šauer P at al.: Environ. Int., 178, 107957 (2023).*
3. *Mikusova P. et al.: J Hazard Mater 471 (2024)*

ACKNOWLEDGEMENT:

The authors acknowledge the Czech Science Foundation grant No. GACR 20-04676X "Holistic exposure and effect potential assessment of complex chemical mixtures in the aquatic environment".

ThO-14: Ambient ionization mass spectrometry provides screening of performance-enhancing drugs in clandestine products

Alžběta Nemeškalová^{1*}

1. Department of Analytical Chemistry, University of Chemistry and Technology, Prague, Czech Republic

Muscle-enhancers, also referred to as performance-enhancing drugs or supplements, can pose significant health risks, especially when used outside medical supervision. Despite being banned by most European jurisdictions, medicinal products containing Anabolic-Androgenic Steroids or Selective Androgen Receptor Modulators can be obtained through online retailers, often with poor or even completely absent quality control. The presented work highlights the application of Ambient Ionization Mass Spectrometry (AI-MS) for a fast screening of these compounds in bodybuilding food supplements. Two AI-MS techniques are compared, namely Desorption Atmospheric Pressure Photoionization and Dielectric Barrier Discharge Ionization, both offering fast detection of target compounds in samples with minimal processing. The AI-MS results were further validated against a gold-standard LC-UV-MS/MS method performed in multiple reaction monitoring mode with an external calibration for each analyte. The results demonstrate that the reliability of AI-MS screening depends on applying several specific criteria, including mass accuracy, proper isotopic distribution, signal intensity, and consistency across multiple scans. When following a set of consistent rules, a good agreement was found between the tested AI-MS and LC-UV-MS/MS methods, with AI-MS being effective at detecting the target compounds at pharmacologically relevant doses, i.e., approximately above 1 mg per capsule. It is demonstrated that when adhering to a set of clear and consistent criteria, ambient mass spectrometry can be employed as a qualitative technique for the screening of illegal SARMS with sufficient confidence and without the necessity to perform a regular LC-MS analysis.

* Correspondence: nemeskaa@vscht.cz

ThO-15: Ultraviolet LAESI/LAESCI: a novel approach for ambient MSI

Filip Gregar^{1*}, Barbora Papoušková¹, Andrea Horniaková², Petr Fryčák¹, Karel Lemr¹, Tomáš Pluháček¹

1. *Department of Analytical Chemistry, Faculty of Science, Palacký University, Czechia*

2. *Faculty of Pharmacy, Comenius University, Bratislava, Slovakia*

The development of ambient molecular mass spectrometry techniques has significantly advanced the analysis of complex biological surfaces under native conditions. Among these techniques, laser ablation electrospray ionization (LAESI) has shown great promise due to its ability to directly sample surfaces with minimal preparation [1]. However, conventional LAESI systems often face challenges such as poor spatial resolution and a limited range of detected analytes [2]. This study introduces a novel deep UV LAESI/LAESCI dual ionization source with an adjustable interface that enables the simultaneous acquisition of complementary ESI and APCI mass spectra. The experimental setup included a 193 nm Analyte G2 laser ablation unit hyphenated to a hybrid Q-TOF mass spectrometer. The technique was tested with analytes of varying molecular masses and polarities, demonstrating high sensitivity. The method also proved highly effective in molecular mass spectrometry imaging (MSI), using the Ilaps software [3]. LAESI/LAESCI-MSI enables the visualization of latent fingerprints from volunteers exposed to new psychoactive substances, such as butylone, naphyrone, cathinone, and phedrone. The final images were able to identify individual volunteers through dactyloscopic analysis and to quantitatively detect trace amounts of drugs on their fingers. Thus, the proposed technique demonstrated its capability of capturing images without distortion or alterations during MSI experiments that lasted over 12 hours. The deep UV LAESI/LAESCI dual ion source offers advantages in minimal sample preparation, enhanced analyte coverage, and improved spatial resolution ($\leq 3 \mu\text{m}$). It holds potential for applications in biomedicine, environmental analysis, and materials science.

* *Correspondence: filip.gregar01@upol.cz*

REFERENCES:

1. *Nemes P. et al.: Anal. Chem. 79 (2007) 8098*
2. *Nielen M. W. F. et al.: Anal. Bioanal. Chem. 406 (2014) 6805*
3. *Faltusova V. et al.: J. Anal. At. Spectrom. 37 (2022) 733*

ACKNOWLEDGEMENT:

The financial support of the research by the Internal Grant Agency of Palacký University (project no. IGA_PrF_2024_026) is gratefully acknowledged.

ThO-16: Photoemission spectroscopy of liquids

Lukáš Tomaník^{1*}

1. University of Chemistry and Technology, Prague

Photoemission (or photoelectron) spectroscopy (PES) has been used for decades. Thanks to recent advancements, the technique has been extended to the realm of liquids. This enabled us to study a full electronic structure of solvents as well as solutes. Furthermore, it became evident recently that PES measurements of liquids provide more complex information that could be extracted together with electronic structure. Specifically, one can probe molecular structure, intermolecular interactions, or a specific orientation of solvent molecules around the solute. I will introduce the experimental technique and demonstrate its capabilities in various chemical systems.

* Correspondence: tomanikl@vscht.cz

FrO-17: The Longer, The Higher? ESI Responses of Model Oxo-Compounds labeled with Hydroxylamine Reagents Possessing Variable Alkyl Chain on a Quaternary Ammonium Moiety

Stanislav Opekar ¹, Zdeněk Chval ², Martin Moos ¹, Petr Šimek ^{1*}

1. CAS, Biology Centre, Institute of Entomology

2. Faculty of Health and Social sciences, University of South Bohemia

A number of compound classes exhibit poor ionization efficiency in LC coupled with electrospray mass spectrometry (ESI MS). To improve the MS response, derivatization of suitable targets in their structures may offer some advantages over a direct analytical approach, not only to improve their MS response, but also to improve their stability, separation and quantification. Many oxo-metabolites represent a class with these properties, typically reactive alkyl aldehydes, oxo-carboxylic acids such as pyruvate or oxaloacetate, which are difficult to detect and quantify directly without derivatization [1,2].

Four cationic tetraalkylammonium hydroxylamine reagents with alkyl chains of different lengths (methyl, hexyl, dodecyl and hexadecyl) were prepared and investigated for the targeted LC-MS analysis of the four model oxo compounds. Acetone, pyruvate, decanal and testosterone were converted to the corresponding oximes and prepared in milligram quantities for further studies. The MS-ESI ionization efficiency of the synthetic products was investigated and compared using a linear ion trap and two quadrupole-based MS spectrometers. The physicochemical properties of the prepared oximes were calculated using quantum chemical methods and compared with their MS responses to find an effective future derivatization strategy for their LC-MS quantification in complex biological matrices.

* Correspondence: opekar@bclab.eu

REFERENCES:

1. Deng P. et al: *The Analyst* 143(1), 311-322 (2018).
2. Star-Weinstock M. et al: *Anal. Chem.* 84(21), 9310-9317 (2012).

ACKNOWLEDGEMENT:

This work was supported by the Czech Science Foundation (project GAČR, No. 23-06600S).

FrO-18: Non-Targeted Analysis of PFAS in Environmental Samples Using HPLC coupled with Drift Tube Ion Mobility and Quadrupole Time-of-Flight Mass Spectrometry

Markus Wierer ^{1*}, Christian Klampfl ¹, Markus Himmelsbach ¹

1. JKU Linz

Per- and polyfluoroalkyl substances (PFAS) are a diverse group of fluorinated compounds commonly found in consumer and industrial products such as rain jackets, nonstick cookware, and firefighting foams. Recent research indicates that 31% of groundwater samples worldwide exceed PFAS levels deemed hazardous to human health by the EPA [1].

Of the more than 14,000 described PFAS, only a small subset is routinely analyzed in environmental and biological studies. While many legacy PFAS have been phased out, a variety of newer PFAS compounds have been introduced as substitutes. Due to their diversity those are often not included in standard PFAS screenings. This is evident in human serum samples, where recent years have shown decreasing concentrations of detectable PFAS, while total extractable organic fluorine levels remain relatively stable, suggesting the presence of undetected PFAS that are not captured by conventional targeted screening approaches [2].

High-resolution mass spectrometry has been employed for non-targeted PFAS detection and characterization. However, in low-concentration environmental samples, standard data-dependent acquisition approaches often fail to fragment and identify many low-abundance species.

This presentation introduces an advanced method for non-targeted PFAS analysis that combines high-performance liquid chromatography with drift tube ion mobility spectrometry and quadrupole time-of-flight mass spectrometry. This approach adds a separation dimension based on an ion's collision cross section. Additionally, data-independent acquisition generates MS/MS spectra by fragmenting all ions in the collision cell, with fragments realigned according to their drift times, enhancing the detection and of PFAS in complex, low-concentration samples.

* Correspondence: markus.wierer@jku.at

REFERENCES:

1. Ackerman Grunfeld, D., et al. *Nat. Geosci.* 17 (1), 340-346 (2024).
2. Miaz, L. T., et al. *Environ. Sci.: Processes Impacts* 22 (4), 1071-1083 (2020).

ACKNOWLEDGEMENT:

This work was conducted under the frame of "Programm Interreg ATCZ52 Österreich – Tschechische Republik: Infrastruktur für Metabolomik-Forschung und Klinische Chemie".

FrO-19: Products of photochemical degradation of the antidiabetic drug metformin

Martina Poncarová ¹*, Veronika Oušková ¹, Pavla Fojtíková ¹, Šárka Klementová ¹

1. Jihočeská univerzita, Přírodovědecká fakulta, katedra chemie

Metformin is a drug used to treat type II diabetes ranking as No. 2 in the list of prescribed drugs in the U.S. in 2022 [1] and, according to data from the State Institute of Drug Control [2], even No. 1 in the Czech Rep. in the years 2014 – 2018 (more recent data are not available).

Since metformin is not metabolized in the human body [3], it enters wastewater and subsequently surface waters where it can undergo photochemical degradation.

In this study, photochemical degradation under light conditions relevant to surface waters was performed in the presence of a quinone sensitizer (sodium salt of anthraquinone-2-sulfonic acid as a model of natural quinone sensitizers).

The identification of the products formed in the photochemical reaction was studied by UHPLC-Q-TOF. Eight major products were found in the reaction mixture, and for six of them, structures were proposed.

* Correspondence: poncam00@prf.jcu.cz

REFERENCES:

1. ClinCalc: Metformin - Drug Usage Statistic 2013-2022 (2024).
2. SÚKL: Hodnocení výdejů léčivých prostředků (2018).
3. Szymczak-Pajor I. et al.: *Pharmaceuticals* 15(7), 810 (2022)

ACKNOWLEDGEMENT:

Sincere thanks go to Karel Hořejší for his valuable technical support in the measurement on UHPLC-Q-TOF.

FrO-20: Bimodal imaging reveals a characteristic immune response to bacterial infection of the central nervous system

Gabriela Lokočová ^{1*}, Jan Šlauf ¹, Miloš Petřík ², Andrea Palyzová ¹, Vladimír Havlíček ¹, Dominika Luptáková ¹

1. *Institute of Microbiology of the Czech Academy of Sciences, Vídeňská 1083, 14200 Prague 4, Czechia*

2. *Institute of Molecular and Translational Medicine, Palacký University, Olomouc, Czechia*

Infection of the CNS induces expression of inflammatory mediators, exerting region-specific influences on brain function [1]. By combining MALDI MSI and histology, this study profiles brain immune response and bacteria interaction.

The neuroinfection was induced in immunocompetent Lewis rats by intracranial injection of 5×10^3 *Escherichia coli* 5172 suspension. After 24 h, brains (5 infected, 5 control) were collected and cryosectioned at 12 μ m at the -0.72 mm coronal level from bregma. H&E staining was used to examine morphological changes. For molecular profiling, sections were sprayed with 1,2-DAN matrix (7 mg/mL in 70% ACN) using M3+ sprayer, followed by MS data acquisition in negative ion mode using a 12T XR-2 ω FTICR solariX mass spectrometer. MS data of deprotonated molecules were processed in SCLab v.2024b.

E. coli primarily invaded the ventricular system, prompting a strong immune response characterized by neutrophils surrounding bacteria and cerebral arteries. In infected brains, MALDI MSI revealed compounds at *m/z* 1113.7416, 1115.7569, 1131.7538, and 1133.7683 that were exclusively present in the *E. coli*-neutrophil interaction zone. Increase of phosphatidic acids (PA), e.g., PA (40:6) (*m/z* 747.4977), PA (O-32:0) (*m/z* 633.4864), and PA (O-36:4) (*m/z* 681.4860) were detected in ventricles. Glycerophosphoethanolamines (PE), e.g., PE (O-36:1), PE (O-38:5), and PE (O-40:7) at *m/z* 730.5753, 750.5441, and 774.5436, exhibited higher abundance in the choroid plexus.

The bimodal imaging enabled morphological-molecular profiling of the interaction interface between *E. coli*, immune, and neuronal cells. Changes in the profile of PAs indicate the regulation of inflammation [2], while the accumulation of PEs suggests lipid metabolism rewiring.

* Correspondence: gabriela.lokocova@biomed.cas.cz

REFERENCES:

1. Klein R. et al.: *Nat Immunol* 18, 132–141 (2017).
2. Lim H.-K. et al.: *J Biol Chem.* 278(46):45117-27 (2003).

ACKNOWLEDGEMENT:

Czech Science Foundation: 22-06771S.

FrO-21: Animal Behaviour Through the Lens of Mass Spectrometry

Klára Švihovcová^{1*}, Filip Štrach¹, David Kahoun¹, Roman Vodička², Martin Moos³,
Petra Berková³, Stanislav Opekar³, Martina Konečná¹, František Vácha¹

1. Faculty of Science, University of South Bohemia in České Budějovice, Branišovská 1760, České Budějovice 37005, Czech Republic
2. The Prague Zoological Garden, U Trojského zámku 3/120, 171 00 Prague, Czech Republic
3. Biology Centre, Czech Academy of Sciences, České Budějovice 37005, Czech Republic

Efforts to protect endangered species require an understanding of their behaviour, which has traditionally been achieved through observation, but can be enhanced by new techniques that allow its study at the molecular level. One of the objectives of this project was to develop an LC-MS/MS method for the determination of estrone, estradiol, progesterone, cortisol and corticosterone in faecal samples of *Hyaena brunnea* to elucidate the estrous cycle of females. However, the method in its primary design was successfully validated only for the determination of estrone, estradiol and progesterone with MQL in the range of 10-50 ng/g. The results showed that hyenas are polyestrous, helping caretakers in optimizing reproductive conditions in captivity.

The study further explores the development of a method for assessing stress and sex hormones in the faeces of *Gorilla gorilla gorilla*, supporting behavioural study on the effects of relocation and group division on stress. Starting from the previously mentioned method, the SPE was added to the sample preparation and three new analytes were added - cortisone, 11 β -hydroxyetiocholanolone, 11-oxoetiocholanolone. The method was successfully validated already for all analytes with MQLs in the range of 8-80 ng/g. However, when analysing a set of 22 samples covering a three-month period, it unfortunately appeared that all analytes were below the MQL. In collaboration with the Biological Centre CAS, a more sensitive triple quadrupole mass spectrometer was used, which newly detected only progesterone. Considering the potential solution options, the next method development strategy has been focused on derivatization of the analytes using the reagent QAO, whose early preliminary results look promising.

* Correspondence: svihok00@jcu.cz

ACKNOWLEDGEMENT:

We extend our sincere gratitude to MVDr. Roman Vodička, Ph.D., for his invaluable advice on the zoological aspects of this project, as well as to the staff at the Prague Zoo for their crucial assistance in sample collection. We are also deeply thankful to Ing. Martin Moos, Ph.D., Ing. Petra Berková, Ph.D., and Mgr. Stanislav Opekar, Ph.D., for facilitating the triple quadrupole measurements, providing the derivatization reagent, and offering their continuous support throughout this project. This work was supported by the Ministry of Education, Youth and Sports of the Czech Republic and by the Student Grant Agency of the Faculty of Science of the University of South Bohemia.

FrO-22: Glycosphingolipidomics: Mass spectrometry profiling of glycosphingolipids in biological samples

Karel Hořejší^{1,2*}, Zuzana Vaňková², Denisa Kolářová², Susann Teneberg³, Chunsheng Jin⁴, Robert Jirásko², Michaela Chocholoušková², Denise Wolrab², Robert Jirásko², Michal Holčápek²

1. *Department of Chemistry, Faculty of Science, University of South Bohemia in České Budějovice*

2. *Department of Analytical Chemistry, Faculty of Chemical Technology, University of Pardubice*

3. *Sahlgrenska Academy, Department of Medical Biochemistry, University of Gothenburg, Sweden*

4. *University of Gothenburg, Sahlgrenska Academy, Proteomics Core Facility, Göteborg, Sweden*

Glycosphingolipids (GSLs) are a diverse and structurally complex class of lipids that play crucial roles in various biological processes, including cellular signaling, recognition, and adhesion. They are integral components of cellular membranes and are implicated in the pathophysiology of numerous diseases, including cancer. The structural diversity of GSLs given by both the hydrophobic and hydrophilic part of the molecule poses significant challenges for their comprehensive analysis. High performance liquid chromatography coupled to mass spectrometry techniques have proven to be the best suited for the detection, structural elucidation, and quantitation of GSLs. Specifically, hydrophilic interaction liquid chromatography (HILIC) coupled with electrospray ionization tandem mass spectrometry (ESI-MS/MS) have enabled detailed structural profiling of GSL species from complex biological matrices such as human plasma/serum or tissues together with the separation of glycan isomers using porous graphitic carbon columns. Despite these advancements, efficient isolation and detection of especially low-abundance GSLs species still remain demanding. Addressing these issues is crucial for high-throughput GSL analysis, which holds promise for advancing our understanding of GSL roles in health and disease and for the development of novel diagnostic and therapeutic approaches.

* Correspondence: khorejsi@prf.jcu.cz

REFERENCES:

1. Horejsi K. et al.: *Metabolites* 11(3), 140 (2021).
2. Horejsi K. et al.: *J. Biol. Chem.* 299(3), 102923 (2023)
3. Horejsi K. et al.: *Trac-Trends. Anal. Chem.* 178, 117827 (2024)

ACKNOWLEDGEMENT:

This research was supported by the Czech Science Foundation (GAČR, grant No. 18-12204S, No. 21-20238S), the Czech Health Research Council (No. NU21-03-00499), the Swedish Cancer Foundation (No. 20 0759 PjF 01 H), and the Ministry of Education, Youth and Sports, Czech Republic (project OP JAK No. JA344644).

FrO-23: MALDI MSI Reveals Brain Region-Specific Changes in Lipids as Mediators of Inflammation in an Experimental Model of Bacterial Infection of the Central Nervous System

Jan Šlauf^{1*}, Gabriela Lokočová¹, Miloš Petřík², Andrea Palyzová¹, Vladimír Havlíček¹, Dominika Luptáková¹

1. Institute of Microbiology of the Czech Academy of Sciences, Vídeňská 1083, 14200 Prague 4, Czechia

2. Institute of Molecular and Translational Medicine, Palacky University, Olomouc, Czechia

Lipids play critical role in redox homeostasis, energy storage, cell signaling, and the induction and resolution of acute and chronic inflammation [1]. Bacterial invasion to the CNS induces an inflammatory response leading to neuronal injury. Using MALDI MSI, this study reveals brain region-specific lipid changes underlying the mechanisms of CNS infection.

Neuroinfection was induced in immunocompetent Lewis rats by intracranial injection of 5000 *E. coli* 5172 suspension. Collected brains (5 infected, 5 control brains) were cryosectioned coronally at 12 μm at the -0.72 mm level from bregma. Tissues were sprayed with an internal standard solution of deuterated lipids followed by 1,2-DAN matrix (7 mg/mL in 70% ACN) using M3+ sprayer. Data were collected in negative ion mode using 12T XR-2 ω FTICR solariX and subsequently visualized and processed using SCiLS Lab software. CNS infection induced changes in lipid distribution in white and gray matter, particularly in the corpus callosum. The most significant changes common to all regions were found in fatty acids, mainly arachidonic acid (m/z 303.2327), palmitic acid (m/z 255.2329), oleic acid (m/z 281.2489) and stearic acid (m/z 283.2641), cyclic glycerophosphatidic acids (CPA), e.g. CPA (16:0) (m/z 391.2253), CPA (18:0) (m/z 419.2562) and CPA (18:1) (m/z 417.241), and lysophosphatidic acids (LPA), e.g. LPA (18:0) (m/z 437.367). Certain long-chain sulfated hexosylceramides with polyunsaturated chains (SHexCer), e.g. SHexCer (d40:1) (m/z 862.6082), SHexCer (d41:1) (m/z 876.6254), and SHexCer (t40:2) (m/z 876.5891), were significantly upregulated in corpus callosum.

E. coli induced a complex cascade of host lipidome changes that may be critical signaling mediators of infection-induced neuronal injury.

* Correspondence: janslauf1@seznam.cz

REFERENCES:

1. Hornburg, D. et al.: *Nat Metab* 5, 1578–1594 (2023).

ACKNOWLEDGEMENT:

Czech Science Foundation: 22-06771S.

FrO-24: Quantification of Lipid Fatty Acids by Transmethylation, Isotope-Coded Derivatization and GC-PICI-MS Analysis

Lucie Řimnáčová^{1*}, Petr Vodrážka¹, Stanislav Opekar¹, Petra Berková¹, Helena Zahradníčková¹, Petr Šimek¹, Martin Moos^{1,2}

1. Laboratory of Analytical Biochemistry and Metabolomics, Institute of Entomology, Biology Centre

2. Department of Applied Chemistry, University of South Bohemia

Fatty acids (FAs) are essential components of lipids and an important source of energy for organisms. Polyunsaturated fatty acids (PUFA), especially omega-3, -6 and -9, play a key role in the structure of cell membranes, immune function and the development of the nervous system. The determination of the omega-3 index (DHA and EPA levels in the blood) is an important medical indicator of health status and is usually measured by GC-MS quantification of transmethylated fatty acyl methyl esters (FAME) using one or more appropriate internal standards. In this study, we show that GC-PICI-MS in combination with isotope-coded derivatization (ICD) is an efficient method for the determination of lipid-bound FAs. Internal FAME standards with a D3 mass shift were synthesized by esterification of FA standards with D3-labeled methyl chloroformate and mixed with FAME samples prepared by transmethylation of lipid extracts with sodium methoxide. The method was investigated by FAME analysis in urine using GC-MS with electron ionization (EI) and chemical isobutane ionization (PICI-MS). The latter PICI-MS proved to be advantageous, especially for PUFA analysis. While EI-GC-MS produces complex fragment mass spectra due to double bond migration, PICI-GC-MS yields intense pseudomolecular [M+H]⁺ ions and a distinct +3 mass shift in D3 standards, allowing robust quantification of FAME against FAME-D3 standards in biological samples.

The new GC-PICI-MS method has been validated in accordance with established FDA and EIA guidelines. The method was successfully applied for the analysis of 37 FAs in dried blood spots, human serum and bird semen. These results underline the importance of the GC-PICI-MS technique for the future quantification of lipid FAs, especially in complex biological matrices.

* Correspondence: rimnacova@bclab.eu

FrO-25: Feature-Based Molecular Networking Guided Discovery and Isolation of Novel Capsaicinoids from the chili pepper (*Capsicum* sp.) and their TRPV1 activation

Joshua Smith^{1,2}, Vendula Stillerova³, Martin Dračinský¹, Hannah Gaustad¹, Quentin Lorenzi¹, Helena Smrčková¹, Jakob Reinhardt⁴, Marjorie Lienard^{5,6}, Pavel Šácha¹, Tomáš Pluskal^{1*}

1. Institute of Organic Chemistry and Biochemistry CAS
2. First Faculty of Medicine Charles University
3. CZ-OPENSSCREEN
4. Chemistry & Chemical Biology of Northeastern University
5. GIGA Institute
6. Broad Institute

Chilis contain capsaicin, a renown molecule in both science and cooking. Capsaicin's target protein, the transient receptor potential cation channel subfamily V member 1 (TRPV1), has been linked to many post-activation metabolic effects including changes in metabolism and pain sensation. Beneficial effects of TRPV1 activation via capsaicin have been explored through clinical trials in the form of dietary supplements or as analgesic creams or patches. Capsaicinoids, other than capsaicin, also bind to the target, TRPV1, but current studies often disregard non-capsaicin interactions. To fill in these gaps, we screened 40 different chili varieties derived from 4 *Capsicum* species with untargeted metabolomics and a rTRPV1 calcium influx activation assay. Capsaicinoid profiles were specific to each variety and not fully consistent in each of the species. Based on TRPV1 activation from crude chili extracts, capsaicinoids act in a synergistic manner and a capsaicinoid profile can gauge this activation. In addition, we isolated 18 capsaicinoids, with 5 new-to-nature molecular structures. 16 of the 18 structures were confirmed with NMR and the other two with MS/MS in combination with NMR structure confirmation of its isolated analog. We tested TRPV1 activation by 23 capsaicinoids and 3 related compounds. We found most deviation from the capsaicin structure reduces potency, with a single hydroxylation on the acyl tail reducing potency to TRPV1 by over 100-fold. Lastly, we tested non-activating analogs and weakly activating capsaicinoids in combination with capsaicin on TRPV1 activity. We found that non-activating analogs act as a positive modulator to capsaicin TRPV1 compounds. In addition, weakly activating capsaicinoids also act as positive modulators but to a lesser extent.

* Correspondence: tomas.pluskal@uochb.cas.cz

ACKNOWLEDGEMENT:

J.D.S. was supported by the Charles University Grant Agency (GA UK) and the First Faculty of Medicine of Charles University, project ID: 252182 376322 Smith. T.P. was supported by the Czech Science Foundation (GA CR) grant 21-11563M and by the European Union's Horizon 2020 research and innovation programme under Marie Skłodowska-Curie grant agreement No. 891397. V.T.S. was supported by the Ministry of education and health of the Czech republic (LM2023052). We want to greatly thank The Botanical Garden of the Faculty of Tropical AgriSciences, the Czech University of Life Sciences Prague (CZU) for donation of the chilis and the continuous collaboration. Members of the Pluskal lab were also a great help in this project, in particular Teo Hebra for the continuous support and Roman Bushuiev for the help with Python scripts.

WeS-01: Removing contaminating ions in Q0 region to increase robustness of MS analyzer

Tomáš Korba ¹*

1. AMEDIS

The role of the interface in mass spectrometer with atmospheric ionization is to remove neutral particles and focus as many ions as possible into mass analyzer. Beside ions of interest, also ions of background are focused. In triple quadrupole analyzer (QQQ) quadrupole 1 (Q1) selects ions that continue to collision cell (Q2) and quadrupole 3 (Q3). Unused ions are discharged by collisions with Q1 causing a deposit of contamination. This leads to the decrease of performance, especially the sensitivity.

Novel bandpass filter, Mass Guard, in SCIEX Triple Quad 7500+, allows to remove unwanted ions already in the interface region, namely quadrupole 0 (Q0). T shape electrodes are inserted between rods of Q0. Applied voltage adds octapole component to the radial trapping field leading to the destabilization of bands of ions outside of the range of interest.

The outcome is improved robustness when working with complex matrices. Cleaning of analyzer parts is less frequent.

* Correspondence: korba@amedis.cz

WeS-02: Mapping Chemical Space Sampled by Three Passive Samplers in Wastewater Effluent Using LC-HRMS and Suspect Screening

Pavla Fialová ^{1*}, Kateřina Šverclová ², Kateřina Grabicová ¹, Mats Tysklind ³, Branislav Vrana ², Roman Grabic ¹

1. University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters
2. Masaryk University, Faculty of Science, RECETOX
3. Umeå University, Department of Chemistry

Passive sampling is a valuable tool for monitoring micropollutants in water, providing time-weighted average concentrations over a sampling period and enabling the detection of low-level contaminants. Combining passive sampling with non-targeted analysis (NTA) or suspect screening broadens the chemical space of identified compounds. This study aims to map the chemical space captured by three passive samplers deployed in wastewater effluent using suspect screening and chemical space modeling. The samplers, with different constructions and sorbents, were deployed side-by-side in the treated effluent of a municipal wastewater treatment plant (500,000 pe) in Brno (Modřice), Czech Republic. Extracts were analyzed by liquid chromatography-high resolution mass spectrometry (LC-HRMS) with full scan and data-independent MS/MS acquisition in both ionization modes. Data were processed in Compound Discoverer 3.3 and screened against an in-house library of over 1500 compounds including pharmaceuticals, pesticides and biocides. Suspect hits were manually processed, and confidence of compound identification was assigned according to the Schymanski scale [1]. Compounds identified at level 1 (confirmed structure by reference standard) and level 2 (probable structure by library spectrum match) were used for chemical space mapping. Suspect screening identified 127 compounds, 66 of which overlapped with the 155 detected by target analysis, allowing a more accurate assessment of the sampled chemical space. All three samplers provided similar chemical space coverage. Combining passive sampling with suspect screening and chemical space modeling offers enhanced insights into the sampled chemical space, providing valuable information for using passive samplers in water environmental monitoring.

* Correspondence: fialovap@frov.jcu.cz

REFERENCES:

1. Schymanski, E.L. et al.: *Environ Sci Technol* 48, 2097–2098 (2014).

ACKNOWLEDGEMENT:

The authors acknowledge the Czech Science Foundation grant No. GACR 20-04676X “Holistic exposure and effect potential assessment of complex chemical mixtures in the aquatic environment”. This work was carried out with the support of VVI CENAKVA Research Infrastructure (ID 90238, MEYS CR, 2023–2026), The authors also thank Research Infrastructure RECETOX RI (LM2023069) financed by The Ministry of Education, Youth and Sports, and Operational Programme Research, Development and Education – project CETOCOEN EXCELLENCE (No CZ.02.1.01/0.0/0.0/17_043/0009632) for supportive background.

WeS-03: Assessing Pesticide Levels in Soils of the Czech Republic: Methods and Findings

Alina Sadchenko ^{1*}, Radka Kodešová ², Ganna Fedorova ¹, Helena Švecová ¹, Aleš Klement ², Miroslav Fér ², Antonín Nikodem ², Roman Grabic ¹

1. University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters

2. Czech University of Life Sciences Prague, Faculty of Agrobiolology, Food and Natural Resources

In modern agriculture, pesticides are vital for pest, weed, and crop management but carry environmental and health risks. Monitoring pesticide levels in food, water, and soil is crucial. While mass spectrometry coupled with chromatography enables multi-residue quantification, the constantly evolving list of pesticides and their transformation products (TPs) requires broader analysis beyond targeted methods.

Within this study, we analyzed soil samples collected from 14 agricultural fields used for planting vegetables in the Czech Republic in July 2023. We aimed to identify the specific pesticides present and determine their levels in these complex matrices.

Initially, a list of 110 pesticides and their TPs found in surface and groundwater was analyzed with the targeted LC/QqQ method, revealing that 73 compounds exceeded the limit of quantification in at least one soil sample. We then employed non-targeted liquid chromatography high-resolution mass spectrometry (LC-HRMS) with full-scan data-independent MS² (DIA) to identify unknown pesticides and their TPs. In total, 75 compounds were identified at Szymanski level 2, and 28 overlapped the targeted analysis. In the next phase, 23 routinely quantified compounds were added to the list of relevant substances, together with 45 pesticides identified through non-targeted screening. The resulting quantification method was validated and applied for more focused research on the fate of the selected pesticides in soil/plant/water systems.

Combining targeted and non-targeted mass spectrometric methods provides valuable insights for pesticide management and environmental protection while reducing costs by eliminating the need for expensive broad multi-residual standards.

* Correspondence: asadchenko@frov.jcu.cz

ACKNOWLEDGEMENT:

The study was conducted within the project of the Ministry of Agriculture of the Czech Republic, National Agency for Agricultural Research (project no. QK23020018).

WeS-04: Transitioning from User-Based Applications to Cloud Computing: The Python-Based COVAIN v2.0 Toolbox for Metabolomics Data Analysis on Google Colab

Jana Schwarzerová ^{1,2,6}*, Jiahang Li ³, Jakub Idkowiak ^{4,5}, Steffen Waldherr ², Wolfram Weckwerth ^{2,5}

1. *Department of Biomedical Engineering, Faculty of Electrical Engineering and Communication, Brno University of Technology, Technická 12, 616 00 Brno, Czech Republic*
2. *Molecular Systems Biology (MOSYS), University of Vienna, Vienna, Austria*
3. *School of Mathematical Sciences, Nankai University, Tianjin 300071*
4. *University of Pardubice, Faculty of Chemical Technology, Department of Analytical Chemistry, Studentská 573, 532 10 Pardubice, Czech Republic*
5. *Vienna Metabolomics Center (VIME), University of Vienna, Vienna, Austria*
6. *Department of Molecular and Clinical Pathology and Medical Genetics, University Hospital Ostrava, Ostrava, Czech Republic;*

With the increasing demand for more flexible and scalable tools for metabolomics data analysis, the transition from desktop-based software like MATLAB to cloud-based platforms such as Google Colab has become a key focus. In this study, we present COVAIN 2.0, a comprehensive toolbox for metabolomics data processing in a Python-based environment, enabling broader accessibility and collaborative use through Google Colab.

The newly encoded COVAIN toolbox offers a powerful suite for uni- and multivariate statistical analysis, time-series analysis, and correlation network analysis, as well as advanced methods for inverse estimation of the differential Jacobian from metabolomics covariance data. The python-based toolbox ensures greater flexibility in the analysis pipeline. A key advantage of this approach is the integration with Colab's cloud infrastructure, which allows users to run computationally intensive tasks. Users can access the toolbox directly through a web browser, facilitating collaborative research and sharing of workflows across different platforms.

In addition to basic and advanced preprocessing features, the COVAIN 2.0 also supports dynamic visualizations, clustering techniques and integration of multiomics data. The transition from MATLAB to Python via Google Colab is based on several GitBook resources for Omics Data Visualization in R and Python. Our work offers an all-in-one Google Colab notebook where users can directly access pre-implemented scripts, allowing for faster and easier use.

COVAIN 2.0 in Google Colab not only broadens accessibility for users but also enables integration with popular data science libraries like Pandas, NumPy, SciPy, and Matplotlib, making the toolbox more adaptable to future research needs.

* Correspondence: Jana.Schwarzerova@vut.cz, wolfram.weckwerth@univie.ac.at

WeS-05: Comprehensive study of the effect of oat grain germination on the content of avenanthramides

Michal Jágr^{1*}, Andreas Hofinger-Horvath², Petra Hlásná Čepková¹, Regine Schönlechner², Václav Dvořáček¹

1. Výzkumný ústav rostlinné výroby

2. University of Natural Resources and Life Sciences

Avenanthramides (AVNs) are a group of phenolic compounds found exclusively in oats, and in processed oat products. AVNs are found to provide many health benefits in mammals (including humans), such as anti-oxidation, anti-inflammation, anti-atherosclerosis, anti-diabetic, anti-hypertensive, and anti-cancer properties(1). The chemical profile and the levels of AVNs in oat varieties after germination have been examined. In the present study, 12 distinct oat varieties were germinated for 0–192 h and a total of 28 AVNs and 3 AVN-hexosides were determined in these samples. Among them, three novel AVNs were synthesized (AVN 1a, AVN 2a, and AVN 2ad), characterized using NMR techniques (1D- and 2D-NMR), and assessed in real samples for the first time. The most abundant AVNs in the samples were AVN 2c, AVN 2p, AVN 2f, and their long-chained analogues AVN 2 cd, AVN 2pd, AVN 2fd, together representing 75–85 % of the total AVNs content. The highest total AVN level was observed on average after 48–72 h of germination time and it reached a value 1–1.2 mg/g (2).

* Correspondence: jagr@vurv.cz

REFERENCES:

1. Wise M. L. In: Chu Y ed. *Oats Nutrition and Technology*. John Wiley& Sons. Ltd., pp. 195–226 (2014).
2. Jágr M. et al.: *Food Chem.* 437, 137807 (2024).

ACKNOWLEDGEMENT:

This work was financed by the project NAZV QK1810102 of the National Agency for Agricultural Research of the Ministry of Agriculture of the Czech Republic, and partially funded by the Ministry of Agriculture of the Czech Republic, institutional support MZE-RO0423. This work was also financed by the Czech Science Foundation Grant 21- 10845S, and by the Ministry of Agriculture of the Czech Republic, Subsidy Programme - the National Program for the Conservation and Use of Plant Genetic Resources and Agrobiodiversity (no. MZE-62216/ 2022-13113/6.2.14). The cooperation of the Austrian and Czech project team was financially supported by the Austriás Agency for Education and Internationalisation, programme WTZ (project Nr. CZ 01/2022) and by the project 8J22AT020 (IK 1702) of the Czech Ministry of Education Youth and Sports.

WeS-06: Fast photochemical oxidation of protein: data-independent workflow and impact of the peptide identification framework

Dmitry Loginov ^{1*}, Marek Zakopcanik ^{1,2}, Daniel Kavan ¹, Petr Novak ¹

1. *Institute of Microbiology, CAS*

2. *Department of Biochemistry, Faculty of Science, Charles University*

Structural mass spectrometry methods, including FPOP, are crucial for studying protein folding and interactions. Accuracy of assignment of modification sites is vital for FPOP analysis. This is achieved using different search engines with varying techniques for spectrum identification and scoring systems. In this study, we assessed the reliability of peptide identifications from Mascot and PEAKS X+ search engines and evaluated the potential of spectral libraries combined with data-independent acquisition (DIA) to enhance precision in assigning FPOP modifications.

Comparing Mascot and PEAKS X+ using ProteomeXchange dataset PXD021621, we found that the choice of search engine significantly influences the assignment of FPOP modifications to detected peptides, with only 31% of overlapping identifications. PCA revealed distinct clusters of modified peptides based on score, site probabilities, and intensities. Mascot demonstrated more confident site determination, correlating well with fragmentation spectrum quality and assigned site confidence resulted in less manual validation time.

We propose an innovative approach that integrates validated spectral libraries with DIA data. Initially, data-dependent acquisition data processed by FragPipe is validated using Skyline software to create a spectral library. This library is then applied to filter identifications from the DIA dataset. Out of 421 accepted identifications in the model HbHp dataset, 346 contained FPOP modifications. Comparison with manually validated library results and Mascot searches revealed that the library offers a broader range of unique modifications, improved spatial resolution, and enhanced coverage of biologically relevant regions of protein complexes.

* Correspondence: dmitry.loginov@biomed.cas.cz

ACKNOWLEDGEMENT:

This work was supported by Czech Scientific Foundation (22-27695S), Technology Agency of the Czech Republic (ODEEP-TH86010001) and by Programme Johannes Amos Comenius Photomachines (CZ.02.01.01/00/22_008/0004624).

WeS-07: Ion Exchange Trapping: A Solution for Excess Derivatization Reagent Removal

Michal Baka ^{1,2*}, Stanislav Opekar ¹, Martin Moos ¹, Petr Šimek ¹

1. *Czech Academy of Sciences, Biology Centre, Laboratory of Analytical Biochemistry and Metabolomics, B*

2. *Department of Food Analysis and Nutrition, Faculty of Food and Biochemical Technology, University of*

Derivatization is a common technique in the sample preparation step prior to mass spectrometric (MS) analysis, as it can improve analytical properties involving detectability, stability and particularly quantification of analytes in matrices. Derivatization offers distinct advantages, it has certain limitations. A major problem is introduction of the reagent excess in the sample, which often contributes to additional matrix effect and thus ameliorate validation of the developed analytical method. The removal of the reagent and its products expect the analytes is an unpopular measure, because it is not straightforward, laborious and, as a result, some purification step, such as liquid-liquid microextraction (LLME) or solid phase extraction on cartridges (SPE) is required. The sample preparation process involving simultaneous removal of the interfering components during the sample preparation process is therefore highly desired.

This study addresses this issue and its impact on analytical performance of quantification reactive aldehydes, such as 4-hydroxynonenal (4-HNE) or malondialdehyde (MDA) in human serum by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Quaternary aminoxy (QAO) reagents, introduced in 2012 [1], are also suitable for the derivatization of aldehydes but removal of the reagent is difficult to achieve with LLME or SPE. Several derivatization reagents (2QAO, 3QAO, QDA and QHDA) reacted with the tested aldehydes and formed stable oximes. The key to removing the excess derivatization reagent was chemical modification of the derivatization reagent followed by ion exchange trapping. The results of LC-MS analysis showed that the proposed method effectively removed the excess reagent and significantly improved the quality of the data obtained.

* Correspondence: michal.baka@bclab.eu

REFERENCES:

1. *Star-Weinstock, M., Williamson, B. L., Dey, S., Pillai, S., & Purkayastha, S. (2012). LC-ESI-MS/MS Analysis of Testosterone at Sub-Picogram Levels Using a Novel Derivatization Reagent. Analytical Chemistry, 84(21), 9310–9317. doi:10.1021/ac302036r*

ACKNOWLEDGEMENT:

This work was supported by the Czech Science Foundation (project GAČR, No. 23-06600S).

WeP-01: Targeted profiling of serum oxysterols by UHPLC–MS/MSMichal Kaleta^{1,2*}, Jana Oklešťková¹, Miroslav Kvasnica¹, Ivan Petřík¹, Ondřej Novák¹1. *Laboratory of Growth Regulators, Palacký University and IEB CAS, Olomouc*2. *Department of Neurology, Faculty of Medicine and Dentistry, Palacký University, Olomouc*

Oxysterols are a significant group of oxygenated cholesterol derivatives believed to naturally regulate cellular signaling, exhibit immunomodulatory properties, and play physiological roles in processes such as steroid hormone biosynthesis, fatty acid metabolism, and cholesterol homeostasis. They are also likely involved in the pathogenesis of various cardiovascular (e.g., atherosclerosis), neurodegenerative (e.g., Alzheimer's disease), oncological (e.g., breast, lung, stomach cancers), and other diseases. Oxysterol production in the human body primarily occurs through intracellular enzymatic oxidation of cholesterol but can also result from non-enzymatic auto-oxidation. However, comprehensive studies on oxysterol metabolism require reliable analytical approaches. This work presents a high-performance method based on time-efficient sample preparation and sensitive detection using ultra-high-performance liquid chromatography coupled with tandem mass spectrometry (UHPLC–MS/MS), enabling the metabolic profiling of several target oxysterols in human serum. The key sample preparation step involves the precipitation of serum proteins, followed by filtration to remove them. High-efficiency chromatographic separation on a reverse phase allows for simultaneous profiling of ten metabolically related oxysterol compounds in a single experiment, even from serum samples as small as a few microliters. The developed and validated method holds potential for use in targeted metabolomic studies aimed at evaluating changes in oxysterol metabolic pathways under various physiological or pathological conditions. The insights gained could contribute to the discovery of biomarkers for disease prevention, diagnosis, or monitoring progression, as well as improving therapeutic strategies.

* Correspondence: michal.kaleta@upol.cz

REFERENCES:

1. Borah K. et al.: *Redox Biol.* 36, 101595 (2020).
2. Chen L. et al.: *Chromatographia* 82, 553–564 (2019).
3. de Freitas F. A. et al: *Cells* 11(8), 1251 (2022).

ACKNOWLEDGEMENT:

This work was financially supported by the Czech Science Foundation (project GA23-05389S).

WeP-02: DIGging into the DIGestion

Jasmína Portašiková ^{1,2*}, Zuzana Kalaninová ^{1,2}, Daniel Kavan ¹, Petr Novák ¹, Petr Man ¹

1. Institute of Microbiology, Czech Academy of Sciences, BioCeV, Prague, Czech Republic

2. Charles University, Faculty of Science, Prague, Czech Republic

Protein digestion is an essential tool in both classical bottom-up proteomics and structural proteomics approaches such as chemical cross-linking, radical labelling or hydrogen/deuterium exchange. While proteomics uses specific proteases with predictable digestion patterns and minimal missed cleavages, HDX relies on non-specific or semi-specific acidic proteases. These are highly promiscuous in terms of amino acid preferences and tend to generate a lot of overlaps. Although this hampers predictability and adds complexity, it also has several advantages, such as a higher number of experimental data points and increased spatial resolution. As more proteolytic columns become available, the need for rigorous methods to compare digestion results increases. To meet these needs, we have developed a Java-based software tool that takes the exported raw search results (e.g. csv from MASCOT) and the corresponding database and performs an instant extraction of the key digestion parameters. This tool, called DigDig, provides digestion metrics, reproducibility assessment, coverage maps, extraction of cleavage preferences up to the N-th position, peptide length distribution, missed cleavage analysis and data export for IceLogo. It works with single proteins or complex mixtures, where it can plot data for either selected protein(s) or the entire identified ensemble. We will show how this software has helped us to identify novel cleavage preferences, optimise the HDX-MS workflow, but also its applicability in the evaluation of specific proteases, the key tool of the proteomic pipeline.

* Correspondence: portasij@natur.cuni.cz

ACKNOWLEDGEMENT:

Financial support from the MEYS/EU OP JAK project PHOTOMACHINES - CZ.02.01.01/00/22_008/0004624 is gratefully acknowledged.

WeP-03: In-situ enrichment of serum proteins on functionalized immunoaffinity chips

Josef Dvořák ^{1*}, Ljubina Adámková ¹, Dmitry Loginov ¹, Petr Pompach ², Michael Volný ¹, Petr Novák ¹

1. Institute of Microbiology, The Czech Academy of Sciences, Prague, Czech Republic

2. Institute of Biotechnology, The Czech Academy of Sciences, Věstec, Czech Republic

Mass spectrometry is an indispensable tool for identifying disease biomarkers. While immunohistochemistry and immunoassays remain the standard of clinical protein analysis, limitations in standardization and reproducibility hinder them. Immunoaffinity chips represent a promising alternative. These chips utilize ion soft landing technology (AISL), which allows conductive surface modification with biologically active proteins, e.g., protein G, A, or L, for a non-covalent immobilization of antibodies specific to target proteins. The immunoaffinity chips were used to quantify serotransferrin and haptoglobin, acute phase proteins relevant to diagnosing anemia, hemolysis, and others.

The chips were prepared by depositing protein G (or L or A) onto a 96-well plate using AISL. Subsequently, antibodies specific to haptoglobin, serotransferrin, or isotype control were immobilized on the modified chips. Human serum was then incubated on the chip to achieve enrichment of the desired biomarker. In the following, bottom-up proteomic analysis of the enriched serum fraction was performed on the Bruker timsTOF SCP in the data-dependent acquisition mode and statistically evaluated using Perseus software.

The in situ enrichment method was successfully optimized for detecting and quantifying human plasma/serum biomarkers. The chips modified with protein G effectively enabled the detection of haptoglobin and serotransferrin. In this study, we lay the foundation stone for a multipurpose protein chips assay that is compatible with various antibodies. In addition, compatibility with pipetting robots and LC-MS systems (Evosep) represents a potential to transfer the enrichment protocol into the high throughput scale for efficient and automated patient sample analysis in clinical labs.

* Correspondence: dvorakjos@natur.cuni.cz

REFERENCES:

1. Jia Y, Wood F, Buehler PW, Alayash AI (2013) Haptoglobin Preferentially Binds β but Not α Subunits Cross-Linked Hemoglobin Tetramers with Minimal Effects on Ligand and Redox Reactions. *PLoS ONE* 8(3): e59841.
2. Huttlin EL, Bruckner RJ, Paulo JA, Cannon JR, Ting L, Baltier K, Colby G, Gebreab F, Gygi MP, Parzen H, Szpyt J, Tam S, Zarraga G, Pontano-Vaites L, Swarup S, White AE, Schweppe DK, Rad R, Erickson BK, Obar RA, Guruharsha KG, Li K, Artavanis-Tsakonas S, G

ACKNOWLEDGEMENT:

We acknowledge support from Talking Microbes - understanding microbial interactions within the One Health framework (CZ.02.01.01/00/22_008/0004597).

This work was mainly supported by the research grants NW24-09-00464 provided by the Czech Health Research Council. This research has been also supported by the Ministry of Education, Youth and Sports of the Czech Republic grant Talking microbes - understanding microbial interactions within One Health framework (CZ.02.01.01/00/22_008/0004597).

WeP-04: Visualization of Secretion Kinetics in Microbes by CycloBranch

Jiří Novák^{1,2*}, Jiří Houšť^{1,3}, Dominika Luptáková¹, Vladimír Havlíček^{1,3}

1. Institute of Microbiology of the CAS, v.v.i., Vídeňská 1083, 142 00 Prague 4

2. Faculty of Information Technology, CTU in Prague, Thákurova 9, 160 00 Prague 6

3. Faculty of Science, Palacký University, 17. listopadu 12, 771 46 Olomouc

CycloBranch is an open-source and cross-platform tool focused on the annotation of high-resolution mass spectra of natural products (<https://ms.biomed.cas.cz/cyclobranch/>). The tool supports multiple functions such as the annotation of product ion mass spectra of nonribosomal peptides having cyclic, branched, and branch-cyclic structures [1], the database and database-free annotation of fine isotopic structures of natural products in liquid chromatography/mass spectrometry (LC-MS) and imaging mass spectrometry datasets [2], and quantification of small molecular weight natural products in LC-MS datasets [3]. Here, we show how CycloBranch can be used to study small molecular weight natural products in the kinetics of *in vitro* experiments using a sample dataset of siderophores produced by microorganisms. The biological samples were collected at several different time points, and LC-MS datasets were recorded by a 12T Solarix FT-ICR mass spectrometer. The latest CycloBranch version can visualize kinetics graphs and calculate descriptive statistics. It has also been optimized, making the annotation of novel compounds significantly faster.

* Correspondence: jiri.novak@biomed.cas.cz

REFERENCES:

1. Novák J. et al.: *J. Am. Soc. Mass Spectrom.* 26(10), 1780-1786 (2015).
2. Novák J. et al.: *Anal. Chem.* 92(10), 6844-6849 (2020).
3. Novák J. et al.: *Eur. J. Mass Spectrom.* 29(2),102-110 (2023).

ACKNOWLEDGEMENT:

This work was supported by the Czech Science Foundation project 22-06771S.

WeP-05: HILIC as an alternative separation method for RNA-caps and short oligonucleotides analysis

Anton Škríba¹, Hana Cahová^{1*}

1. *Ústav organické chemie a biochemie AV ČR, v. v. i.*

Recent expansion of RNA chemical modifications field opened new questions regarding post-transcriptional gene regulation. Apart from internal modifications, in our group we mostly focus on 5'-RNA caps. These caps are typically derived from various metabolites such as cofactors [1] and dinucleotide polyphosphates [2]. Although their presence is believed to influence RNA stability, cellular metabolism, and mRNA translation, their exact role remains poorly understood. The physicochemical properties of RNA caps, which include high hydrophilicity, acidic phosphate functional groups, and nucleobases, make their liquid chromatography mass spectrometry (LC-MS) detection challenging. Currently, these molecules are analyzed by reversed-phase chromatography with ion-pairing agents, such as alkylammonium salts. This technique is well established in the oligonucleotides field, however the presence of high salt concentration suppresses the ionization and lowers the sensitivity of mass spectrometry detection. We have employed an alternative method - HILIC (hydrophilic interaction chromatography), which does not need such strong ion-pairing agents and can be used even for analysis of longer oligonucleotides [3].

This work presents an overview of HILIC chromatography used in qualitative and quantitative analysis of canonical and non-canonical 5'-RNA caps in bacteria and mammalian cells. As examples, we present application in detection of NAD cap in RNA from *Bordetella pertussis* bacteria, hypermethylation of canonical cap in small nuclear RNA and oligonucleotides analysis in viroid.

* Correspondence: hana.cahova@uochb.cas.cz

REFERENCES:

1. Cahova, H., Winz, M. L., Hofer, K., Nubel, G. and Jaschke, A. (2015), *Nature*, 519, 374.
2. Hudeček, O., Benoni, R., Reyes-Gutierrez, P.E., Culka, M., Šanderová, H., Hubálek, M., Rulíšek, L., Cvačka, J., Krásný, L. and Cahová, H. (2020), *Nature Comm.*, 11, 1052.
3. Lobue, P.A., Jora, M., Addepalli, B., Limbach, P.A. (2019), *J. Chromatogr. A.*, 1595, 39.

ACKNOWLEDGEMENT:

European Research Council Executive Agency (ERCEA) under the European Union's Horizon Europe Framework Programme for Research and Innovation (ERC starting grant agreement No 101041374-StressRNAction. Project OP JAC CZ.02.01.01/00/22_008/0004575 RNA for therapy, Co-Funded by the European Union and the MEYES.

WeP-06: Lipid dynamics in plasma and extracellular vesicles in the acute adaptive response to aerobic exercise

Dominika Olešová ^{1*}, Aleš Kvasnička ^{2,3}, Martin Schon ¹, Igor Straka ⁴, Zuzana Kosutzká ⁴, Peter Valkovič ⁴, David Friedecký ^{2,3}, Jozef Ukropec ¹, Barbara Ukropcová ¹

1. Institute of Experimental Endocrinology, Biomedical Research Center, Slovak Academy of Sciences

2. Department of Clinical Biochemistry, University Hospital Olomouc

3. Laboratory for Inherited Metabolic Disorders, Faculty of Medicine and Dentistry, Palacký University

4. IIInd Neurology Clinic, Faculty of Medicine Comenius University & University Hospital Bratislava

During exercise, skeletal muscles and adipose tissue communicate with other vital organs through the spectrum of molecules, including myokines and adipokines, metabolites, lipids, and microRNAs. This complex molecular crosstalk helps the body maintain allostasis and possibly contributes to exercise-enhanced neurogenesis and synaptic plasticity. The exact molecular patterns that mediate this communication are still largely unknown.

Blood samples were collected from 12 healthy young physically active adults before, directly after, and 60 min after a 90-min monitored outdoor run at ~75% HRmax. Targeted lipidomic analysis of plasma was carried out by a pseudotargeted approach, using a liquid chromatography-tandem mass spectrometry system consisting of ExionLCTM, and QTRAP® 6500+ for MS/MS (Sciex, USA).

Our results showed that directly after exercise, plasma and PEG-isolated EV lipidome was dominated by elevated fatty acids indicating activation of fatty acid oxidation, and decreased levels of several lysoglycerophospholipids, and phosphatidylethanolamine species. One hour after the run, the levels of fatty acids began to decrease, returning slowly to the baseline levels, while lysophospholipids and phosphatidylethanolamines continued to further decrease. On the other hand, SEC-isolated EVs showed different lipid profiles, dominated by increased glycerophospholipids and lysophosphatidylcholines immediately after run.

These preliminary findings help us elucidate the complex molecular dynamics of the adaptive response to physical activity and the mechanisms behind the health benefits of exercise. Moreover, it highlights that detailed characterization of different isolation approaches is of great importance.

* Correspondence: dominika.olesova@savba.sk

ACKNOWLEDGEMENT:

Grant support: APVV 20-0466, APVV 19-0411, VEGA 2/0076/22, VEGA 2/0164/20

WeP-07: Comprehensive analysis of the redox proteome reveals survival pathways activated upon resistance to Pevonedistat

Michaela Myšáková^{1*}

1. 1. *Lékařská Fakulta, Univerzita Karlova, BIOCEV*

Pevonedistat (PEVO) is a selective inhibitor of neddylation-dependent protein degradation and has been shown to enhance the efficacy of the hypomethylating drug 5-azacytidine (AZA) in leukemia therapy. However, despite promising results, even combination therapy fails due to the emergence of resistance. Our recent data showed that AZA alters redox homeostasis and oxidative modifications of proteins, which further contributed to the selective pressure of AZA. PEVO activates the main antioxidant defense pathway by inhibiting the degradation of nuclear factor erythroid 2-related factor 2 (NRF2), a substrate of ubiquitin ligase Kelch-like ECH-associated protein 1 (KEAP1) and thus also affects cellular redox homeostasis. Redox changes are sensed by protein thiol groups which undergo various oxidative modifications leading to modulation in protein function. Therefore, in the presented work we investigated the impact of PEVO on redox homeostasis and redox proteome with the aim to reveal the role of redox homeostasis in the response of MDS/AML cells to the combined therapy.

We used cell line models of MDS/AML myeloblasts sensitive and resistant to AZA and PEVO developed in our laboratory. We performed quantitative proteomic analysis using TMT10-plex and Orbitrap Fusion Tribrid MS instrument on PEVO-sensitive cells treated with PEVO and PEVO-resistant cells. We identified and quantified 6,886 proteins. PEVO treatment affected the level of 98 proteins, and resistance was associated with changes in levels of 566 proteins. Principal component analysis separated the analyzed groups, with differing levels of NRF2 target proteins contributing most to separating the PEVO-treated sample. Indeed, the activity of NRF2, determined by its accumulation in the nucleus, was significantly increased in the PEVO-treated sample. This was associated with changes in the oxidative state monitored by flow cytometry using fluorescent probes. Interestingly, enhanced activity of NRF2 was detected in untreated PEVO-resistant cells as well. Mass spectrometry-based redox proteomic analysis of reduced and oxidized cysteine thiol groups using iodoTMT isobaric labelling and Orbitrap Fusion Tribrid MS instrument identified 26,265 cysteine-containing peptides from 6,634 proteins and showed that PEVO resistance is accompanied by changes in the redox state of 228 proteins. We identified increased oxidation on cysteines 289 and 290 of Sequestosome-1, previously shown to be important for the autophagic clearance of ubiquitinated proteins.

We have identified prominent modifications in proteins with roles in autophagy, indicating a role in autophagy regulation that could contribute to the adaptive mechanisms leading to the emergence of resistance. Our results indicate that the altered redox state has a significant role in PEVO and AZA resistance.

* Correspondence: mysakovamichaela@gmail.com

WeP-08: Radiolarite artefacts provenance: multimodal elemental analysis

Tomáš Pluháček ^{1 *}, Martin Moník ², Filip Gregar ¹, Zdeňka Nerudová ³, Petr Hamrozi ³, Jitka Součková ¹

1. *Department of Analytical Chemistry, Faculty of Science, Palacky University, Czechia*

2. *Department of Geology, Faculty of Science, Palacky University, Czechia*

3. *Centre for Cultural Anthropology, Moravian Museum, Czechia*

Radiolarites, siliceous sedimentary rocks composed mainly of SiO₂ (chalcedony) and micro-remains of radiolarians (marine plankton), have been considered important raw materials in the Palaeolithic assemblages of Central Europe [1]. It was used predominantly for the manufacture of flaked stone artefacts, which were transported over large distances, thus providing a unique material for studying the mobility of Upper Palaeolithic cultures: the Aurignacian (ca. 42 - 34 thousand years BP) and the Gravettian (ca. 34 - 24 thousand years BP). The multimodal approach combining petrological evaluation, X-ray fluorescence (XRF) and laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) was used to determine the characteristic chemical fingerprint of the radiolarite artefacts. [2] The presented study focused on the mobility of hunters from the Northern Calcareous Alps to the eastern fringes of the Pieniny Klippen Belt. The multimodal approach was applied to 151 radiolarites collected in six different areas, namely: 1. Northern Calcareous Alps (NCA; AUT), 2. St. Veit Klippenbelt in and around Vienna (AUT), 3. Gerecse Mts. (HUN), 4. White Carpathians (SVK), 5. Pieniny Mts. (POL) and 6. Bakony Mts. (HUN), and 2 x 30 radiolarite artefacts from the Aurignacian and Gravettian sites in Moravia (Czech Republic). A statistical method, Linear Discriminant Analysis (LDA), was applied for classification and dimensionality reduction. The categorical response variable consisted of distinct geographical radiolarite groups, while the features included Pb, Ba, Sr, Cu, Mg, Li, As, and Cr. The LDA analyses allowed us to trace radiolarite artefacts from Moravian sites to their source areas: mostly Slovakian/Polish, but in two cases also the Gerecse/Bakony Mts. group. [3]

* *Correspondence:* tomas.pluhacek@upol.cz

REFERENCES:

1. M. Brandl, C. Hauenberger, W. Postl, M. M. Martinez, P. Filzmoser, G. Trnka, *Quaternary International*, 75, 29-40, 2001
2. F. Gregar, M. Moník, P. Hamrozi, J. Součková, Z. Nerudová, T. Pluháček submitted to *Spectrochimica Acta Part B: Atomic Spectroscopy*.
3. M. Moník, Z. Nerudová, F. Gregar, T. Pluháček, J. Součková, P. Hamrozi, submitted to *Archaeological and Anthropological Sciences*.

ACKNOWLEDGEMENT:

The research was funded by Czech Science Foundation project 22-05547S.

WeP-09: Dyeing palette of Czech aristocracy from a mass spectrometric perspective – advantages and pitfalls of a comprehensive UHPLC-HDMS^E method

Volodymyr Pauk ^{1*}, Kateřina Cichrová ², Kateřina Hlavničková ³

1. *Univerzita Palackého v Olomouci*
2. *National Heritage institute, UPS České Budějovice, Department of presentation, installations and exhibitions*
3. *National Heritage institute, State Castle and Chateau Český Krumlov*

The unique collection of historical clothes, from servant liveries to noblemen wedding costumes, from hunting outfits to opera gowns, is hidden in the vaults of Český Krumlov Castle. An unexplored treasure counting over 500 items reflects the luxury yet thoughtful lifestyle of prominent aristocratic houses of the Central Europe, e.g. the Rosenbergs, the Eggenbergs and the Schwarzenbergs. The analysis of those costumes uncovers over half-millennium timespan of developments in textile industry as well as insights into natural dyestuff manufacture or later advances in organic synthesis.

A comprehensive analytical method was developed to deal with immense chemical diversity of encountered dyes and their degradation products, ranging from benzoic acids, indigoids, anthraquinoids, flavonoids and their glycosides, naphthoquinoids, coumarins, polyenes, tannins to early synthetic dyes including triphenylmethane, azo or xanthene derivatives. A two-step extraction protocol involving DMSO and methanol:acetone:water:oxalic acid mixture is followed by UHPLC separation with PDA and MS detection (Waters Acquity I-Class with Synapt G2-S). The full scan data are first screened using an automatic targeted method based on our in-house dye database currently comprising about 130 analytical standards complemented by reference dyeings with natural dyestuffs. Subsequently, HDMS^E mode (data-independent all precursor MS/MS aided by ion mobility separation) can be used for substance confirmation or structure elucidation of unknown dye components.

The discussion of the method performance showcases insufficiency of “limit of detection” as the only indicator of sensitivity in qualitative methods.

* Correspondence: volodymyr.pauk@upol.cz

ACKNOWLEDGEMENT:

The research was financed from the funds of the Programme for the support of applied research in the field of national and cultural identity for the years 2023 to 2030 (NAKI III) of the Ministry of Culture of the Czech Republic within the project “From the Aristocratic Court to the Theatre Stage. Historical Wardrobe in the Collections of Czech Castles and Chateaux in the European Context”(DH23P03OVV046).

WeP-10: Psychoactive Drug Effect on Fish Reproductive System: A Proteomic Perspective

Filip Dyčka^{1*}, Serhii Boryshpolets², Vitaliy Kholodnyy², Anatolii Sotnikov², Ján Štěrba¹, Ganna Fedorova²

1. Faculty of Science, University of South Bohemia, Czech Republic

2. Faculty of Fisheries and Protection of Waters, CENAKVA, University of South Bohemia, Czech Republic

The presence of psychoactive drugs in surface waters poses a global threat to aquatic life. Several studies have reported behavioral changes in fish as a result of exposure to these compounds [1]. These substances interact with neurotransmitters and neuroreceptors in neuron cells, which are well-conserved across species. This indicates that psychoactive drugs may affect fish through mechanisms similar to those in humans [2]. Neurotransmitter receptors are not only crucial for brain function but also play an integral role in activating signal transduction pathways in sperm. The presence of neuroreceptors in fish sperm and gonads suggests that psychoactive drugs could impact reproductive success.

Our study aims to investigate the presence of neurotransmitters in the reproductive tissues of fish. Additionally, we examined the effect of the psychoactive drug methamphetamine on the fish reproductive system. In this work, sperm and gonad proteins of *Perca fluviatilis* were identified using nanoLC-timsTOF-MS/MS. Functional analysis revealed that the most abundant proteins are involved in cytoskeleton organization, with a significant number also participating in signaling and nervous system processes. Several neurotransmitter receptors, including acetylcholine, muscarinic, and gamma-aminobutyric acid (GABA) receptors, were identified in the fish sperm and gonad proteome, consistent with previous studies on mammalian sperm [3]. However, these receptors were found in very low protein abundance.

* Correspondence: fdycka@prf.jcu.cz

REFERENCES:

1. Hossain M. S. et al.: *Sci Total Environ.* 751, (2021).
2. Gunnarsson L. et al.: *Environ Sci Technol.* 42(15), 5807-5813 (2008).
3. Meizel S.: *Biol Rev Camb Philos Soc.* 79(4), 713-732 (2004).

ACKNOWLEDGEMENT:

This work was supported by the Czech Science Foundation, GA CR (grant no. GA22-03754S) and South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses CENAKVA.

WeP-11: Hydrogen/deuterium exchange mass spectrometry for complex samples

Tomáš Smrčka^{1,2*}, Dmitry Loginov¹, Petr Novak¹, Petr Pompach³, Pavla Vankova³, Alan Kadek¹, Petr Man¹

1. *Institute of Microbiology (BIOCEV), The Czech Academy of Sciences, Prague, Czech Republic*
2. *Faculty of Science, Charles University, Prague, Czech Republic*
3. *Institute of Biotechnology, The Czech Academy of Sciences, Prague, Czech Republic*

Hydrogen/deuterium exchange mass spectrometry (HXMS) is a well-established method providing insights into the dynamic structure of proteins. Its main advantage is the ability to examine proteins directly in solution. In addition, HXMS can be applied on small peptides as well as large non-covalent protein assemblies. Recent goal is to bring HXMS to a proteome-wide scale. Hence, we took human serum as a test sample in which we can look into the binding of protein, peptide and/or small molecule ligands.

To do so, we chose two model protein systems: human haptoglobin-haemoglobin (HP-HB) complex and α -1-acid glycoprotein (A1AGP), a known binder and transporter of numerous drugs. Our first goal was to characterise these systems individually and subsequently in complex systems. Haptoglobin exists in three different phenotypes – 1-1; 1-2 and 2-2. The static X-ray structure of human HP dimer (1-1) in complex with haemoglobin and additional stabilizing proteins has been already revealed, but the structure dynamics of oligomeric HPs (2-1, 2-2) and their binding to HB remains unknown and was investigated in this work. Next, the interaction of A1AGP with its famous and medically important interactor, warfarin, was probed together with its previously suggested pH-dependency that may provide insight into the dynamics of the A1AGP binding cavity.

Our initial findings presented here will be further exploited to understand the A1AGP pharmacokinetics of cyanobacterial secondary metabolites with biotherapeutic potential, namely Nostatin A and its analogues. Overall, the work presented here provides valuable insights into two isolated model systems that we hope will pave the way towards advanced HXMS applications in the highly heterogeneous environment of the whole human serum.

* Correspondence: smrckat99@gmail.com

ACKNOWLEDGEMENT:

This work was supported by the Ministry of Education, Youth, and Sports of the Czech Republic, OP JAK project „Photomachines“ (CZ.02.01.01/00/22_008/0004624).

WeP-12: Evaluation of MALDI-TOF MS and FTIR spectroscopy for typing of clinically relevant *Acinetobacter baumannii* clones isolated from hospital environments

Violetta Shestivska ^{1*}, Jan Tkadlec ², Martina Maixnerová ¹, Gabriela Jakubcová ²,
Alexandr Nemeč ^{1,2}

1. *Laboratory of Bacterial Genetics, National Institut of Public Health, Prague*

2. *Department of Medical Microbiology, Charles University, 2nd Faculty of Medicine and Motol University Hospital*

Question: The bacterium *Acinetobacter baumannii*, a member of the taxonomically diverse genus *Acinetobacter*, has emerged as an opportunistic human pathogen associated with multidrug resistance and epidemic spread in hospitals. Outbreaks of multidrug-resistant *A. baumannii* are linked to increased patient mortality. Reliable tracking of such outbreaks is essential for effective medical treatment. Rapid typing methods can assist in tracing transmission routes and managing outbreaks. This study evaluates whether MALDI-TOF MS or FTIR spectroscopy can complement the gold-standard macrorestriction analysis of genomic DNA by pulsed-field gel electrophoresis (PFGE) for strain typing of clinical isolates of *A. baumannii*.

Methods: We analyzed 20 clinical isolates collected between 2019 and 2021 from four Czech hospitals. Initially, PFGE was used to determine clonal diversity, followed by clustering analyses using MALDI-TOF MS and FTIR spectroscopy. Mass spectra were recorded in positive linear mode using the Microflex LT/Bruker Biotyper RTC (Bruker Daltonics, Germany), IR spectra (1300 cm⁻¹ to 4000 cm⁻¹) were processed with IR-Biotyper 3.1 (Bruker Daltonics, Germany).

Results: The 20 isolates were classified into five subtypes (four isolates per subtype) using PFGE and epidemiological data. FTIR analysis successfully identified three of these PFGE subtypes, but two subtypes remained undifferentiated. MALDI-TOF MS, in automatic mode, failed to discriminate between the isolates.

Conclusion: FTIR and MALDI-TOF MS can be employed as rapid pre-screening methods to complement routine typing techniques for clinical *A. baumannii* isolates. However, the use of MALDI-TOF MS for strain typing should be considered only in combination with bioinformatic data analysis tools.

* Correspondence: shestivsk@seznam.cz

REFERENCES:

1. Nemeč A. *Acinetobacter*. In: Trujillo ME, Dedysh S, DeVos P, Hedlund B, Kämpfer P, Rainey FA, Whitman WB, editors. *BMSAB*. Wiley., (2022).

WeP-13: Analysis of tau protein biology and interactome using crosslinking mass spectrometry

Jakub Sinsky ^{1*}, Petra Majerova ¹, Lenka Hornakova ^{1,2}, Juraj Piestansky ^{1,3}, Rostislav Skrabana ¹, Ondrej Cehlar ¹, Jozef Hanes ¹, Andrej Kovac ¹

1. Institute of Neuroimmunology, Slovak Academy of Sciences, Bratislava, Slovakia

2. Department of Biochemistry, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia

3. Department of Pharmaceutical Analysis and Nuclear Pharmacy, Comenius University in Bratislava, Slovakia

Mass spectrometry-based methods have emerged as a powerful tool in proteomic workflows for protein identification, quantitative proteomics, analysis of protein complexes, protein-protein interactions, etc. We have employed protein crosslinking mass spectrometry (XL-MS) pipeline to study tau protein interactions and kinetics to give insight into its biology and pathological processes of tauopathies. Since many protein-protein interactions are transient or weak, the chemical crosslinking allows fixation and capture of such interactions and facilitates their identifications. Therefore, XL-MS enables the acquisition of a highly reliable data for determination of stable, as well as transient protein-protein interactions including their exact interaction site. Furthermore, implementation of distance restraints of individual crosslinkers and their visualization in 3D protein model serves as useful tool for solving structure of proteins or protein complexes.

* Correspondence: jakub.sinsky@savba.sk

ACKNOWLEDGEMENT:

The work was supported by VEGA 2/0146/24, VEGA 2/0078/22, APVV-21-0254, Schwarz fund no. 2021/OV2/005

WeP-14: Deciphering species diversity and trophic preferences of phlebotomine sand flies using MALDI-TOF mass spectrometry

Vít Dvořák¹, Daniel Kavan², Kristýna Hlavačková¹, Alexandra Chaskopoulou³, Petr Volf¹, Petr Halada^{2*}

1. *Department of Parasitology, Faculty of Science, Charles University, Prague, Czech Republic*

2. *Institute of Microbiology of the Czech Academy of Sciences, BioCeV, Vestec, Czech Republic*

3. *USDA-ARS, European Biological Control Laboratory, Thessaloniki, Greece*

Leishmaniasis caused by *Leishmania* protozoans and transmitted by females of phlebotomine sand flies (Diptera: Psychodidae) belong to very important yet still neglected vector-borne diseases. When evaluating the epidemiological role of sand flies, both reliable species identification and knowledge of trophic preferences towards reservoir hosts are required to understand local transmission cycles and design efficient disease control strategies in endemic regions.

During the last decade, MALDI-TOF MS protein profiling and MALDI-TOF MS peptide mass mapping have been successfully employed in sand fly research. Protein profiling was proved as a method of choice for conclusive, time and cost-effective species identification of large sets of field-caught sand flies from various endemic regions of the Old World including the Mediterranean, East Africa or Southeast Asia, creating a reference database that currently includes more than 40 sand fly species. Standardized protocol of specimen trapping, storage and sample preparation ensures to acquire reproducible species-specific protein profiles that serve as useful tool in integrative taxonomy, supporting formal description of new species, discriminating among sibling species and challenging validity of established taxons while allowing utilization of a single sand fly specimen for other purposes (DNA barcoding, morphological analysis, blood meal identification, pathogen screening). MALDI-TOF MS peptide mass mapping of host-specific haemoglobin peptides in engorged females enables efficient and reliable blood meal origin identification, including mixed blood meals, up to 48 hours post feeding, clearly outperforming other currently used methods.

* *Correspondence: halada@biomed.cas.cz*

ACKNOWLEDGEMENT:

Funding: Czech Science Foundation (GA23-06299S)

WeP-15: Detection of human growth hormon using affinity MALDI mass spectrometry chips

Beata Pradlovská ^{1*}, Josef Dvořák ², Petr Novák ², Alžbeta Nemeškalová ¹, Michael Volný ¹

1. Department of Analytical Chemistry, University of Chemistry and Technology, Prague, Czech Republic

2. Institute of Microbiology of the Czech Academy of Sciences, Prague, Czech Republic

Human Growth Hormone (hGH) is a peptide hormone that stimulates growth, cell reproduction, and cell regeneration. It is thus important in human development. hGH is a 191-amino acid, single-chain polypeptide that is synthesized, stored and secreted by somatotrophic cells within the lateral wings of the anterior pituitary gland. Its recombinant form is called somatropin, which can have primary sequences different from the natural hGH. Somatropin is used as a prescription drug to treat children's growth disorders and adult growth hormone deficiency. In its role as an anabolic agent, hGH has been used by in sports since 1980s and has been banned by all major sport doping agencies. However, illegal formulations are widely available and abused as different dietary supplements.

We present a development of a technique for fast detection of hGH using MALDI affinity chips prepared by ambient ion soft landing. Affinity MALDI chips provide sample clean-up and preconcentration of the analyte prior MALDI analysis. Here, the enrichment step is being utilized for two different methodologies: One is to obtain clean enough sample so MALDI top-down sequencing (MALDI-TDS) can be achieved in order to determine the exact primary sequence that can potentially distinguish sources of origine of different illegal somatotropins. The second assay workflow is focused on achieving detection limits low enough to provide screening for hGH in serum.

* Correspondence: beapradlovska@gmail.com

WeP-16: Optimization of the SPE Protocol for Oligonucleotide Sample Preparation

Kristína Spustová ¹*, Anton Škríba ¹, Hana Cahová ¹

1. *Ústav organické chemie a biochemie AV ČR*

The solid-phase extraction (SPE) method is often used as one of the best options for sample preparation to extract the analyte of interest from complex samples.

This work focuses on the development of an efficient purification protocols for the 5'-RNA caps and oligonucleotides using (SPE) with a semi-automated positive pressure manifold, Otto (Waters). Issues with oligonucleotides, due to their large and complex nature, arise from insufficient sample flow through the sorbent bed or from using an eluent that is not strong enough to effectively elute the desired oligonucleotides from the sorbent. The developed SPE protocol, utilizes a special mixed-mode weak anion exchanger (WAX) sorbent facilitating the removal of nucleosides, salts, and enzymes. Samples were applied to WAX sorbent, washed stepwise with water and 50% MeOH in water and eluted using 73% acetonitrile, 0,2% ammonium hydroxide and water. In the course of development, the elution step proved to be insufficient, particularly for the elution of larger oligonucleotides. To address this, alternative elution buffer compositions were tested to avoid the use of TEAA (triethylammonium acetate). Elution buffers were tested on oligonucleotides ranging from 2 to 35-mers, including combinations such as 10-75% methanol with 0.5-5% ammonium hydroxide, etc. The results from these experiments were compared both to each other and to control oligonucleotide samples without SPE purification. All elution buffer experiments following SPE were analyzed using hydrophilic interaction chromatography (HILIC) as an alternative to ion-pairing chromatography. For untargeted or qualitative detection, the Xevo G2-XS qTOF mass spectrometer (Waters) was employed, offering precise mass identification and detailed structural information.

* Correspondence: kristina.spustova@uochb.cas.cz

WeP-17: A novel cross-linking strategy for aromatic residues

Michael Karpíšek^{1,2*}, Lukáš Fojtík^{1,2}, Zdeněk Kukačka¹, Petr Novák^{1,2}

1. *Institute of Microbiology, the Czech Academy of Sciences, Prague*

2. *Faculty of Science, Charles University, Prague*

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

The cross-linking reaction is based on a two step FFAP mechanism which includes activation of hypervalent iodine using a proton as a Lewis acid and a subsequent attack of aromatic residues by the generated fluoroalkyl radical. The studied model proteins (such as horse heart myoglobin) were analyzed by bottom up approach using mass spectrometry (timsTOF SCP, Bruker Daltonics). Samples were digested by trypsin protease, separated on a reverse phase column online coupled to a mass spectrometer.

The obtained results demonstrate the ability of our cross-linking strategy to bridge aromatic amino acids as well as cysteines and their combinations. These cross-links of uncommon amino acid residues provide information not attainable by lysine-specific cross-linkers.

* *Correspondence: karpisem@natur.cuni.cz*

ACKNOWLEDGEMENT:

This work was mainly supported by the Czech Science Foundation (grant number 22-27695S) and European Commission H2020 (The European Proteomics Infrastructure Consortium providing access EPIC-XS – project agreement No. 823839).

WeP-18: Detection of Selective Androgen Receptor Modulators and Anabolic-Androgenic Steroids in Dietary Supplements by Ambient Ionization Mass Spectrometry

Jana Knytlová ^{1*}, Iveta Šilhánková ¹, Petra Dinisová ², Lukáš Plaček ², Roman Hájek ², Jan Buček ³, Michael Volný ^{1,4}, Alžběta Nemeškalová ¹

1. Department of Analytical Chemistry, University of Chemistry and Technology, Prague, Czech Republic

2. Pragolab s.r.o. Nad Krocínkou 285, 190 00 Prague, Czech Republic

3. Plasmion GmbH, Am Mittleren Moos 48, 86167, Augsburg, Germany

4. Institute of Microbiology of the Czech Academy of Sciences, Vídeňská 1083, 142 00, Prague 4, Czech Republic

Ambient ionization mass spectrometry allows for analysis of samples in their natural state. Here we present an application of commercially available implementation of Dielectric Barrier Discharge Ionization (DBDI) ion source, for the analysis of dietary supplements for Selective Androgen Receptor Modulators and Anabolic Androgenic Steroids, two different classes of anabolic-acting molecules, which represent common compounds of abuse. For the direct analysis using the DBDI source, samples from homogenized capsules content were dispersed in methanol. A tip of a syringe needle was briefly submerged in the solution and inserted into the GCSPME module of plasma-based Sicrit ion source (Plasmion, Switzerland). The DBDI ion source was operated using voltage of 1600 V and frequency of 15 kHz. These values are vendor recommended and to provide trade-off between ionization efficiency and softness. For sample introduction, the GCSPME module was heated and continuously flushed with nitrogen to isolate the system from the outer environment. The ion source was mounted on the Orbitrap Exploris 120 Mass Spectrometer (Thermo Scientific), operating in positive mode. The spectra were processed using FreeStyle 1.18. Same samples were also analyzed by LC-MS/MS using an Agilent 1290 Infinity II liquid chromatography system and a 6470 triple quadrupole mass spectrometer (Agilent Technologies) operating in MRM mode to provide confirmation. While LC-MS/MS outperformed the AI-MS in quantitative analytical figures of merit, the results indicate that there is a good qualitative agreement between the results obtained by ambient ionization and by the golden standard LCMS technique and that AI-MS could be thus used for rapid screening of dietary supplements for illegal anabolic compounds.

* Correspondence: j.knytlova@volny.cz

WeP-19: Heptafluorobutyl Chloroformate Mediated Derivatization for Chiral Analysis of Secondary Amino Acids via GC-MS

Petr Vodrážka^{1,2*}, Stanislav Opekar¹, Helena Zahradníčková¹, Martin Moos^{1,3}, Lucie Řimnáčová¹, Petr Šimek¹

1. *Laboratory of Analytical Biochemistry and Metabolomics, Biology Centre CAS*
2. *Department of Chemistry of Natural Compounds, University of Chemistry and Technology*
3. *Department of Applied Chemistry, University of South Bohemia*

Heptafluorobutyl chloroformate (HFBCF) mediated dispersive liquid-liquid microextraction (DLLME) has proven to be an efficient method for fast and cost-effective GC-MS analysis of both chiral and nonchiral amino acids [1]. This work extends its application to the chiral analysis of secondary amino acids, including cyclic ones like proline and pipercolic acid, which play critical roles in protein folding and exhibit unique conformational rigidity. Sample preparation involves in-situ derivatization with HFBCF, followed by DLLME into iso-octane and amidation with methylamine. After evaporation and redissolution, chiral GC-MS is performed using a Chirasil-L-Val column. The method effectively separates enantiomers of eight biologically significant secondary amino acids, including azetidine-2-carboxylic acid, pipercolic acid, nipecotic acid, and several hydroxyproline isomers. This two-step derivatization process has been successfully applied to various biological matrices such as human biofluids, peptide hydrolysates, and collagen [2]. It was also utilized for the chiral analysis of Nostatin A (NosA), a ribosomally synthesized and post-translationally modified peptide (RiPP) from cyanobacteria. After hydrolysis of NosA, the chirality of individual amino acid residues was determined, revealing that all except D-trans-4-isobutylproline were in the L-configuration [3]. The method provides robust enantiomeric separation even for challenging secondary amino acids and enables determination of enantiomeric ratios down to 0.1%. This technique is highly sensitive, rapid, cost-effective, and compatible with aqueous biological samples, making it an invaluable tool for chirality analysis in complex biological matrices.

* Correspondence: vodrazka@bclab.eu

REFERENCES:

1. Šimek P. et al. *Amino acid analysis methods and protocols*, 2nd Edn. Springer, Silver Spring, pp. 237–251 (2019)
2. Opekar S. et al. *Amino Acids* 53, 347–358 (2021)
3. Delawska K. et al. *Org. Biomol. Chem.* in review

ACKNOWLEDGEMENT:

The financial support of the Grant Agency of the Czech Republic, project No. 17-22276S is greatly appreciated.

WeP-20: Development of Software for LC-MS Data Processing of Oligonucleotides

Evgeniya Biryukova ^{1,2*}, Marek Polák ^{1,2}, Petr Novák ¹

1. *Mikrobiologický ústav AV ČR*

2. *Department of Biochemistry, Faculty of Science, Charles University, Czech Republic*

The development of advanced software for processing LC-MS data, particularly for oligonucleotides, represents a critical step forward in biomedical research and therapeutic applications. Our work focuses on creating a Python-based tool designed to streamline the analysis of oligonucleotide sequences. Upon input of an oligonucleotide sequence, the algorithm generates a library of monoisotopic masses for the sequence fragments across all charge states. These theoretical masses are then matched with experimentally measured masses within a defined ppm range using LC-MS data exported from Bruker Data Analysis.

The algorithm has potential applications in inflammation and cancer diagnostics through measuring miRNA from liquid body fluids, enabling early detection of disease progression. This could offer a non-invasive diagnostic approach. Additionally, oligonucleotide therapeutics are becoming a key class of drugs for targeted therapy. Accurate interpretation of complex mass spectra remains a challenge, and this software will address it by offering efficient tools for analysis, improving oligonucleotide characterization.

Our methodology will enable quantitative and qualitative analysis of the complex biological matrix. The software's graphical user interface (GUI) will simplify interaction, making it accessible to researchers with varying levels of computational expertise.

This software can aid ongoing efforts in oligonucleotide research and demonstrate the integration of computational tools in modern biomedical sciences, thereby fostering innovation and improving healthcare outcomes.

* *Correspondence: biryukova777@seznam.cz*

WeP-21: How electrospray tuning counteracts the matrix effect

Ivan Petřík^{1*}, Michal Kaleta¹, Jitka Šíroková¹, Ondřej Novák¹

1. *Laboratoř růstových regulátorů, UP Olomouc & ÚEB AV ČR v. v. i., Šlechtitelů 27, 779 00 Olomouc*

Most targeted mass spectrometry-based bioanalyses tend to be burdened by weaker or stronger matrix effects due to the presence of non-targeted substances such as salts, electrolytes or lipids in a biological sample [1]. As a result, the signal of a targeted analyte is suppressed or, conversely, enhanced, which can lead to a biased quantification. A common approach to overcome this problem is both signal normalization using an internal standard and more or less complex sample preparation. In our work, we present another way to mitigate the potential consequences of the matrix effects, by means of the electrospray tuning. Using the Xevo TQ-S triple quadrupole (Waters, Manchester, UK), we selected seven electrospray parameters that may affect the MS signal of the small acidic molecules. We searched for a statistically significant interaction between these parameters and the plant matrix using the fractional factorial experimental design and the linear regression analysis [2]. It turned out that the spraying geometry, desolvation gas temperature and capillary voltage are the key players that can influence the matrix effect magnitude. Obviously, this observation is related to other parameters of the HPLC-MS/MS instrument, such as the composition and flow rate of the mobile phase and the structure of an analyte, all of which still need to be further investigated.

* Correspondence: ivan.petrik@upol.cz

REFERENCES:

1. Nováková L, Douša M, Česla P, Urban J. (2024) *Modern HPLC separations in theory and practice. Czech Chromatographic School.*
2. Montgomery DC. (2021) *Design and Analysis of Experiments, Tenth Edition EMEA Edition. John Wiley & Sons Inc.*

ACKNOWLEDGEMENT:

This work was supported with the financial support of the Internal Grant Agency of Palacky University (IGA_PrF_2024_013) and the Czech Science Foundation (GACR) (22-17435S).

WeP-22: HPLC-MS/MS method for simultaneous determination of six anti-cancer drugs in serum of lymphoma patients

Laura Zellner^{1*}, Soyoung Lee², Susanna Zierler³, Clemens Schmitt⁴, Christian Klampfl¹, Markus Himmelsbach¹

1. *Institute of Analytical and General Chemistry, Johannes Kepler University Linz*

2. *Institute of Tumor Biology, Faculty of Medicine, Johannes Kepler University Linz*

3. *Institute of Pharmacology, Faculty of Medicine, Johannes Kepler University Linz*

4. *Department of Hematology and Oncology, Kepler University Hospital, Johannes Kepler University Linz*

Non-Hodgkin lymphoma is an invasive hematologic malignancy and is among the top 10 causes of cancer-related mortality [1]. The standard treatment for this disease is the CHOP regimen—a combination chemotherapy consisting of cyclophosphamide, doxorubicin, vincristine, and prednisolone [2]. In our experiments, this traditional regimen is extended by incorporating bortezomib and ibrutinib. To understand the pharmacodynamics and -kinetics of this combination therapy it is essential to monitor the plasma concentrations of these drugs over time. Each drug exhibits distinct concentration profiles, that eventually decline to sub ng/mL levels [3]. Thus, an ultrasensitive analytical method for simultaneous quantification of these six drugs at very low concentrations in patient samples is needed.

To meet these criteria, we developed a liquid chromatography-tandem mass spectrometry method. The serum samples were prepared by protein precipitation followed by an enrichment step. Due to significant matrix effects, matrix-matched calibration with bovine serum was chosen. Low limits of detection and quantification in the pg/ml range were achieved, and the method showed good correlation coefficients ($r > 0.997$) for all of the drugs. Additionally, the stability of the analytes in serum and extract was investigated.

This assay will eventually be used to analyze samples from patients undergoing the mentioned combination therapy. Given its very low quantification limits, the method seems promising for monitoring plasma levels even after microdosing. Administering doses that are approximately 1000 times lower than conventional levels could be particularly advantageous in cancer therapy, potentially reducing adverse effects while maintaining therapeutic efficacy.

* Correspondence: laura.zellner@jku.at

REFERENCES:

1. Siegel R.L. et al., *CA Cancer J Clin* 73, 17–48 (2023)
2. Michallet A.S. et al., *Blood Rev* 23, 11–23 (2009)
3. Reece D.E. et al., *Cancer Chemother Pharmacol* 67, 57–67 (2011)

ACKNOWLEDGEMENT:

The infrastructure used for this work was supported by the “Programm Interreg ATCZ52 Österreich – Tschechische Republik: Infrastruktur für Metabolomik-Forschung und Klinische Chemie”.

WeP-23: Subcellular fraction-specific interactomes of the tick-borne encephalitis virus capsid protein

Kateřina Jaklová ^{1,2}*, Filip Dyčka ², Hana Tykalová ^{1,2}, Martin Selinger ², Ján Štěrba ²

1. *Institute of Parasitology, Biology Centre, Czech Academy of Sciences*

2. *Department of Chemistry, Faculty of Science, University of South Bohemia in České Budějovice*

The *Orthoflavivirus encephalitis* (*Flaviviridae* family; *Orthoflavivirus* genus), previously known as tick-borne encephalitis virus (TBEV), is an arthropod-borne virus transmitted by ticks. This virus attacks the central nervous system causing a disease called tick-borne encephalitis whose incidence has been steadily increasing in Europe and Asia in recent years. Since there is a lack of a specific treatment for TBEV infection, attempts have been made to identify prospective candidates for drug targeting. One of these is the capsid protein (TBEV C), responsible for the formation of nucleocapsids. Its ability to bind the viral RNA and function in promoting the proper assembly of infectious particles makes it a crucial regulatory viral protein in infected cells. Even more attention has been paid to this protein since its nuclear localization was documented. Almost all TBEV C functions take place in the cytoplasm or endoplasmic reticulum, and to this day, only a few details are known about the role of TBEV C in the nucleus. For these reasons, the primary aim of this work was to identify the TBEV C interaction partners in subcellular fractions, with an emphasis on the nuclear, organellar, and cytoplasmic binding partners using co-immunoprecipitation and mass spectrometry. To eliminate the identification of false-positive nucleic acid-mediated binding partners, benzonase was used to ensure the degradation of all DNA and RNA in samples. This protein was found to bind histones amongst others during the infection process which indicates an important role for TBEV C in the nucleus; however, further research is needed to verify our findings.

* Correspondence: jaklok01@prf.jcu.cz

ACKNOWLEDGEMENT:

This study was supported by the Ministry of Education, Youth and Sports of the Czech Republic INTER-ACTION project LTAUSA18040, the Grant Agency of the Czech Republic (18-27204S, 22-25042S) and SGA FSc USB (2022).

WeP-24: Middle-up quantification of infliximab in pharmaceutical matrix by capillary electrophoresis-mass spectrometry

Jana Havlíková^{1*}, Katarína Maráková¹, Peter Mikuš¹

1. Univerzita Komenského v Bratislave

Infliximab (IFX) is a chimeric mouse-human therapeutic monoclonal antibody (mAb) against tumor necrosis factor (anti-TNF α), commonly used in treatment of inflammatory bowel disease. IFX is, structurally, a large protein of size approximately 150 kDa consisting of two types of subunits – two heavy (~50 kDa) and two light (~25 kDa) chains connected by disulfide bonds. Typically, bottom-up approach is employed for mAb analysis, where mAbs are enzymatically digested into smaller peptides and subsequently analysed. This work makes use of an alternative, middle-up (MU) approach, where the mAb disulfide bonds are reduced by a reducing agent into heavy and light chains. An MU procedure is used together with capillary electrophoresis-mass spectrometry (CE-MS) for quantitative analysis of IFX in pharmaceutical matrix. Stock solution of IFX reference standard (Sigma-Aldrich, US) (1 mg/mL in water) was prepared and IFX disulfide bonds were reduced by tris(2-carboxyethyl)phosphine (TCEP). The reduced IFX was subsequently analysed by Agilent 7100 CE system coupled to an Agilent 6410 triple quadrupole mass spectrometer (Agilent, Santa Clara, US). Background electrolyte systems (BGE) containing 0,5 M and 1 M formic acid and acetic acid in the range of 2 – 4 M were tested together with different separation voltages applied (18 – 25 kV). Various concentrations of IFX stock solutions (0,005 – 0,04 mg/mL) were analysed to plot a calibration curve. The obtained results show that the most suitable BGE for analysis of reduced mAb IFX is 1 M formic acid and the optimal separation voltage to be 25 kV. Furthermore, a calibration curve was plotted from the acquired data showing that the proposed proof-of-concept quantitative method requires further optimisation.

* Correspondence: jana.havlikova@uniba.sk

ACKNOWLEDGEMENT:

This project was funded by VEGA 1/0514/22, VEGA 1/0483/20, FaF/36/2022 and by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under the project No. 09I03-03-V04-00208.

WeP-25: Simple UPLC-MS amino acid analysis approach for quality control of KLH

Juraj Piestansky ^{1,2*}, Petra Majerova ², Andrej Kovac ^{1,2}

1. *Department of Galenic Pharmacy, Faculty of Pharmacy, Comenius University*

2. *Institute of Neuroimmunology, Slovak Academy of Sciences*

Keyhole limpet hemocyanin (KLH) is an extracellular respiratory protein which is obtained from the Californian giant keyhole limpet *Megathura crenulata*. It is known that the KLH protein consists of two structurally and physiologically distinct isoforms – KLH1 and KLH2, each based on a subunit with molecular weight of approximately 400 kDa [1, 2]. KLH acts as a potent immunoactivator and therefore it is widely used as hapten carrier and immune stimulant in research and clinical studies. To ensure high quality, efficacy, and safety of the protein carrier, it is necessary to dispose with effective analytical approaches.

One of the usually used method necessary for characterization and quantification purposes of biopharmaceuticals is amino acid analysis. In this work, an advanced analytical method based on precolumn derivatization and reversed-phase ultra high-performance liquid chromatography in combination with single quadrupole mass spectrometer was developed for amino acid analysis in different batches of KLH. The method used isotopically labeled internal standards, and was validated according to the International Council for Harmonisation guideline. The developed method offers favorable performance and validation parameters, i.e., time of analysis (6 min), specificity, linearity ($r^2 \geq 0.99$), limit of detection (0.009 – 0.822 μM), limit of quantification (1 – 2.5 μM), accuracy and precision. The proposed amino acid analysis approach is suitable for routine quality control of KLH with the potential to be implemented into the laboratories of biopharmaceutical companies.

* *Correspondence: piestansky@fpharm.uniba.sk*

REFERENCES:

1. Harris J.R. et al.: *Micron*. 30, 597-623 (1999).
2. Harris J.R. et al.: *Eur Urol*. 37 (Suppl 3), 24-33 (2000).

ACKNOWLEDGEMENT:

Acknowledgements: This work was funded by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under the project No. 09I03-03-V04-00622.

AUTHORS INDEX

Adámková Ljubina.....	WeP-03
hmed Summra.....	ThO-06
Baka Michal.....	WeS-07
Berková Petra.....	FrO-21, FrO-24
Birner-Gruenberger Ruth.....	ThO-11
Biryukova Evgeniya.....	WeP-20
Boryshpolets Serhii.....	WeP-10
Brinsa Vítězslav.....	ThO-07
Buček Jan.....	WeP-18
Busch Florian.....	ThO-05
Cahová Hana.....	WeP-05, WeP-16
Cehlar Ondrej.....	WeP-13
Chaskopoulou Alexandra.....	WeP-14
Chmelík Josef.....	ThO-06
Chocholoušková Michaela.....	FrO-22
Chval Zdeněk.....	FrO-17
Cichrová Kateřina.....	WeP-09
Dinisová Petra.....	WeP-18
Dračinský Martin.....	FrO-25
Dvořáček Václav.....	WeS-05
Dvořák Josef.....	WeP-03, WeP-15
Dvořák Vít.....	WeP-14
Dyčka Filip.....	ThO-11, WeP-10, WeP-23
Fedorova Ganna.....	WeS-03, WeP-10
Fér Miroslav.....	WeS-03
Fiala Jan.....	ThO-05
Fialová Pavla.....	WeS-02
Fojtík Lukáš.....	WeP-17
Fojtíková Pavla.....	FrO-19
Frantová Helena.....	ThO-11
Friedecký David.....	WeP-06
Fryčák Petr.....	ThO-15
Gaustad Hannah.....	FrO-25
Grabic Roman.....	WeO-02, ThO-13, WeS-02, WeS-03
Grabicová Kateřina.....	WeO-02, ThO-13, WeS-02
Gregar Filip.....	ThO-15, WeP-08
Greisch Jean-Francois.....	ThO-05
Hájek Roman.....	WeP-18
Halada Petr.....	WeP-14
Hamrozi Petr.....	WeP-08
Hanes Jozef.....	WeP-13
Havlíček Vladimír.....	ThO-06, FrO-20, FrO-23, WeP-04
Havlíková Jana.....	WeP-24
Heck Albert J. R.....	ThO-05

Hilscherová Klára.....	ThO-13
Himmelsbach Markus.....	FrO-18, WeP-22
Hlásná Čepková Petra.....	WeS-05
Hlavačková Kristýna.....	WeP-14
Hlavničková Kateřina.....	WeP-09
Hnízda Aleš.....	ThO-07
Hofinger-Horvath Andreas.....	WeS-05
Holčapek Michal.....	FrO-22
Horn Martin.....	ThO-11
Hornakova Lenka.....	WeP-13
Horniaková Andrea.....	ThO-15
Hořejší Karel.....	FrO-22
Houšť Jiří.....	WeP-04
Idkowiak Jakub.....	WeS-04
Jágr Michal.....	WeS-05
Jaklová Kateřina.....	WeP-23
Jakubcová Gabriela.....	WeP-12
Jankevics Andris.....	ThO-05
Ječmen Tomáš.....	ThO-10
Jelinkova Anna.....	WeO-01
Jin Chunsheng.....	FrO-22
Jirásko Robert.....	FrO-22
Jirečková Barbora.....	ThO-12
Kádek Alan.....	ThO-06, ThO-07, WeP-11
Kahoun David.....	FrO-21
Kalaninová Zuzana.....	ThO-12, WeP-02
Kaleta Michal.....	WeP-01, WeP-21
Kamenik Zdenek.....	WeO-01
Karpíšek Michael.....	WeP-17
Kavan Daniel.....	ThO-12, WeS-06, WeP-02, WeP-14
Kholodnyy Vitaliy.....	WeP-10
Klampf Christian.....	FrO-18, WeP-22
Klement Aleš.....	WeS-03
Klementová Šárka.....	FrO-19
Knytlová Jana.....	WeP-18
Kodešová Radka.....	WeS-03
Kolářová Denisa.....	FrO-22
Konečná Martina.....	FrO-21
Konik Peter.....	ThO-09
Kopáček Petr.....	ThO-11
Korba Tomáš.....	WeS-01
Kosutzká Zuzana.....	WeP-06
Kovac Andrej.....	WeP-13, WeP-25
Kozelková Tereza.....	ThO-11
Krynická Vendula.....	ThO-09

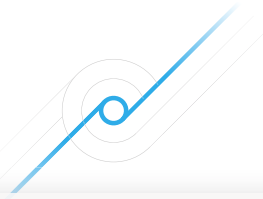
AUTHORS INDEX

Kukačka Zdeněk.....	WeP-17
Kvasnica Miroslav.....	WeP-01
Kvasnička Aleš.....	WeP-06
Lee Soyoung.....	WeP-22
Lemr Karel.....	ThO-15
Li Jiahang.....	WeS-04
Lienard Marjorie.....	FrO-25
Loginov Dmitry.....	WeS-06, WeP-03, WeP-11
Lokočová Gabriela.....	FrO-20, FrO-23
Lorenzi Quentin.....	FrO-25
Lu Stephen.....	ThO-11
Lubeck Markus.....	ThO-05
Luptáková Dominika.....	ThO-06, FrO-20, FrO-23, WeP-04
a Hynek.....	ThO-06
Maixnerová Martina.....	WeP-12
Majerova Petra.....	WeP-13, WeP-25
Man Petr.....	ThO-12, WeP-02, WeP-11
Maráková Katarína.....	WeP-24
Mikuš Peter.....	WeP-24
Mikušová Petra.....	ThO-13
Mistrík Robert.....	Pl-02
Moník Martin.....	WeP-08
Moos Martin.....	FrO-17, FrO-21, FrO-24, WeS-07, WeP-19
Musilová Karolína.....	ThO-07
Myšáková Michaela.....	WeP-07
Nejadebrahim Shiva.....	ThO-10
Nemec Alexandr.....	WeP-12
Nemeškalová Alžběta.....	ThO-14, WeP-15, WeP-18
Nerudová Zdeňka.....	WeP-08
Nikodem Antonín.....	WeS-03
Novák Jiří.....	WeP-04
Novák Ondřej.....	WeP-01, WeP-21
Novák Petr.....	ThO-07, ThO-12, WeS-06, WeP-02, WeP-03, WeP-11, WeP-15, WeP-17, WeP-20
Nováková Jana.....	ThO-12
Oklešťková Jana.....	WeP-01
Olešová Dominika.....	WeP-06
Ollivier Simon.....	ThO-05
Opekar Stanislav.....	FrO-17, FrO-21, FrO-24, WeS-07, WeP-19
Oušková Veronika.....	FrO-19
Palyzová Andrea.....	FrO-20, FrO-23
Papoušková Barbora.....	ThO-15
Pauk Volodymyr.....	WeP-09
Pengelly Stuart.....	ThO-05
Petrík Ivan.....	WeP-01, WeP-21
Petrík Miloš.....	FrO-20, FrO-23

Piastansky Juraj.....	WeP-13, WeP-25
Plaček Lukáš.....	WeP-18
Pluháček Tomáš.....	ThO-15, WeP-08
Pluskal Tomáš.....	FrO-25
Polák Marek.....	ThO-12, WeP-20
Pompach Petr.....	WeP-03, WeP-11
Poncarová Martina.....	FrO-19
Portašiková Jasmina Mária.....	ThO-12, WeP-02
Pradlovská Beata.....	WeP-15
Raether Oliver.....	ThO-05
Randák Tomáš.....	WeO-02
Raška Milan.....	ThO-06
Reinhardt Jakob.....	FrO-25
Řimnáčová Lucie.....	FrO-24, WeP-19
Sadchenko Alina.....	WeS-03
Selinger Martin.....	WeP-23
Shestivska Violetta.....	WeP-12
Schittmayer-Schantl Matthias.....	ThO-11
Schmitt Clemens.....	WeP-22
Schon Martin.....	WeP-06
Schönlechner Regine.....	WeS-05
Schuster Dina.....	ThO-05
Schwarzer Martin.....	WeO-01
Schwarzerová Jana.....	WeS-04
Sinsky Jakub.....	WeP-13
Skotnicová Petra.....	ThO-09
Skrabana Rostislav.....	WeP-13
Smith Joshua.....	FrO-25
Smrčka Tomáš.....	WeP-11
Smrčková Helena.....	FrO-25
Sobotka Roman.....	ThO-09
Sojka Daniel.....	ThO-11
Sotnikov Anatolii.....	WeP-10
Součková Jitka.....	WeP-08
Spustová Kristína.....	WeP-16
Stefani Tommaso.....	WeO-01
Stillerova Vendula.....	FrO-25
Straka Igor.....	WeP-06
Šácha Pavel.....	FrO-25
Šilhánková Iveta.....	WeP-18
Šimek Petr.....	FrO-17, FrO-24, WeS-07, WeP-19
Široká Jitka.....	WeP-21
Škríba Anton.....	WeP-05, WeP-16
Šlauf Jan.....	FrO-20, FrO-23
Štěrba Ján.....	WeP-10, WeP-23

AUTHORS INDEX

Štrach Filip.....	FrO-21
Švecová Helena.....	WeS-03
Švercová Kateřina.....	WeS-02
Švihovcová Klára.....	FrO-21
Teneberg Susann.....	FrO-22
Tkadlec Jan.....	WeP-12
Tomaník Lukáš.....	ThO-16
Toušová Zuzana.....	ThO-13
Tykalová Hana.....	WeP-23
Tysklind Mats.....	WeS-02
Ukropcová Barbara.....	WeP-06
Ukropec Jozef.....	WeP-06
Urbanová Veronika.....	ThO-11
Vácha František.....	FrO-21
Valkovič Peter.....	WeP-06
Vaněk Ondřej.....	ThO-10
Vankova Pavla.....	WeP-11
Vaňková Zuzana.....	FrO-22
Vodička Roman.....	FrO-21
Vodrážka Petr.....	FrO-24, WeP-19
Vojs-Staňová Andrea.....	ThO-13
Volf Petr.....	WeP-14
Volný Michael.....	WeP-03, WeP-15, WeP-18
Vrana Branislav.....	WeS-02
Waldherr Steffen.....	WeS-04
Weckwerth Wolfram.....	PI-01, WeS-04
Wierer Markus.....	FrO-18
Wolrab Denise.....	FrO-22
Zahradníčková Helena.....	FrO-24, WeP-19
Zakopcanik Marek.....	WeS-06
Zellner Laura.....	WeP-22
Zhang Ning.....	WeO-02
Zierler Susanna.....	WeP-22
Žáček Petr.....	WeO-03



Hmotnostní spektrometry s vysokým rozlišením

Řada Orbitrap Exploris (QqOT)



Exploris 120

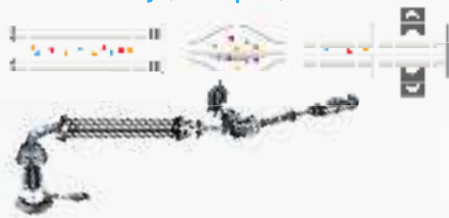


Exploris 240



Exploris 480

Řada Tribridy (QOTqLIT)

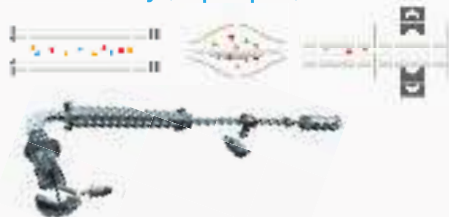


Orbitrap Eclipse



Orbitrap IQ-X

Řada Tribridy (QqOTqLIT)

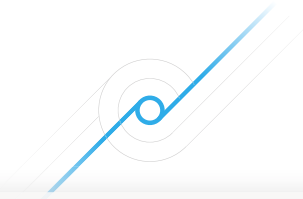


Orbitrap Ascend

Řada Tribridy (QOTqToF)



Orbitrap Astral



Hmotnostní spektrometry s nízkým rozlišením

Jednoduchý kvadrupól (Q)



ISQ EM



ISQ EC

Trojitý kvadrupól (QqQ)



TSQ Altis Plus

TSQ Quantis Plus

TSQ Fortis Plus

Kvadrupól s lineární iontovou pastí (QqLIT)



Stellar



TIMS-MS

timsTOF Series

Next generation timsTOF platforms for high-throughput, high-speed and high-sensitivity 4D-Proteomics™, 4D-Multiomics and SpatialOMx®

Defy the odds with the timsTOF. Introducing the next generation of ion mobility mass spectrometry. Utilizing trapped ion mobility spectrometry (TIMS) unlocks an additional dimension of separation and delivers revolutionary improvements in enhanced specificity and robustness.

- **timsTOF Ultra** – Make the invisible visible – Navigate the unknown with precision and accuracy using PASEF®
- **timsTOF SCP** – Cell by cell; proteomics takes on new meaning with timsTOF
- **timsTOF HT** – Expanding the capabilities of depth and high-throughput 4D-Proteomics
- **timsTOF Pro 2** – The platform of choice for 4D-Multiomics applications with its proven robustness, high sensitivity and fast MS/MS acquisition
- **timsTOF flexX** – With **MALDI-2** and **microGRID** towards enhanced sensitivity at high spatial resolution
- **timsTOF MALDI PharmaPulse®** – Taking label-free high-throughput screening to the next level



For Research Use Only. Not for use in clinical diagnostic procedures.

For more information please visit www.bruker.com



MERCK

BIG SCIENCE SMALL FOOTPRINT

Sustainable lab products,
solutions & services
for responsible science

Ensuring a sustainable future together

Our mission is to ensure a more sustainable future for everyone. With our growing portfolio of greener products, programs and services, we now offer even more ways for you to practice responsible science.

Our solutions combine enhanced sustainability with exceptional quality, so they are better for the planet, and for your work.

Your Opportunities

- Sustainable & less harmful alternatives
- Biodegradable & greener chemicals
- Waste reduction & sustainable packaging



Much more on
SigmaAldrich.com



The life science business of
Merck operates as MilliporeSigma
in the U.S. and Canada.

© 2024 Merck KGaA, Darmstadt, Germany and/or its affiliates. All Rights Reserved. Merck, Millipore, Sigma-Aldrich, and Supelco are trademarks of Merck KGaA, Darmstadt, Germany or its affiliates. All other trademarks are the property of their respective owners. Detailed information on trademarks is available via publicly accessible resources.

Sigma-Aldrich®
Lab & Production Materials

Supelco®
Analytical Products

Millipore®
Preparation, Separation,
Filtration & Monitoring Products