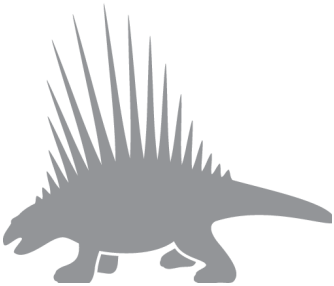

6. KONFERENCE ČESKÉ SPOLEČNOSTI PRO HMOTNOSTNÍ SPEKTROMETRII

Olomouc, 29. – 31. března 2017

SBORNÍK PŘÍSPĚVKŮ

Sborník příspěvků z 6. konference České společnosti pro hmotnostní spektrometrii

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Česká společnost pro hmotnostní spektrometrii

Olomouc 2017

Sborník příspěvků z 6. konference České společnosti pro hmotnostní spektrometrii

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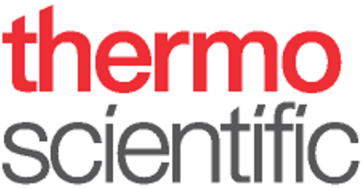

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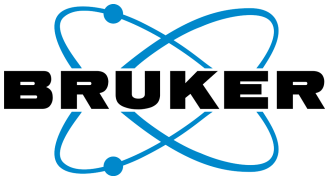

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6. konference České společnosti pro hmotnostní spektrometrii

Datum konání

29. – 31. března 2017

Místo konání

BEA campus Olomouc

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Pořadatel

Česká společnost pro hmotnostní spektrometrii, Olomouc

Výbor ČSHS

Předseda: Petr Fryčák (Univerzita Palackého v Olomouci)

Místopředseda: Petr Man (Mikrobiologický ústav AV ČR, v.v.i.)

Členové:

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Pavel Řehulka (Univerzita obrany v Hradci Králové)

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Středa 29. března 2017

12:00 – 17:00	Registrace
15:00 – 15:10	Zahájení konference ČSHS
15:10 – 16:10	**Plenární přednáška I. (Prof. Andrea Sinz)**
PL-1	*The power of cross-linking/mass spectrometry for protein structure analysis*
16:10 – 16:20	Přestávka
16:20 – 16:50	Workshop firmy ChemAxon
16:50 – 17:10	Přestávka na kávu a čaj
17:10 – 18:40	**I. Hmotnostní spektrometrie v iontové chemii a organické analýze**
	(Předsedající: Anton Škríba)
17:10 – 17:50	Prof. Richard O'Hair (Detlef Schröder lecture)
WeO-001	*Teaching an old dog new tricks: Application of the twin ion method to drug metabolism, insecticide resistance and toxicology*
17:50 – 18:10	Jiří Váňa
WeO-002	*Determination of rate constants by ESI-MS: extension of the delayed reactant labeling method*
18:10 – 18:40	Jana Roithová
WeO-003	*Použití iontové spektroskopie v organické chemii*
18:40 – 18:50	Přestávka
18:50 – 19:20	Workshop firmy AMEDIS
19:25 – 23:00	Párty a sekce plakátových sdělení

Čtvrtek 30. března 2017

09:00 – 10:40	**II. Vývoj metod, instrumentace a softwaru**
	(Předsedající: Pavel Řehulka)
09:00 – 09:20	Josef Cvačka
ThO-004	*Iontové zdroje APCI a APPI vyvíjené pro malé průtoky vzorku*
09:20 – 09:40	Antonín Bednařík
ThO-005	*TLC – DLTV ICP MS: Chromatografie na tenké vrstvě ve spojení s hmotnostní spektrometrií indukčně vázaného plazmatu s tepelným odpařováním diodovým laserem*
09:40 – 10:00	Lukáš Najdekr
ThO-006	*Bottlenecks in metabolomic data processing*
10:00 – 10:20	Jaroslava Jáčová
ThO-007	*Dimenzionalita vzorku v optimalizaci 2D separací*
10:20 – 10:40	Volodymyr Pauk
ThO-008	*Peak broadening in supercritical fluid chromatography coupled to atmospheric pressure chemical ionization mass spectrometry*
10:40 – 11:00	Přestávka na kávu a čaj
11:00 – 11:30	Workshop firmy Shimadzu
11:30 – 12:30	Oběd
12:30 – 14:10	**III. Hmotnostní spektrometrie v biologii**
	(Předsedající: Jan Preisler)
12:30 – 12:50	Vladimír Havlíček
ThO-009	*Molecular and elemental mass spectrometry imaging on track of infectious diseases*
12:50 – 13:10	Petr Novák
ThO-010	*Transcription factor-DNA interaction studied by structural mass spectrometry*
13:10 – 13:30	Marek Stiborek
ThO-011	*Studium vlivu mědi na cytotoxicitu wedelolaktonu v buňkách karcinomu prsu pomocí (SALD) ICP MS*

13:30 – 13:50	Petra Darebná
ThO-012	*Detection and quantification of carbohydrate deficient transferrin by MALDI-compatible protein chips prepared by ambient ion soft landing*
13:50 – 14:10	Jiří Novák
ThO-013	*CycloBranch as a tool for dereplication and visualization of organic compounds in LC-MS and MSI datasets*
14:10 – 14:30	Přestávka na kávu a čaj
14:30 – 15:50	**IV. Hmotnostní spektrometrie v potravinářství, průmyslu a ochraně prostředí**
	(Předsedající: Petr Novák)
14:30 – 14:50	Kevin A. Schug
ThO-014	*Characterization of chemical and microbial contamination in proximity to unconventional oil and gas extraction processes*
14:50 – 15:10	Eliška Čechová
ThO-015	*Látky s vývojovou neurotoxicitou: Lidská expozice novými environmentálními polutanty*
15:10 – 15:30	Roman Mareček
ThO-016	*Ultratrace speciation with GC-ICP-MS*
15:30 – 15:50	Ondřej Lacina
ThO-017	*Hledání jehly v kupce sena: Snadněji pomocí kombinace omics přístupů*
15:50 – 16:10	Přestávka na kávu a čaj
16:10 – 16:50	**Předání ceny Zdeňka Hermana Nadačním fondem Rezonance a přednáška vítězné práce**
16:50 – 17:20	Workshop firmy Merck
17:20 – 18:00	Schůze ČSHS
18:00 – 19:30	Volno
19:30 – 23:00	Párty a sekce plakátových sdělení

Pátek 31. března 2017

09:00 – 11:00	**V. Hmotnostní spektrometrie v klinické a farmaceutické analýze a metabolomice**
	(Předsedající: Vladimír Havlíček)
09:00 – 09:20	David Friedecký
FrO-018	*Metabolomická analýza tauopatie*
09:20 – 09:40	Alessandro Visco
FrO-019	*Therapeutic drug monitoring using fully automated sample preparation LC/MS/MS system*
09:40 – 10:00	Zdeněk Spáčil
FrO-020	*Profilování metabolických biomarkerů v biologických vzorcích pomocí tandemové hmotnostní spektrometrie*
10:00 – 10:20	Kateřina Mičová
FrO-021	*Orbitrap-based HRMS for new biomarker and drug metabolites study*
10:20 – 10:40	Zuzana Demianova
FrO-022	*SWATH® ako novým prístup ku presnejšej identifikácii metabolitov*
10:40 – 11:00	Karel Chalupský
FrO-023	*Metabolic phenotyping of mouse models*
11:00 – 11:20	Přestávka na kávu a čaj
11:20 – 11:50	Workshop firmy Thermo Fisher Scientific
11:50 – 12:50	**Plenární přednáška II. (Prof. Michal Holčapek)**
PL-2	*Mass spectrometry based lipidomic quantitation and applications in cancer biomarker research*
12:50 – 13:00	Závěr konference
13:00 – 14:00	Oběd

PL-1:
The power of cross-linking/mass spectrometry for protein structure analysis

Andrea Sinz [1 *]

1. Martin Luther University Halle-Wittenberg

During the last 15 years, chemical cross-linking combined with mass spectrometry (MS) and computational modeling has advanced from investigating 3D-structures of isolated proteins to deciphering protein interaction networks. Chemical cross-linking relies on the introduction of a covalent bond between functional groups of amino acids within one protein, to gain insight into the conformation of a protein, or between interaction partners to elucidate interfaces in protein complexes. Based on the distance restraints derived from the chemical cross-linking data, three-dimensional structural models of proteins and protein complexes can be constructed.

One of our goals is to extend the arsenal of existing cross-linkers to obtain complementary structural information of proteins. To facilitate the identification of cross-linked products, we have designed MS cleavable cross-linkers creating characteristic marker ions in the fragment ion mass spectra [1]. We have evaluated different fragmentation methods available on an Orbitrap Fusion in combination with a dedicated software tool, MeroX, for conducting fully automated analyses of cross-linked products. An alternative, more exploratory strategy relies on a novel cross-linking reagent, containing a TEMPO and a benzyl group. The aim for designing this cross-linker was to facilitate the assignment of cross-linked products by FRIPS [2].

A direct way to probe protein-protein interactions is by site-specific incorporation of genetically encoded photo-reactive amino acids or by non-directed incorporation of photo-reactive amino acids. These photo-reactive amino acids contain benzophenone or diazirine groups and are activated upon UV-A irradiation [3]. As such, they possess the potential to study *in vivo* protein interactions.

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LITERATURA:

1. *Müller MQ. et al: Anal. Chem. 82, 6958-6968 (2010).*
2. *Hage C. et al: J. Am. Soc. Mass Spectrom. 28, 56-68 (2017).*
3. *Piotrowski C. et al: Methods 89, 121-127 (2015).*

PL-2:
Mass spectrometry based lipidomic quantitation and applications in cancer biomarker research

Michal Holčapek [1 *], Miroslav Lísa [1], Eva Cífková [1], Robert Jirásko [1], Maria Khalikova [1], Bohuslav Melichar [2], David Vrána [2]

1. University of Pardubice, Faculty of Chemical Technology, Pardubice
2. Palacký University, Medical School and Teaching Hospital, Olomouc

Lipids are major building blocks of membranes of cells and intracellular compartments and they also fulfil numerous crucial cell functions. Their dysregulation is related with some serious human diseases including cancer, because tumor and normal cells have different proteomic, metabolomic and lipidomic composition. We demonstrate that the lipidomic composition of tumor and normal cell lines shows statistically significant differences for several tumor types [1]. The same differences are also found in tumor and normal tissues obtained after the oncological surgery and tumor/normal differentiation is verified for plasma samples of inbred mice. The last and the most difficult step is the search for statistically relevant lipidomic differences for human plasma. This type of research requires high-throughput, robust and validated MS assays for the quantitation of large number of lipids from multiple lipid categories and classes. Shotgun ESI-MS/MS is used for the direct quantitation of several phospholipid and sphingolipid classes in total lipid extracts without a chromatographic separation. Separation - MS approaches can increase the sensitivity due to the reduction of matrix effects and improve the confidence of lipid identification. HILIC [2] or ultrahigh-performance supercritical fluid chromatography [3] methods are convenient for the separation of various lipid classes, where the coelution of class internal standards and analytes guarantee the same ionization efficiencies. Negative-ion MALDI is used for semi-quantitation of some negatively charged lipids, such as sulfatides and related compounds. Finally, the multivariate data analysis using nonsupervised and supervised methods is performed to find the main differences between tumor and normal samples.

* *Korespondence: Michal.Holcapek@upce.cz*

LITERATURA:

1. *Cífková E. et al.: Rapid Commun. Mass Spectrom. 31(3), 253-263 (2017).*
2. *Cífková, E. et al.: J. Chromatogr. B 1000, 14-21 (2015).*
3. *Lísa, M. et al.: Anal. Chem. 87, 7187-7195 (2015).*

PODĚKOVÁNÍ:

This work was supported by ERC CZ project No. LL1302 (MSMT, Czech Republic).

WeO-001:

Teaching an old dog new tricks: Application of the twin ion method to drug metabolism, insecticide resistance and toxicology

Richard A. J. O'Hair [1 *], Michael G. Leeming [1], Roberto Fusetto [1], Philip Batterham [1], William A. Donald [2]

1. Bio21 Institute of Molecular Science and Biotechnology, The University of Melbourne, Australia
2. School of Chemistry, The University of New South Wales, Sydney, Australia

The metabolic fate of an endogenous compound can determine the success of a new drug lead or help unravel the molecular basis of insecticide resistance or toxicological effects. Thus, significant effort is directed to identifying the metabolites formed from a given molecule. Here, an automated and non-targeted procedure is introduced for detecting metabolites of drugs, insecticides and known carcinogens without authentic metabolite standards using stable isotope labelling, liquid chromatography mass spectrometry (LCMS), and high performance computing.

LCMS of blood plasma extracts from rats that were administered a 1:1 mixture of acetaminophen (APAP) and $^{13}C_6$-APAP resulted in mass spectra that contained “twin” ions for drug metabolites that were not detected in control spectra . By developing a program (High-resolution Twin-Ion Metabolite Extraction; HiTIME) that can identify twin-ions in raw high-resolution mass spectra, 9 doublets corresponding to APAP metabolites were identified.

We next applied this methodology to identify the protein targets of chemically reactive metabolites thought to be responsible for acute drug toxicity or carcinogenesis. As a proof-of- concept study, 1:1 APAP and $^{13}C_6$-APAP were oxidised in liver microsomes. Following trypsin digestion and LCMS analysis, HiTIME scoring revealed twin-ion signals corresponding to peptides that had undergone reaction with NAPQI/$^{13}C_6$-NAPQI. The site of protein modification was then identified via collision induced dissociation.

These methodologies can be used to rapidly detect the metabolites of small molecules and the protein targets of reactive metabolites without prior knowledge of their identity.

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WeO-002:
Determination of rate constants by ESI-MS: extension of the delayed reactant labeling method

Jiří Váňa [1,2] *, Vladimir Petrović [2,3], Thibault Terencio [2], Jana Roithová [2]

1. University of Pardubice
2. Charles University in Prague
3. University of Kragujevac

In recent years we have focused our attention on development of a new method for determination of rate constants of reactions proceeding in solution by means of ESI-MS[1,2]. The Delayed reactant labeling approach is based on mixing of isotopically labeled and unlabeled reactants in different reaction times. The mutual evolution of equivalent, labeled and unlabeled signals in the ESI-MS spectrum reflects the kinetics of the relevant species in solution.

We present here an extension of this methodology with employing differently substituted reactants instead of isotopically labeled ones. We have investigated a palladium catalyzed reaction and followed formation of palladium containing complexes. Hence, introduction of substituents with different masses helped to overcome a problem of a signal overlap caused by the rich isotopic pattern of palladium. The different substitution of reactants causes a different ESI response of analogous complexes (i.e. the same concentrations of the given complexes in solution does not automatically lead to the same signals in the ESI-MS spectrum). This problem is accounted for in the method and can be separated out in the extraction of the rate constants.

We demonstrate this approach on palladium catalyzed C–H activation reaction of substituted acetanilides. The Hammett analysis of the rate constants obtained by our method for a series of meta and para substituted acetanilides provides the ρ value of -1.4, which is in agreement with values reported for similar C–H activations. Comparison of the results with those obtained from UV-Vis measurements and theoretical calculations shows a nice agreement and were further used for deeper analysis of reaction mechanism.

* *Korespondence: jirkavopic@seznam.cz*

LITERATURA:

1. *Jašíková L. et al.: J. Am. Chem. Soc. 137, 13647-13657 (2015).*
2. *Schulz J. et al.: Chem. Eur. J. 22, 9827-9834 (2016).*

PODĚKOVÁNÍ:

This work was supported by the Czech Science Foundation (14-20077S), European Research Council (ERC CoG No. 682275), and the COST action CHAOS.

WeO-003:
Použití iontové spektroskopie v organické chemii

Jana Roithová [1 *]

1. Univerzita Karlova

Kombinace hmotnostní spektrometrie s optickou spektroskopií hmotově vybraných iontů je mocný nástroj ke studiu struktury iontů. UV/Vis a IR spektra v kombinaci s teoretickými výpočty umožňují jednoznačně přiřadit strukturu nebo studovat změny struktury spojené např. se změnou teploty nebo s elektronickou excitací. V přednášce ukážu použití iontové spektroskopie k charakterizaci reaktivních intermediátů v organometalických reakcích.

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PODĚKOVÁNÍ:

Tento výzkum byl financován ERC CoG IsoMS.

ThO-004:
Iontové zdroje APCI a APPI vyvíjené pro malé průtoky vzorku

Josef Cvačka [1,2 *], Vladimír Vrkoslav [1], Barbora Rumlová [2], Pavlína Nekvasilová [2], Miloslav Šulc [3], Timotej Strmeň [1,2]

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2. Přírodovědecká fakulta Univerzity Karlovy v Praze
3. Česká zemědělská univerzita v Praze

Při chemické ionizaci za atmosférického tlaku (APCI) a fotoionizaci za atmosférického tlaku (APPI) dochází k ionizaci analytů v plynné fázi. Ionizační mechanismus je zcela odlišný od elektrospreje, což umožňuje analýzu sloučenin, pro které je elektrosprejová ionizace málo účinná nebo zcela nevhodná. Techniky APCI a APPI lze použít pro celou řadu nízkomolekulárních látek, např. pro léčiva, metabolity, přírodní látky, lipidy nebo pesticidy. Hmotnostní spektra obsahují vedle molekulových aduktů většinou i fragmenty, což lze v závislosti na aplikaci považovat za nevýhodu, nebo naopak za přednost umožňující strukturní charakterizaci bez nutnosti MS/MS. Komerční iontové zdroje pro APCI a APPI vycházejí z tradiční konstrukce, která poskytuje optimální výsledky pro průtoky vzorku kolem 1 ml/min. Zejména v oblasti bioanalytické chemie se však dnes běžně používají chromatografie s průtoky v řádech stovek nanolitrů či několika mikrolitrů za minutu. V těchto průtokových režimech je však prakticky jediným dostupným iontovým zdrojem nanoelektrosprej.

V naší laboratoři se pokoušíme vytvořit iontové zdroje pracující při nízkých průtocích mobilní fáze. V současné době vyvíjíme a testujeme otevřený iontový zdroj, který ke zmlžení kapalného vzorku využívá skleněný mikrofluidní čip [1] nebo křemennou kapiláru vyhřívanou pomocí odporového či indukčního topení. Geometrické uspořádání jednotlivých komponent zdroje hraje klíčovou roli a ovlivňuje citlivost i stabilitu signálu. Iontový zdroj je testován pro lipidy a další látky, a to v režimu APCI i APPI.

* *Korespondence:* cvacka@uochb.cas.cz

LITERATURA:

1. *Saarela V. et al.: Lab Chip 7, 644-646 (2007).*

PODĚKOVÁNÍ:

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ThO-005:

TLC - DLTV ICP MS: Chromatografie na tenké vrstvě ve spojení s hmotnostní spektrometrií indukčně vázaného plazmatu s tepelným odpařováním diodovým laserem

Antonín Bednařík [1], Pavla Foltýnová [1], Viktor Kanický [1,2], Jan Preisler [1,2] *

1. Ústav chemie, Přírodovědecká fakulta, Masarykova Univerzita, Brno
2. CEITEC – Středoevropský technologický institut, Brno

Tepelné odpařování diodovým laserem (DLTV) je cenově dostupná technika zavádění vzorků pro hmotnostní spektrometrii s indukčně vázaným plazmatem (ICP MS) [1]. DLTV využívá kontinuálních infračervených laserových diod (808 nm) k vyvolání pyrolýzy vhodného substrátu nesoucího kapalný vzorek ve formě vyschlých kapek o objemu pod 1 µl. Jako substrát lze s výhodou použít běžný filtrační papír přetištění pomocí komerčních tiskáren vrstvou běžného černého inkoustu, který slouží jako absorbér laserového záření. Absorbovaná energie laseru dostačuje k zahřátí substrátu se vzorkem a vyvolání jeho pyrolýzy. Vznikající aerosol je následně veden v proudu nosného plynu do ICP MS. Klíčové výhody této techniky zahrnují jednoduchou přípravu vzorků se složitou matricí (např. krev), minimální spotřebu vzorku, rychlost analýzy, využití předpřipravených kalibračních sad, možnost transportu a archivace vzorku.

V tomto příspěvku prezentujeme DLTV ICP MS jako detekční techniku pro chromatografii na tenké vrstvě (TLC). Jako substrát zde posloužily hliníkové TLC desky s vrstvou celulózy nebo silikagelu. Vyvinutý chromatogram je přetištěný proužkem inkoustu, vystřižen z TLC desky a skenován laserem ve skleněné tubulární komoře. Tento koncept byl ověřen na modelovém systému čtyř kobalaminů a jejich kvantifikaci ve vitaminovém doplňku stravy. Technika poskytla citlivost o pět řádů vyšší, než v případě optické detekce (LOD ~ 2 pg) [2]. Druhá prezentovaná aplikace techniky je speciace sloučenin obsahujících selen v kvasnicích a řasách.

* *Korespondence: buhbedna@gmail.com*

LITERATURA:

1. *Foltýnová, P. et al.: J. Anal. At. Spectrom. 29, 1585-1590 (2014).*
2. *Bednařík, A. et al.: J. Chromatogr. A 1364, 271-275 (2014).*

PODĚKOVÁNÍ:

Děkujeme za finanční podporu Grantové agentuře České republiky (GA15-05387S) a projektu CEITEC 2020 (LQ1601).

ThO-006:
Bottlenecks in metabolomic data processing

Lukáš Najdekr [1] *, David Friedecký [1], Tomáš Adam [1]

1. Univerzita Palackého v Olomouci (ÚMTM) a Fakultní nemocnice Olomouc

Many different metabolite signals are detected in the single metabolomic analyses when studying complex biological matrices. Nowadays, mass spectrometric instruments are capable of various ways of data acquisition (DIA, DDA, SRM, MS/MS, MSn, etc.). Such a vast amount of data creates a necessity of finding the comprehensive software capable extracting the important pieces of information.

Basically, two solutions are available: a commercial software (Compound Discoverer, MarkerView, MarkerLynx, etc.) with all the vendor support and training, although restricted only the specific file type. The second choice is to go to way of the open source software which is capable of processing multiple file types, but the support and available training is limited. Most people whose doing LC-MS analyses are using open source software and/or commercial software bundled with the mass spectrometry instrument. Where the open source field is dominated by XCMS and MZmine 2 (or MZmine).

The main bottleneck in the metabolomic data processing is a peak-picking. The individual chromatograms, based on the mass traces, are detected through all the analyses files and chosen for further data treatment. The number of features in the final table can vary vastly depending on the algorithm settings such as mass precision (ppm), the retention time window settings and signal-to-noise ratio. The pre-processing steps such as smoothing, noise-cut-of values, play also an important role. The final step is deconvolution. Its settings influencing the difference between detecting the “real” peaks, but not the noise.

Depending on your data file structure the proper software and peak-picking algorithms should be chosen carefully. Unwise decision might devalue the data and/or some information may be overlooked.

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ThO-007:
Dimenzionalita vzorku v optimalizaci 2D separací

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Dimenzionalita vzorku je parametr nezbytný pro optimalizaci 2D separací. Je definována jako počet nezávislých proměnných, kterými lze popsat chemické vlastnosti analytů přítomných ve vzorku [1]. 2D separace je optimalizována pomocí veličiny nazývané ortogonalita. Čím je ortogonalita 2D systému vyšší, tím efektivněji je využíván 2D separační prostor. Existují dva typy ortogonality: globální, popisující rozsah využitého separačního prostoru, a lokální ortogonalita, charakterizující rovnoměrnost jeho pokrytí.

Cílem práce je pomocí modelových chromatogramů obsahujících 2500 bodů generovaných in silico a reálných 2D separací plasmy a moči prokázat vliv dimenzionality vzorku na optimalizaci 2D separací. Lokální ortogonalita byla stanovena pomocí geometrického přístupu [2], globální ortogonalita pomocí aritmetického průměru eukleidovských vzdáleností nejbližších sousedů [3].

Bylo prokázáno, že ortogonalitu nízkodimenzonálních vzorků, lze popsat jak globálními tak lokálními deskriptory. Oproti tomu u vysokodimenzionálních vzorků nebyl deskriptor globální ortogonality funkční, lze je tedy optimalizovat jen částečně (na nepřítomnost shluků). Dimenzionalita vzorku však může být snížena výběrem detekčních podmínek, např. v případě MS detekce aplikací m/z filtru či výběrem selektivního detektoru (např. AFID). Pak se vzorek jeví jako nízkodimenzionální a jeho separaci lze pro cílovou skupinu látek optimalizovat.

Dimenzionalita vzorků představuje komplexní problematiku, která musí být při optimalizaci 2D separace brána v potaz.

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LITERATURA:

1. *Giddings J.C.: J. Chromatogr. A 703, 3-15 (1995).*
2. *Gilar M. et al.: Anal. Chem. 77, 6426-6434 (2005).*
3. *Nowik W. et al.: Anal. Chem. 85, 9449-9458 (2013).*

PODĚKOVÁNÍ:

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ThO-008:

Peak broadening in supercritical fluid chromatography coupled to atmospheric pressure chemical ionization mass spectrometry

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Supercritical fluid chromatography (SFC) is well suited for analysis of low molecular weight substances of low polarity. Atmospheric pressure chemical ionization (APCI) may be beneficial for such analytes. However, a significant peak broadening effect was observed in APCI detection mode after SFC separation of several flavor compounds using an Acquity UPC^2 system with Isocratic Solvent Manager and UPLC eLambda 800 nm photodiode array (PDA) detector coupled to a Xevo TQS triple quadrupole mass spectrometer with a Z-spray ESCi source (all Waters, Manchester, UK). Neither PDA nor electrospray ionization (ESI) traces suffered from such phenomenon. We conducted a series of experiments using a set of ten test compounds covering a broad range of polarity and volatility. Influence of ion source parameters (desolvation gas flow, temperature, cone gas flow, nebulizer gas pressure and makeup liquid flow) on their peak shape and intensity was investigated in APCI and ESI mode. Ratio of chromatographic peak widths obtained with APCI and PDA was used as a measure of peak broadening. The broadening effect was more pronounced for nonpolar volatile compounds while polar ones showed only tailing at peak base. Desolvation gas flow and its temperature affected peak shape to a greater extent than other ion source settings. Tuning of these two parameters dramatically reduced peak distortion and provided higher sensitivity. A mechanism of SFC-APCI peak broadening based on diffusion and adsorption in the ion source is proposed.

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ThO-009:

Molecular and elemental mass spectrometry imaging on track of infectious diseases

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Recently, the invasive aspergillosis accounts for 450 000 deaths per year. It complicates cancer chemotherapy, especially leukaemia, transplantation, exacerbations of chronic obstructive pulmonary disease and other immunosuppressed patients, including those with HIV infection. In this work we used the rat model of experimental aspergillosis. Laser ablation inductively coupled plasma mass spectrometry imaging has revealed a dramatic iron increase in fungi-affected areas of lungs, which were attributed to microbial siderophores. The iron, silver and gold mass spectrometry images revealed that particularly gold can be used as excellent diagnostic element with high dynamic range useful for sensitive tracking the Aspergillus infection. The limit of detection was determined as 30 $ng.g^{-1}$ (at 5 μm laser focus). The same fungal distribution was obtained at molecular level. The sodiated and potassiated adducts of intracellular siderophore ferricrocin were detected by MALDI imaging on FTICR mass spectrometer in CASI mode employing Continuous Accumulation of Siderophore Ions in a quadrupole preceding to the FTICR cell. This mode enabled us to visualize the fungal siderophore molecules in lungs and to compare this MSI distributions with histology and PET. We also detected intracellular ferricrocin and extracellular triacetylfusarinine C (TAFC) in urine samples of infected rats. Detection limits of ferricrocin and TAFC in urine were 5 pg/mL and 200 fg/mL, respectively defining them as non-invasive markers of microbial infection. In infected animals the siderophore concentrations were in ng/mL range contrary to controls, in which no siderophores were detected. The presentation will be concluded with the recent results achieved with other microorganisms pathogenic to humans.

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ThO-010:
Transcription factor-DNA interaction studied by structural mass spectrometry

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Transcription factors (TF) regulate gene expression through interactions with DNA and other regulatory proteins. Our understanding of this regulation depends on the knowledge of the TF-DNA complex structure, which is accessible by the conventional methods (X-ray, NMR), yet the process is still very challenging. Thus, the development of faster alternative approaches allowing detailed description of a TF-DNA complex is beneficial. To design a reliable mass spectrometry technology, a palette of MS based approaches, including H/D exchange, protein-protein and protein-DNA cross-linking was applied to investigate the TF-DNA complex structure in solution.

DNA binding domain of TF was expressed and purified and the oligonucleotide was synthesized. H/D exchange (HXMS) was followed for TF alone or in mixture with the oligonucleotide. Chemical cross-linking (CXMS) included both protein-protein (amino reactive reagents DSS and DSG in non- and deuterated form) and protein-DNA cross-linking (trans-Platinum(II)diammine dichloride). CXMS analyses were performed by reverse-phase chromatography coupled to FT-ICR mass spectrometer. HXMS and CXMS data were processed using own proprietary software DeutEx and LinX, respectively.

H/D exchange results were in agreement with the known high resolution structures of TFs obtained by NMR or X-ray. Protein-protein chemical cross-linking revealed structural differences in several regions, especially in the unstructured part where the data span further beyond the known high-resolution structures. Further, several protein-DNA cross-links were identified explaining structural changes induced by ligand binding.

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ThO-011:
Studium vlivu mědi na cytotoxicitu wedelolaktonu v buňkách karcinomu prsu pomocí (SALD) ICP MS

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V této práci byl studován vliv mědi na cytotoxicitu wedelolaktonu v modelových vzorcích buněčných linií prsního karcinomu *in vitro*.

Wedelolakton je polyfenolický kumestan s kancerostatickými účinky na solidní tumory *in vitro* a *in vivo [1]*. Domníváme se, že cytotoxicita wedelolaktonu může být zvýšena v přítomnosti iontů Cu^{2+} kvůli schopnosti wedelolaktonu redukovat je na Cu^{+}, což může mít za následek poškození DNA in vitro [2].

K monitorování hladiny mědi v buňkách byla použita technika SALD ICP MS (ICP MS s laserovou desorpcí za účasti substrátu) [3] a roztoková nebulizační ICP MS. SALD je technika zavádění kapalných vzorků do plazmatu, která je založena na nanesení kapalného vzorku na UV-absorbující substrát a následné kompletní desorpci vyschlých kapek pomocí pulzního UV laseru.

K vyhodnocení vlivu mědi na cytotoxicitu wedelolaktonu byly připraveny dva experimenty. První experiment spočíval v odlišné době přidání wedelolaktonu k buňkám ošetřených ionty Cu^{2+}. Ve druhém experimentu byla míra cytotoxicity vyhodnocena po transfekci buněk expresním vektorem hCtr1 (lidský transportér mědi) nebo prázdným vektorem cmv jako kontrolou. Počet apoptických buněk byl stanoven pomocí propidium jodidového barvení a průtokové cytometrie.

Provedené experimenty demonstrovaly, že postupné ošetření buněk ionty Cu^{2+} a wedelolaktonem může signifikantně zvýšit cytotoxicitu wedelolaktonu, přičemž výrazný nárůst cytotoxicity byl zaznamenán u buněk s transfektovaným genem hCtr1. Na druhou stranu, simultánní ošetření buněk vede ke snížení cytotoxicity wedelolaktonu, což připisujeme neschopnosti komplexu Cu^{2+}-wed penetrovat přes cytoplazmatickou membránu buňky.

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LITERATURA:

1. *Beneš P. et al.: Cancer Lett. 303(1), 29-38 (2011).*
2. *Beneš P. et al.: Environ. Mol. Mutagen. 53(7), 515-524 (2012).*
3. *Peš O. et al.: Anal. Chem. 80, 8725-8732 (2008).*

PODĚKOVÁNÍ:

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ThO-012:

Detection and quantification of carbohydrate deficient transferrin by MALDI-compatible protein chips prepared by ambient ion soft landing

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Immobilization of proteins is of fundamental interest in biochemistry, biosensor, and material science as well as bioanalytical chemistry. We present a technology that allows the preparation of MALDI-compatible protein affinity surfaces by ambient ion landing of proteins which can be used for the development of bioanalytical assays. These assays are based on the interaction between the immobilized protein and the sample analyte directly on the protein chip and subsequent analysis by MALDI mass spectrometry.

The electrosprayed proteins are immobilized on various conductive surfaces from dry metal to metal oxide surfaces. The ion landing is performed under atmospheric pressure, which leaves proteins in native form, by automated ion landing apparatus that can manufacture protein affinity surface with the predefined array of sample positions. The protein surfaces prepared by this technique are conductive and durable enough to be used directly as MALDI plates.

The glycoprotein transferrin is synthetized in the liver and is the most important carrier for iron transport in the human body. Severe alcohol consumption leads to alterations in glycosylation of transferrin. From its discovery, carbohydrate deficient transferrin (CDT) is routinely used for clinical screen of excessive alcohol abuse. Current CDT detecting methods include HPLC and capillary electrophoresis. A need exists for a reliable method for high-throughput clinical applications. In principle, mass spectrometry enables fast detection and quantification of transferrin isoforms since they have different molecular masses. In this study we use antibody chips prepared by ambient soft ion landing at atmospheric pressure in combination with MALDI mass spectrometry for detection and quantification of CDT.

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ThO-013:

CycloBranch as a tool for dereplication and visualization of organic compounds in LC-MS and MSI datasets

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CycloBranch (http://ms.biomed.cas.cz/cyclobranch/) is our in-house, open-source and cross-platform software originally dedicated to *de novo* sequencing of nonribosomal peptides (NRPs) from accurate product ion mass spectra [1]. Recently, we extended the tool to support the dereplication and visualization of small molecular weight natural compounds from liquid chromatography/mass spectrometry (LC-MS) and mass spectrometry imaging (MSI) datasets [2]. The plain text, standard mzML/imzML, and native Bruker and Waters file formats are supported. The current compound libraries include microbial NRPs and secondary metabolites, siderophores, lipids, and custom databases.

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LITERATURA:

1. *Novak J. et al.: J. Am. Soc. Mass. Spectrom. 26(10), 1780-1786 (2015).*
2. *Novak J. et al.: Biochim. Biophys. Acta - Proteins and Proteomics, in press, (2016).*

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ThO-014:
Characterization of chemical and microbial contamination in proximity to unconventional oil and gas extraction processes

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The extraction of oil and gas resources from tight shale formations using unconventional techniques is a multi-faceted process. Given the volume of activity, particularly in Texas, there is a need for careful monitoring of environmental impact. We have tailored a series of analytical methods to comprehensively monitor groundwater and soil quality in proximity to oil and gas extraction operations. Among other techniques, featured are the use of gas chromatography – mass spectrometry for analysis of volatile and semi-volatile organic compounds, supercritical fluid extraction – supercritical fluid chromatography - mass spectrometry for analysis of polyaromatic hydrocarbons and their metabolites from soil, and matrix-assisted laser desorption/ionization – mass spectrometry for identification of microbial populations in environmental waters. While some significant contamination events may be attributable to oil and gas extraction operations, some instances were found to be ephemeral. Importantly, our most recent studies lead us to believe that altered microbiomes in mildly contaminated groundwater may be more of a concern to human health than chemicals themselves. An overview of such research performed by our group in Texas will be presented.

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PODĚKOVÁNÍ:

Work was performed using funds solicited through the Collaborative Laboratories for Environmental Analysis and Remediation (CLEAR; http://clear.uta.edu) at the University of Texas at Arlington.

ThO-015:
Látky s vývojovou neurotoxicitou: Lidská expozice novými environmentálními polutanty

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Vzhledem k rostoucímu počtu dětí s neurovývojovými poruchami roste zájem o studium vlivu efektů neurotoxických sloučenin na zdraví dětí. Tyto látky jsou během raného vývoje přenášeny z matky na dítě, proto bylo vybráno ke studiu právě mateřské mléko. Protože jsme exponováni směsmi chemických látek, je žádoucí vývoj multireziduálních metod. Hlavním cílem práce bylo vyvinout a validovat metodu pro současné stanovení neperzistentních pesticidů (pyrethroidy) a alternativních zpomalovačů hoření (AFRs), které nahradily již zakázané organochlorové pesticidy (OCPs) či polybromované difenylethery (PBDEs). Vzhledem ke komplexnosti matrice mateřského mléka obsahující lipidy s podobnými vlastnostmi jako cílové látky, je potřeba pečlivý výběr metodiky přípravy vzorku. K tomuto účelu byla použita nekonvenční metoda čištění, dialýza, která byla poprvé použita při nízkých teplotách a byla aplikována na komplexní směs látek. Dialýza umožnila detekci nepersistentních látek přítomných v mateřském mléce ve stopových koncentracích. Instrumentální metodou, která zajistila citlivou detekci přítomných látek, byla plynová chromatografie s vysokorozlišovací hmotnostní spektrometrií (GC-HRMS).

Vyvinutá metoda byla následně použita pro analýzu více než 500 vzorků mateřského mléka ze tří evropských zemí, Norska, Nizozemí a Slovenska, přičemž bylo provedeno srovnání koncentrací obou skupin látek v těchto zemích. Pro mnoho AFRs představovala data první informaci o přítomnosti těchto látek v lidských matricích vůbec. Byla rovněž vypočítána expozice kojenců jako skupiny nejcitlivější vůči působení neurotoxických látek a byly srovnány jednotlivé expoziční cesty. Data z této studie budou sloužit k epidemiologickým vyhodnocením pro hledání vztahu mezi přítomností neurotoxických látek a zdravím dětí.

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PODĚKOVÁNÍ:

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ThO-016:
Ultratrace speciation with GC-ICP-MS

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Goal is to develop a robust and accurate method for tin or bromin speciation. Often the total elemental concentration of an analyte is not sufficient to accurately assess the impact of the analyte on the enviroment or human health. That is because the toxicity and mobility as well as other physicochemical properties are dependent on the chemical form of the species of the analyte.

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ThO-017:
Hledání jehly v kupce sena: Snadněji pomocí kombinace omics přístupů

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Častým problémem mnoha studií využívajících „omics“ přístupy, je množství a komplexnost generovaných dat. Část generovaných dat jsou reálné biologické signály, které souvisí se zkoumanou skutečností; zbylá data jsou šum, který vzniká náhodně kvůli použité technice nebo z důvodu biologické variability. Rozhodnutí, jaká data představují signál a co je šum, představuje velký problém, protože při použití striktních filtrů se ztratí velké množství relevantních signálů, při použití rozvolněných filtrů je nezbytné tolerovat vysokou míru šumu. Pro zvýšení poměru signál/šum v „omics“ studiích lze využít předchozí znalost dat, nebo soubor komplementárních dat, který zvýší množství získaných informací a zvýrazní tak rozdíly mezi zkoumanými skupinami [1].

Tento princip dokumentujeme na příkladu studie zabývající se autismem, který potenciálně souvisí s polymorfismem na genu CELF6. Transkriptomická data získaná na myších s vypnutým genem a kontrolní skupině při běžně používaném (striktním) zpracování a filtraci vedlo k odfiltrování všech signálů v rámci šumu [2]. Při použití rozvolněných filtrů však „prošlo“ 1326 různě exprimovaných genů, odpovídající 22 různým metabolickým drahám. Metabolomická data získána pomocí LC–QTOF MS systému ze stejných vzorků také nevedla k jednoznačným výsledkům; i zde došlo k odfiltrování všech metabolitů a až při zvýšení *p-value* na 0,2 se ukázalo 11 rozdílně regulovaných metabolitů. Pro zjištění relevantních rozdílů mezi testovanými skupinami byla nezbytná kombinace obou souborů dat mapováním odlišně regulovaných genů a metabolitů na metabolické dráhy. Při kombinaci transkriptomických a metabolomických výsledků se jako nejpravděpodobnější objevily pouze dvě ovlivněné metabolické dráhy – metabolismus jednouhlíkatých zbytků a metabolismus aminokyselin.

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LITERATURA:

1. *Ideker T. et al.: Cell 144(6), 860-863 (2011).*
2. *Dougherty J. D. et al.: J. Neurosci. 33(7), 2732-2753 (2013).*

FrO-018:
Metabolomická analýza tauopatie

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Tauopatie se řadí do skupiny neurodegenerativních onemocnění (včetně Alzheimerovy choroby), které jsou charakterizovány výskytem abnormálně modifikovaného proteinu Tau. V mozkové tkáni, přestává hyperfosforylovaný a zkrácený Tau protein plnit svou funkci, agreguje se do forem neurofibriálních klubek (NTF) narušujících strukturu neuronů. Metabolomická analýza je vhodným nástrojem pro odhalení biomarkerů tauopatie a biochemických změn vyvolaných přítomností NTF - přímo ovlivňujících mozkovou tkáň, nepřímo pak složení cerebrospinalní tekutiny (CSF) a plasmy.

Byla provedena cílená a necílená metabolomická LC/MS analýza vzorků CSF, plasem a mozkových tkání transgenních krys exprimujících Tau protein (SHR72) a kontrol (SHR). Byly aplikovány HILIC pro cílenou analýzu metabolitů (Triple Quad 6500, Sciex) a RP pro necílenou analýzu polárních lipidů (LTQ Orbitrap Elite, Thermo Fisher Scientific). V jednotlivých matricích (CSF/plasma/mozková tkáň) bylo nalezeno 96/163/243 metabolitů a 390/762/422 “features” definovaných přesnou hmotou a retenčním časem. Data byla zpracována a statisticky vyhodnocena (interpolace, clr transformace, PCA, OPLS-DA) v R programu za použití příslušných balíčků.

Byly nalezeny změny v metabolitech citrátového cyklu, metabolismu purinových nukleotidů a hladině glukosy naznačující energetickou depleci u vzorků transgeních krys s tauopatií. Dale byly detekovány významné metabolické rozdíly v metabolismu argininu a hladinách polárních lipidů - fosfatidylcholinů. Byla provedena nezávislá konfirmační studie ve vzorcích CSF, kterou byly potvrzeny výsledky, jež tak lze považovat za potenciální biomarkery procesu tauopatie.

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PODĚKOVÁNÍ:

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FrO-019:
Therapeutic drug monitoring using fully automated sample preparation LC/MS/MS system

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The recent CLAM-2000 (Clinical Laboratory Automated sample preparation Module) coupled with a Shimadzu LC-MS/MS system offers solution for a complete online sample preparation-LC-MS/MS analysis for clinical laboratories.

In this lecture, after a short introduction to the system, five applications for clinical laboratories are presented.

• A fully automated analysis of ten Steroids hormones in serum have been evaluated. Serum samples were directly loaded to the system, avoiding any human intervention until results were obtained.

• Reproducibility, repeatability and accuracy of four immunosuppressant determination in whole blood was tested. Results showed an increase of the analytical performances reducing the risk for human operators and the cost of the analysis.

• Seven antiepileptic drugs and drug active metabolites and two antiarrhythmic drugs with different hydrophilic properties have been determined using the system. The results showed the good performances of the system for a wide range of hydrophilic and hydrophobic drugs.

• The ability to analyze Novel Oral Anticoagulants (NOACs) in plasma samples has been evaluated. Parallel analysis with both manual and automated procedures, for the determination of NOACs in plasma. Results with the two different sample preparation procedures showed good agreement. Furthermore, analyzing the work time for the two sample preparations, the automated one proved to be six times more efficient for every day samples, and three times for STA samples.

• A comparison between manual and automated sample preparation procedure for the analysis of 25-OH Vitamin D2/D3 is showed in the last application. Results demonstrated a good agreement between the two procedures.

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FrO-020:
Profilování metabolických biomarkerů v biologických vzorcích pomocí tandemové hmotnostní spektrometrie

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Expoziční faktory související s nutricí a mikroflórou (symbiotické a patogenní mikroorganismy, které osídlují prostor lidského těla) mají zásadní vliv na hostitele a jsou asociovány s řadou zdravotních dopadů [1]. Nicméně specifické vlivy mikrobiální metabolické aktivity na hostitelské signální dráhy a fyziologické funkce stále nejsou uspokojivě vysvětleny na molekulární úrovni. Předpokládá se, že mikroflóra má vliv, zejména na energetický metabolismus a imunitní systém hostitele [1, 2]. S ohledem na sníženou nebo pozměněnou diverzitu střevního mikrobiomu lze tedy očekávat významné změny v metabolickém fenotypu na úrovni hladin biochemických intermediátů [3]. Metabolické biomarkery (endogenní, nutriční a mikrobiální) byly analyzovány pomocí moderních technik hmotnostní spektrometrie (selected reaction monitoring – SRM, high resolution/accurate mass – HR/MS) s využitím ultra-účinné kapalinové chromatografie (UHPLC). Důraz byl kladen především na intermediáty metabolismu tryptofanu, který je silně ovlivňován střevní mikroflórou. Výsledky zahrnují přípravu SRM knihoven využitelných k profilování metabolitů pomocí hmotnostního detektoru typu trojitého kvadrupólu (QqQ), kvantifikaci za použití strukturně příbuzných vnitřních standardů v kombinaci s koeficienty odezvy (response factor) a aplikaci UHPLC SRM metody na vzorky moči od těhotných matek a novorozenců. Tento přístup vedl ke kvantifikaci intermediátů tryptofanu, které slouží jako biomarkery střevní mikroflóry. Zároveň byly ve vzorcích moči objeveny dosud nepopsané indolové deriváty odvozené od mikrobiálního metabolismu tryptofanu. Nové poznatky přispívají k pochopení interakce mezi hostitelským a mikrobiálním metabolismem na molekulární úrovni a poskytují komplementární informaci např. k metodám 16S rRNA sekvenování.

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LITERATURA:

1. *Belkaid Y. & Hand T.W. Cell.: 157, 121–141 (2014).*
2. *Zhang L.S. & Davies S.S.: Genome Med. 8, 46 (2016).*
3. *Wikoff W.R. et al.: Proc. Natl. Acad. Sci. U. S. A. 106, 3698–3703 (2009).*

PODĚKOVÁNÍ:

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FrO-021:
Orbitrap-based HRMS for new biomarker and drug metabolites study

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Current separation techniques with multistage fragmentation high-resolution mass spectrometry (HRMS) provide a powerful tool for structural elucidation of unknown compounds in complex biological matrices. Determination of molecular structures of uncharacterized m/z signals was performed using Orbitrap instrument to acquire accurate masses and multistage fragmentation spectra. Acquired data led to structural elucidation of new characteristic compounds and drug metabolites, which can be potentially used as biomarkers in diagnostic and follow-up process.

The approach was applied on biomarker study of inborn errors of metabolism and finding new metabolites of drug biotransformation. Plasma samples from phenylketonuria (PKU) and 3-OH-3-methylglutaryl-CoA (HMG-CoA) lyase deficient patients compared to healthy controls. Two new conjugates of glutamyl-glutamyl-phenylalanine and phenylalanine-hexose in PKU and three positional isomers of 3-methylglutaconyl carnitine in HMG-CoA samples were identified, respectively. Detailed imatinib metabolization in the plasma from patients with chronic myeloid leukemia was studied. Mass Frontier and MetWorks software were applied for data evaluation. Overall 90 metabolites were identified and confirmed by the MS2 and MS3 fragmentations. Alternatively, untargeted metabolite profiling by HRMS and data processing with the Compound Discoverer 2.0TM enabled the detection of novel structurally unexpected sulphur-containing metabolites, annotated as cysteine and cystine adducts.

Our results prove, that structural elucidation of unknown metabolites in human biofluids can be done by current accurate mass multistage fragmentation techniques even at submicromolar concentrations.

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PODĚKOVÁNÍ:

NPU I (LO1304), 15-34613L

FrO-022:
SWATH® ako novým prístup ku presnejšej identifikácii metabolitov

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Výskumníci z rôznych odvetví hľadaju v metabolomike odpovede na nové otázky, ale aj dáta nadväzujúce na informácie získané z proteomiky a genetiky. V posledných rokoch, LC-MS technológie zvýšili kvalitu a rôznorodosť metabolomických informácií. Moderné hmotnostné spektrometre vedia presne identifikovať a rýchlo kvantifikovať nespočetné množstvo metabolitov v jednom meraní za využitia datovo nezávislého sberu dát (DIA). DIA prístup umožňuje výskumníkom študovať reguláciu metabolitov v danom čase a ich prítomnosť v danej biologickej ceste (dráhe).

SWATH® akvizícia je DIA prístup adoptovaný z kvantitatívnej protemiky, ktorý stále nie je bežne aplikovaný v metabolomických štúdiách. SWATH® akvizícia kombinuje výhody MS2 s cielenou MRM a MS2 s necielenou identifikáciou metabolitov. Vďaka obsiahlej a nestechiometrickej fragmentácii vo SWATH® akvizícii vieme získať väčšie množstvo fragmentácií meraných metabolitov a preto aj ich obsiahlejšie štrukturálne informácie ako za využitia bežnej MS identifikácii na úrovni MS1. Našim prvým krokom bolo optimalizovať SWATH® metódu za využitia fixných a variabilných okien, taktiež aj vplyv dĺžky separačnej metódy. Využitím SWATH® akvizícii sme identifikovali o 45 percent viac metabolitov v rôznych matriciach ako u bežnej MS identifikácie. Pritom sme nestratili kvantitívne informácie o identifikovaných metabolitoch.

SWATH® akvizícia využíva logiku variabilných okien a poukazuje na výhody cielených a necielených prístupov v MS. Jej veľkou výhodou je, že meranie dát vo vysokom rozlíšení a vysoká rýchlosť skenovania TripleTofu umožňuje kvantitatívne identifikovať stovky metabolitov v priebehu jedného merania.

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FrO-023:
Metabolic phenotyping of mouse models

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Metabolome is connected closely to the phenotype of an organism. Our task is to determine gene function and generate metabolite profiles in mutant mice. Here, we present two examples of the connection between genotype and phenotype in MMP-19 and ADAM10 deficient mice. Previously, metalloproteinases were mainly recognized as the extracellular matrix degrading enzymes. Currently, they are also known as mediators in a variety of processes related to immunity and tissue repair. We have analyzed plasma samples of mice deficient for MMP-19 and ADAM10 using the non-targeted metabolomics approach. Proteins were precipitated by cold acetonitrile, followed by supernatant drying and sample recovery in 40% methanol. 4µl of sample was injected. Zorbax Eclipse Plus C18 column and 5-100% methanol gradient over 15 minutes were used in LC separation. Data were recorded at ESI+ and ESI- polarity on 6550 iFunnel Q-TOF LC/MS system. We have found abnormalities in taurine conjugated bile acids content in MMP-19 and ADAM10 deficient mouse models. The expression of Abcg5/8 genes in the liver was elevated in both mouse models. The increased expression of Abcg5 protein was also confirmed also by Western blotting. Further blood analysis also revealed lower cholesterol concentration in MMP-19 deficient mice although there was no difference in cholesterol level in ADAM10 compared control mice. Altogether, we found a similar bile acid profile in both animal models and we suggest the role of both MMP19 and ADAM10 metalloproteinases in bile acid homeostasis.

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P-001:
Dendritic cell signaling in response to the early phase of *Francisella tularensis* invasion

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Francisella tularensis is a highly infective Gram-negative bacterium, also known as the causative agent of tularemia. *Francisella tularensis* is able to invade and to proliferate inside dendritic cells (DCs) while avoiding their effective activation and maturation. The goal of this work was to map phosphoproteome changes in DCs infected by *Francisella* in order to identify host signaling pathways involved in *Francisella*-DC interaction. The analysis was focused on early intervals (<1 h p.i.) because of the ability of *Francisella* to escape in this time period from the phagosome to the cytosol where it establishes its replicative niche. For comparison, the attenuated *Francisella* strain was used in parallel infection experiments. DC phosphoproteome was analyzed by the means of shotgun LC-MS-based proteomics and relative changes in protein abundance were quantified by the isotope labeling method specially developed for primary bone marrow-derived DCs. In total, more than 17,000 phosphosites from *Francisella*-infected DCs were identified. Phosphosites with similar time profiles were clustered together and potentially involved kinases were deduced from the motif enrichment analysis. Several kinases were found to be activated during the entry of bacteria into the DCs – for example Akt, PAKs or ERKs. In general, there were no significant differences between virulent and attenuated *Francisella* strains in phagocytosis-induced DC signaling. However, strain-specific host signaling profiles, centered mostly on mTOR and MAPKs, started to emerge in later time points (60 min p.i.).

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P-002:

Deeper clarification of the interaction between plasma membrane and matrix protein of human immunodeficiency virus 1

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The matrix protein plays a key role in the retroviral life cycle since it mediates the transport and binding of the viral structural proteins to the plasma membrane, where the formation of the new viral particles takes place. Subject of our interest was the matrix protein of human immunodeficiency virus 1. It has been already proven that the interaction of its highly basic region with phosphatidylinositol-4,5-bisphosphate (PI-4,5-P2) is essential for the binding of viral structural proteins to the plasma membrane. For the deeper clarification of the matrix protein interaction with the membrane, the surface mapping of matrix protein was used. The experimental part of this study was therefore focused on the monitoring of the changes in the surface accessibility of the matrix protein residues after its binding to the liposomes containing phosphatidylserine (PS) and PI-4,5-P2.

Changes of surface accessibility of the residues R19, K21, K25, K26, K29 and W35 were detected. These residues are localized in the highly basic region and obviously mediate the interaction with the PI-4,5-P2 and PS. These results are in a very good agreement with previous studies of other scientific groups, especially with the NMR experiments of the matrix protein with PI-4,5-P2 in solution [1] and with the coarse-grained molecular dynamics analysis of the interaction between matrix protein and the membrane with different phospholipids [2]. The changes of surface accessibility of the residues R38, E39, E41, R42 and E73 localized aside from the highly basic region were also detected. This fact indicates that the matrix protein trimerization proceeds during its interaction with the membrane and may be very important for the finally anchoring the matrix protein into the plasma membrane.

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LITERATURA:

1. *Saad J. S. et al.: Proc Natl Acad Sci U S A. 103(30), 1364-9 (2006).*
2. *Charlier L. et al.: Biophys J. 106(3), 577-85 (2014).*

P-003:

Mass spectrometry-based protein assay for identification and quantification of biomarkers for Huntington's disease in a transgenic minipig model of Huntington´s disease

Jaromír Novák [1 *], Eva Kotrčová [1], Rita Suchá [1], Jiřina Tylečková [1], Martina Žižková [1], Jakub Červenka [1], Ievgeniia Poliakh [1], Hana Kovářová [1], Petr Vodička [1]

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Huntington's disease (HD) is a fatal neurodegenerative disorder. The mutation in the huntingtin gene results in an extended stretch of glutamines in the N terminus of HTT protein. This significantly affects HTT protein conformation, proteolysis, post-translational modifications as well as protein interactions and causes toxicity to striatal neurons.

Minipigs complex neuro-architecture and longer lifespan compared to rodents makes it an attractive model species for gene therapy testing. Our institute established a porcine transgenic model of HD, carrying the first 548 amino acids of human HTT with 124 glutamines in addition to endogenous porcine HTT.

Selected reaction monitoring (SRM) based on mass spectrometry can be used for both relative and absolute quantification on the peptide level. As the protein sequences of human and porcine HTT differ in a couple of amino acids, it is possible to distinguish species-specific peptides of HTT and thus independently quantify mutant and wild-type proteins expressed in our transgenic HD model. We are currently developing and validating a SRM assay for measuring mutant human HTT and endogenous porcine HTT levels. This should allow for a robust and rapid high throughput testing of a therapy efficiency in HD minipig.

As a complement to targeted proteomic approach for HTT detection we are employing untargeted shotgun analysis to identify additional biomarkers of the HD progression. Using this method, we are currently mapping the proteome of HD minipig brain cortex and cerebrospinal fluid (CSF) with the hope to identify new biomarkers for future SRM assay development. We also plan to employ data independent acquisition methods (DIA), such as SWATH-MS, which combines global feature detection with SRM-like data extraction.

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P-004:
Hmotnostní spektrometrie ve spojení s elektrochemií – význam, vztahy, aplikace

Aleš Horna [1,2] *, Kateřina Vyňuchalová [1,2], Barbora Hrvolová [1,2], Michaela Hornová [1,2], Jana Hornová [1,2]

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Hmotnostní spektrometrie stejně jako elektrochemie ve spojení s chromatografií se využívá pro separaci, identifikaci a kvantifikaci látek. Elektrochemické detektory dosahují stejných detekčních limitů jako o řád dražší hmotnostní spektrometry.

Význam hmotnostního spektra pro identifikaci a studium chemické struktury látek se běžně uznává. To, že pro identifikaci látek lze využít hydrodynamický voltamogram, který odráží chemickou strukturu a vlastnosti látky, je známo jen v omezené komunitě specialistů využívajících multikanálové elektrochemické detektory. Elektrochemické detektory s coulometrickou účinností detekce typu Coulochem a CoulArray nalezly široké uplatnění v oblasti klinické biochemie pro stanovení nízkých hladin plazmatických katecholaminů a umožnily výzkum neurotransmiterů již minimálně před více než 20 lety. Dávno před příchodem dnešních LC/MS/MS. Pro většinu odborníků v oboru využívajících analytických separačních věd zůstávaly elektrochemické detektory stranou zájmu. Pro běžného chromatografistu byl elektrochemický detektor „detektorem poslední volby“ a nástup spojení kapalinové chromatografie ve spojení s hmotnostní spektrometrií pro ně znamenal dlouho toužebně očekávaný nástroj stopové analýzy. Na druhé straně elektrochemie principiálně hraje roli ovlivňující podobu hmotnostního spektra látky a jsou patentovány iontové zdroje v kombinaci s elektrochemickou celou pro LC/MS. On-line spojení elektrochemie a hmotnostní spektrochemie představuje efektivní nástroj pro predikci oxidační stability, metabolismu a toxicity látek a jejich metabolitů.

Institut Nutrice a Diagnostiky (IND) v Pardubicích vznikl s podporou projektu MPO EA 4.2PT03/126 VIK RADANAL v letech 2011 až 2014.

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PODĚKOVÁNÍ:

Tato práce vznikla s podporou následujících grantových projektů: LF15006 Introdukce nových odrůd třešní s vysokou kvalitou plodů na evropský trh, NV15-27580 Vnímání chutí, oxidativní poškození a mikroprostředí střeva v kolorektální karcinogeneze: důsledky na riziko nemoci, jeho prognózu a prevenci. QJ1510354 Tvorba a selekce odrůd jabloní s vysokým obsahem zdraví prospěšných látek a prodlouženou skladovatelností plodů

P-005:
Delayed reactant labeling: A useful method for the determination of reactive intermediates kinetics

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Mass spectrometry with electrospray ionization (ESI-MS) is an important method for the investigation of reaction mechanisms. Reactive intermediates that are often present at very low concentrations can be studied by this technique and their reactivity and structure can be determined by collisional experiments or by ion spectroscopy approaches. However, the relative intensities of the peaks in a mass spectrum do not need to reflect the concentrations of the given ions in the sprayed solutions. This makes monitoring of reaction kinetics often a big challenge.

Recently, we invented a new method for the investigation of the reaction kinetics of intermediates in solution by ESI-MS. This method is based on studying of a reaction mixture containing isotopically labeled and unlabeled reactants at different reaction times. We have applied this method for the investigation of the hydration of alkyne catalyzed by gold(I). We have studied rate constants for the degradation of two intermediates, which contain one or two gold atoms. We have determined kinetic isotope effects for the formation and decomposition of these intermediates. We have also studied the effect of the counterion of the gold catalyst on kinetic data of hydration of alkyne.

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LITERATURA:

1. *Jašíková L. et al.; J. Am. Chem. Soc. 137, 13647 (2015).*

PODĚKOVÁNÍ:

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P-006:

Study of pH dependent structural changes of ClC-ec1 chloride/proton antiporter.

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Chloride channels belong to an extensive family of transmembrane proteins, whose dysfunction may cause a wide range of illnesses. A detailed study of the structural changes during ion passage enables us to understand the transport mechanism and can provide valuable information required for effective treatment. Despite the fact that these proteins are named as channels some members of the chloride channel family behaves like transporters. Typical member of the transporter group is bacterial chloride transporter from *E. coli* (ClC-ec1) which antiports two protons for a single chloride anion. This mechanism is widely studied and ClC-ec1 is considered a model protein for chloride transporters. Based on the high-resolution X ray transport mechanism based on amino acid side chain movements was proposed. However the inverted topology repeats organization of ClC-ec1 suggests that larger protein movement encompassing inward and outward facing conformation can be expected. These conformations can be induced by pH changes.

Our aim was to study the ClC-ec1 conformations in the outward (pH 4.5) and inward (pH 7.5) facing state and the transition between them using HDX-MS. The data indicate significant differences in several protein regions. In order to better understand the transition, finer pH stepping was also followed. Based on the data we can conclude that the transitions is gradual through at least one other conformation. Selected mutant forms of ClC-ec1 were designed and will be now expressed, purified and subjected to HDX-MS analyses.

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P-007:
Gas-phase studies of organometallic anticancer ruthenium(II) complexes

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For the last couple of years, organometallic ruthenium(II) complexes have been intensively studied as potential anticancer chemotherapeutics [1]. Biological activity and pharmacological properties of these complexes can be easily tuned by a change of a ligand.

In this study, the binding of two different "piano-stool" ruthenium(II) complexes to thiol containing biomolecules such as *N*-acetylcysteine (NAC) and glutathione (GSH) have been investigated by electrospray ionization mass spectrometry (ESI-MS). The ruthenium(II) complexes of our interest are [RuCYM(*p*-Cl-dkt)]Cl and [RuCYM(*p*-Cl-dkt)(pta)]PF_6 [2]. These complexes show distinct anticancer activity and act differently in the presence of NAC and GSH.

The pta unit prevents the hydrolysis of the [RuCYM(*p*-Cl-dkt)(pta)]PF_6 complex in aqueous solution. The addition of NAC leads to substitution of (*p*-Cl-dkt) ligand with NAC and a new ion is observed, identified as $[RuCYM(NAC\text{-}H)(pta)]^+$ complex. At higher concentration of NAC, an additional species is observed, identified as a $[RuCYM(p\text{-}Cl\text{-}dkt)(pta)NAC]^+$ complex. In this case the coordination sphere around the metal catalyst is preserved with the addition of a NAC unit. No new species were observed by the addition of GSH to a solution of pta complex.

On the other hand, the chloride complex [RuCYM(*p*-Cl-dkt)]Cl is easily hydrolyzed and the addition of thiol molecules to the solution leads to an immediate formation of bigger clusters containing two ruthenium such as $[Ru_2CYM_2(NAC\text{-}H)_3]^+$ or $[Ru_2CYM_2(GSH\text{-}H)_3]^{2+}$.

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LITERATURA:

1. *Ang W. H. et al.: J. Organomet. Chem. 696, 989−998 (2011).*
2. *Seršen S. et al. : J. Med. Chem. 58, 3984−3996 (2015).*

PODĚKOVÁNÍ:

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P-008:
The study of structural changes upon Hsp70 dimmerization

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The ubiquitous ATP-dependent heat shock proteins Hps70 create in coordination with its co-chaperones highly complex cell machinery which assists proper protein folding, prevents their aggregation or mediates degradation. The increased levels of such complexes were identified in cancer cells and thus represents interesting targets in cancer therapy. This project is focused on the study physiologically relevant Hsp70 dimmerization driven by the ATP induced allosteric motions and its subsequent inetraction with Hsp40 co-chaperone.

Hsp70 and its selected mutants (T204A with compromised ATPase activity; N540A and E543A and double mutant N540A-E543A with disturbed dimer stability) were expressed in *E. coli* and purified. Their functionality was tested by luciferase refolding assays. Size exclusion chromatography showed that the wild type Hsp70 forms dimers in the ATP bound state. This dimerization was further stabilized by the T204A mutation whereas N540A and E543A mutation had destabilizing effect. The proteins were then subjected to H/D exchange in the presence or absence of ATP and same conditions were also followed by chemical cross-linking. Finally, the proteins were also analyzed by native ESI.

Interestingly, the proteins failed to electrospray as dimers regardless the ATP addition with the only exception of T204A for which the dimer was detected. H/D data then showed structrual motions accompanying ATP binding and hydrolysis as well as differences between the individual Hsp70 forms. Chemical cross-linking should provide the orientation of the two Hsp70 monomers in the dimer as well as their conformational changes. These analyses are currently underway.

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P-009:

Selected ion flow-drift tube, SIFDT, study of reactions of H_3O^+ and NO^+ with primary alcohols in the presence of water vapour under variable collisional energies.

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Alcohols are often present in foods and other biological media including exhaled breath, urine and cell culture headspace. Their analysis by chemical ionisation techniques such as SIFT-MS, PTR-MS and SIFDT-MS relies on the ion chemistry initiated by the reactions of the reagent ions H_3O^+ and NO^+ with alcohol molecules in the presence of water molecules. Kinetics of such reactions needs to be understood and quantitatively described to facilitate reliable quantitative calculation of absolute concentrations of alcohols in humid air samples. The reactions of H_3O^+ and NO^+ ions have been studied with the primary alcohol molecules in SIFDT analyses (2 mbar He; 0.08 mbar air sample; 300 K; reduced field strength *E/N* up to 28 Td) and over a range of sample gas humidity up to 5.5%. The H_3O^+ reactions leads to the formation of protonated alcohol molecules MH^+ and (MH^+H_2O) fragments. The NO^+ reactions result in the $(M\text{-}H)^+$ product ions. These primary product ions hydrate to $MH^+(H_2O)_{1,2,3}$ and $(M\text{-}H)^+(H_2O)_{1,2,3}$. The variation of the percentages of the hydrated product ions with *E/N* and with the air sample humidity is studied. The experimental results are compared with a reaction system kinetics model based on numerical solution of a complete set of differential equations describing the ion chemistry. The data resulting from this study including the secondary hydrated ion product distributions will facilitate analyses of alcohol vapours in various media.

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P-010:
MALDI-TOF MS profiling as a tool for identification of sand fly juvenile stages

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Phlebotomine sand flies are small blood-sucking insects of high medical and veterinary significance. Females represent well-known vectors of viruses, bacteria, and most importantly parasites of the genus *Leishmania* causing leishmaniasis. To ensure proper vector control and monitoring in epidemiological studies a method for accurate species identification is highly desirable. Recently, we have introduced MALDI-TOF protein profiling as a reliable approach for species differentiation of adult sand flies. Our ongoing research is further focused on sand fly immature stages precise identification of which is practically impossible using classical morphological approaches.

The aim of the study was to evaluate the usefulness of MALDI-MS for identification of different life stages of sand flies and map the changes in their protein profiles during the whole life cycle. Specimens of six different sand fly species from the L2 to L4 larval stages and pupal stage were subjected to MALDI-TOF analysis. For larvae, the spectra were reproducible and easily distinguishable at the species level, and the profiles were stable from the L2 to L4 developmental stages. Therefore only profiles of L4 stages were selected for the creation of juvenile reference database. The performance of the database was tested in a blind study where 123 specimens (from L2 to L4) of six different species were included. The test resulted in 92.7% correct identifications regardless the larval stage. Pupal spectra allow identification at species level as well and surprisingly, they did not change during metamorphosis as observed for other holometabolous insects. The study demonstrates that MALDI-TOF MS is a suitable method for identifying not only adults, but also immature stages of phlebotomine sand flies.

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LITERATURA:

1. *Dvorak V. et al.: Parasit. Vectors 7, 21 (2014).*

PODĚKOVÁNÍ:

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P-011:
Formation of gold-acetonyl complexes

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Gold complexes are attractive catalysts because they can mediate a wide range of transformations [1]. Many studies showed that gold(I)-mediated reactions are catalyzed by cationic gold complex. It therefore represents an ideal field for mechanistic investigations using electrospray ionization mass spectrometry (ESI-MS).

Diaurated complexes and their role as intermediates as well as catalysts or catalysts' precursors are one of the hot topic in gold catalysis [2]. In our contribution, we studied the formation and stability of a digold complex with deprotonated acetone as a counterion ($[Au_2(L)_2(CH_2COCH_3)]^+$, where L is a ligand). The structure of this complex was confirmed by infrared multiphoton dissociation and infrared photodissociation spectroscopy and calculated by density functional theory. This complex is a valuable precatalyst in gold(I)-catalyzed reactions and in silver free catalysis [3].

We studied the kinetics of the gold-acetonyl species formation as well as the effect of the composition of the reaction mixture on the kinetics of its formation. Reaction of a gold(I) complex with a mixture of acetone and acetone-d_6 and water leads to the formation of the labeled and unlabeled diaurated gold-acetonyl complex. Their relative abundance gives the kinetic isotope effect (KIE), directly correlated to the C-H bond activation of acetone. The measurement of KIEs shows that there are two alternative mechanisms for the formation of the complex. With small water concentrations in the solution, the direct keto-enol tautomerization of acetone is the key step, while increasing water concentration leads to the formation of a digold hydroxide that catalyzes the formation of the diaurated gold-acetonyl species.

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LITERATURA:

1. *Hashmi A. S. K.: Top. Organomet. Chem. 44, 143-164 (2013).*
2. *a) Roithová J. et al.: Angew. Chem. Int. Ed. 51, 8378-8382 (2012); b) Jašíková L. et al.: J. Am. Chem. Soc. 137, 13647-13657 (2015); c) Gómez-Suárez A. et al.: Chem. Eur. J. 22, 1125-1132 (2016).*
3. *a) Gasperini D. et al.: Chem. Eur. J. 21, 5403-5412 (2015); b) Gatto M. et al.: ACS Catal. 6, 7363–7376 (2016).*

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P-012:
Spin state controlled photodissociation of iron(III) azide to iron(V) nitride complex in the gas phase

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High-valent iron compounds bearing a very reactive Fe-O or Fe-N bonds are engaged in many important biological and chemical processes [1]. Their involvement in reactions such as oxidation of unactivated hydrocarbons or fixation of nitrogen has attracted attention of many scientists. In nature, these transformations are accomplished by enzymes, which are difficult to study. This is the reason why much smaller compounds – synthetic models – featuring only the ligated active metal species have been developed and studied to aid the understanding of natural systems [2]. There is only a handful of examples of well-characterized iron(V)-nitrido complexes, mainly because of their high reactivity [3]. The primary route for their generation is the photodissociation of iron(III)-azido precursor.

In this work, various mass spectrometry methods coupled with the ion spectroscopy have been used for the investigation of dissociation channels of iron(III) azide to elusive iron(V) nitride in the gas phase. The temperature-dependent photodissociation spectra in the visible range have shown that the iron(III) azide complex is populated mainly in the sextet spin state at higher temperatures and in the doublet spin state at low temperatures. The iron azide and nitride complexes were fully characterized by the helium-tagging infrared photodissociation (IRPD) spectroscopy at 3 Kelvin and by the infrared multiphoton photodissociation spectroscopy at 300 K in the case of iron azide. The IRPD spectroscopy has been shown to be a convenient method for otherwise challenging characterization of Fe-N stretching vibration. All experiments are accompanied with the DFT and the multireference *ab initio* calculations, which support the experimental data.

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LITERATURA:

1. *Abu-Omar M.M. et al.: Chem. Rev. 105, 2227 (2005).*
2. *Hohenberger J. et al.: Nature Commun. 3, 720 (2012).*
3. *Smith J. et al.: Dalton. Trans. 41, 1423 (2012).*

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P-013:
Probing glycosylation pattern of fungal cellulolytic enzymes

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Cellobiose Dehydrogenase (CDH) and Lytic Polysaccharide Monooxygenase (LPMO) are essential parts of the cellulolytic system in wood degrading fungi. CDH is composed of two subunits, larger catalytic C-terminal domain harboring FAD molecule and smaller N-terminal cytochrome domain. Both domains are connected by a flexible linker region. FAD domain oxidizes disaccharides and the generated electrons are forwarded to the cytochrome domain by intramolecular electron transfer (IET). Reduced heme in the cytochrome domain then fuels other enzymes, such as LPMO which oxidatively cleaves microcrystalline cellulose into easily degradable oligosaccharides. Both enzymes have high potential for use in biotechnological applications and thus the understanding of their function is of high importance.

IET is highly dependent on domain distance and post-translational modifications like N- and O-glycosylation, especially in the linker region, can affect the enzyme kinetics. This can be problematic as yeast or fungal expression systems providing high yields of CDH and LPMO tend to over-glycosylate produced enzymes affecting their function and interaction. The goal of this work is to find optimal producing organism and conditions, describe the glycosylation pattern and finally use the most suitable enzymes for structural studies of the CDH-LPMO system during cellulose degradation. Different forms of CDH and LPMO from *Neurosporra crassa* produced in *Pichia pastoris* and CDH from *Crassicarpon thermofilum* produced in *Trichoderma reesei* were studied and compared with respect to their N- and O-glycosylation pattern.

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P-014:

Copper(III)-oxo complexes: Spectroscopic characterization of a terminal Cu-O bond and reactivity in the gas phase

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High-valent metal-oxo complexes are known as active intermediates in hydrogen-atom-transfer and oxygen-atom-transfer reactions in biological and organic systems. These processes produce oxygenated products, such as alcohols or epoxides. Reactions initiated by hydrogen-atom transfer can also result in the substrate desaturation and the water oxidation [1, 2].

In this study, the terminal copper-oxo complex is spectroscopically identified which has previously not been characterized. We present a new approach for the generation and the characterization of the copper(III)-oxo complexes $[(L)CuO]^+$ (L=PQ and ACN). These complexes are formed by electrospraying a methanolic solution of a ligand and copper(II) chlorate salt. The $[(PQ)CuO]^+$ complex has been characterized by infrared photodissociation (IRPD) spectroscopy. In addition, we performed a labelling experiment in order to assign the copper oxygen stretching vibration. The Cu^{III}-O band is found at 718 cm^{-1} and redshifts to 690 cm^{-1} upon ^{18}O labelling. We also employed theoretical calculations to support the interpretation of the experimental results. The gas phase reactivity of the complexes has been probed in reactions with alkane, alkene, and water. The $[(CH_3CN)CuO]^+$ complex is capable of water oxidation and oxygen exchange reaction, whereas $[(PQ)CuO]^+$ provides only oxygen exchange reaction. $[(CH_3CN)CuO]^+$ activates ethane, but reactivity of $[(PQ)CuO]^+$ with alkanes is only moderate (C-H activation has been observed only for propane and larger alkanes) [3]. On the other hand, both complexes readily undergo oxygen-transfer reactions with the C=C bond of ethylene.

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LITERATURA:

1. *Engelmann X. et al.: Angew. Chem., Int. Ed 55, 7632-7649 (2016).*
2. *Gagnon N. et al.: Acc. Chem. Res. 48, 2126-2131 (2015).*
3. *Roithová J. et al.: J. Am. Chem. Soc. 129, 15311-15318 (2007).*

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P-015:
Native mass spectrometry helps of to decipher multimerization rules of G-quadruplex oligonucleotides

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G-quadruplexes can multimerize under certain conditions depending on the sequence combinations. Out of 256 variants of central tetrades, approximately 10% forms dimeric or tetrameric structures. Some mutants could also form heteromultimeric structures. Native ESI mass spectrometry can reveal the oligomeric preference of the given oligonucleotide sequence. We thus applied native ESI mass spectrometry with ion mobility to analyse several quadruplex sequences that form different oligomers on native gel and calculated the oligomeric status from isotopic distribution and charge state of individual ions. We also measured mixed samples of different sequence to see that the oligonucleotides can form heteromultimers.

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P-016:

Low field flow-drift tube study of the H_3O^+, NO^+, O_2^+ ion-molecule chemistry of the environmentally significant volatile organic compounds for their accurate concentration quantification

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Currently several different types of the chemical ionization mass spectrometry instruments are being developed to meet the increasing needs for the on-line volatile organic compound, VOC, concentration measurements. All of them are based on the ion-molecule reactions in the tube reactor, either at carrier gas velocities and energies (SIFT-MS) or at the energies governed by the electric drift field (SIFDT-MS, PTR-MS, SRI-MS and others). The most obvious advantage of the drift tube techniques is the suppression of the ion diffusion losses to the walls of the tube reactor that leads to the high reagent ion signals and thus high sensitivity of the instrument. But it is necessary to pay proper attention to the ion-molecule processes when the drift field is applied due to the elevated interaction energies between reagent ion and neutral analyte of interest. That can change the preferred channels of the reaction, its rate coefficient and product ion fragmentation ratios so they are different than the values available from the thermal studies. All these factors influence calculations of the VOCs concentration.

This poster presents how the reaction rate coefficient of H_3O^+, NO^+, O_2^+ ion-molecule reactions and their product ion ratios depend on the interaction energies up to 0.5 eV. These data were obtained using the selected ion flow-drift tube SIFDT instrument [1], where the ion residence time in the reactor are routinely measured utilizing the Hadamard transformation [2]. This residence time is used to calculate ion velocity and finally the energy of ion-molecule collisions in accordance to the Wanier equations. The impact of the present results on the precision of the VOCs concentration calculation is discussed.

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LITERATURA:

1. *Spesyvyi A. et al: Anal. Chem. 87 (24), 12151-12160 (2015).*
2. *Spesyvyi A. Et al.: Rapid Commun. Mass Spectrom., 29, 1563-1570 (2015).*

PODĚKOVÁNÍ:

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P-017:
Transcription factor – DNA interaction studied by structural mass spectrometry

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Transcription factors (TF) regulate gene expression through interactions with DNA and other regulatory proteins. Understanding of this regulation depends on the knowledge of the TF-DNA complex structure, which is accessible by the conventional methods (X-ray, NMR, EM), yet the process is still challenging. Thus, the development of faster alternative approaches allowing detailed description of a TF-DNA complex is beneficial. To design a reliable mass spectrometry technology, well-characterized model complex was selected. It consisted of the DNA-binding domain of Forkhead-box transcription factor (FOXO4), with its target DNA sequence – DAF-16 DNA native response element. A palette of MS based approaches, including H/D quantitative protein-protein, protein-DNA cross-linking and an ion-mobility measurements, was applied to investigate the FOXO-DNA complex structure in solution.

Based on quantitative chemical cross-linking, structural models for both states, FOXO4 alone and FOXO4/DAF-16 complex, were built by molecular dynamics simulation. These models suggest conformational changes caused by DAF-16 DNA binding in DNA-binding domain nearby regions, that have not been observed yet.

Initial experiments with protein-DNA cross-linking showed trans-platinum (trans-Platinum(II)diammine dichloride) as a suitable cross-linking reagent for double-stranded DNA/TF complexes. Together with the development of protein-DNA cross-linking chemistry a data processing software was created.

All these data clarified the interaction interface region and added new details to up to date structural information of FOXO4/DAF-16 response element complex and also promise a good start point for the development of new *in vivo* protein-DNA interaction identification approach.

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P-018:

Gas-phase infrared spectroscopic study of iron(IV)-oxo complexes: Fe=O stretches, spin-states, and reactivities

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We used Helium-tagging cryogenic ion spectroscopy to determine the Fe=O stretching frequencies of a series of high-valent iron(IV)-oxo complexes with amino(pyridine) ligands [1]. These serve as spectroscopic models for the biologically important non-heme iron enzymes. The gas-phase frequencies are blue-shifted by ~9 cm^{-1} with respect to those reported in solution. In addition, we were able to assign the coordination mode and the spin state of selected complexes by observing shifts in characteristic vibrational modes of coordinated anionic ligands (triflate, nitrate, trifluoroacetate). We also demonstrate the remarkable influence of the anionic ligand on gas-phase reactivities and spin states, which shed a new light on some of the earlier theoretical studies.

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LITERATURA:

1. *Andris E. et al.: J. Am. Chem. Soc. 139, 2757-2765 (2017).*

PODĚKOVÁNÍ:

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P-019:
Proteolytic MALDI compatible chips for structural proteomics

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The effective proteolytical hydrolysis of studied proteins represents essential step in the most proteomics studies. Therefore, proteases with different mechanism of peptide bond hydrolysis are commonly used in some specific application. Aspartic proteases are often used in H/D exchange analysis due to their activity optimum at acidic pH, whereas serine proteases operating around neutral pH are suitable for other proteomics experiments. Firstly, there is a limited proteolysis, but serine proteases are used for protein identification or disulfide bond determination as well. Due to many reasons, proteases have been immobilized on various solid substrates. Enzyme immobilization has usually been mediated by chemical cross-linker, but recently we have used another approach for biochips preparation - electrospray based ambient soft-landing. This approach operates at the atmospheric pressure conditions what enables to form strong and stable protein-surface interaction, while biomolecules still retain their native structure and function.

Each method of structural proteomics requires different conditions for effective protein digestion. Prepared proteolytic Indium Tin Oxide surfaces enable rapid *in situ* digestion directly followed by MALDI FT-ICR mass spectrometric analysis. The functionality of proteolytic surfaces was demonstrated on comparing of two different equine myoglobin conformations. Serine specific protease trypsin and semi-specific chymotrypsin have been used for protein identification and together with unspecific protease from Bacillus subtilis can serve as a novel tool for limited proteolysis experiments. Furthermore, immobilized aspartic proteases pepsin, type XIII and XVI has been used for *in situ* protein H/D exchange products analysis.

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P-020:

Kombinace termální desorpce a hmotnostní spektrometrie v proudové trubici s vybranými ionty, SIFT-MS, pro analýzu izomerických terpenů

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V současné době se ukazuje potřeba nové analytické metody, která by umožnila selektivně a přesně stanovit koncentrace izomerických BVOCs v atmosféře v reálném čase. Je to proto, že správný odhad toků těchto biogenních emisí je nezbytný pro modelování fotochemických dějů v ovzduší, jako je například tvorba troposférického ozonu a organických aerosolů.

Proto nyní pracujeme na vývoji nových analytických metod (viz poster P-016 Mgr. Anatolii Spesyvyi PhD.: Flow-drift tube study of the H_3O^+, NO^+, O_2^+ reaction kinetics with biogenic volatile organic compounds in weak electric fields for their accurate concentration quantification by chemical ionization mass spectrometry techniques.), které by umožnily selektivní monitorování BVOCs, zejména jednotlivých izomerů mono- a seskviterpenů, které jsou pro atmosférickou chemii obzvláště důležité, a to v reálném čase (<doba odezvy 1 s, která odpovídá typické rychlosti změny rychlosti větru). Rozlišení izomerů je nutné zejména proto, že různé terpeny vykazují vysoce variabilní reaktivitu a jejich příspěvky ke vzniku přízemního ozonu a organických aerosolů jsou různé.

Vzhledem k velmi nízkým koncentracím (< 1 objemová miliardtina, ppbv) monoterpenů a seskviterpenů je vhodnou metodou hmotnostní spektrometrie. Pro analýzu odebraných vzorků byla využita hmotnostní spektrometrie v proudové trubici s vybranými ionty v kombinaci s nově vyvinutým zařízením pro termální desorpci. Výsledky ukazují, že je možné rozlišit β-pinene, 3-carene, S-limonene ve směsi na základě proměnlivé desorpční teploty.

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P-021:
Radiosensitization of non-small cell lung cancer cells by autophagy inhibitor Lys05: phosphoproteomic analysis

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Autophagy is a basic cellular process, which enables cells to adapt and survive in stress conditions, including exposure to ionizing radiation. By Recycling of macromolecules cells are able to obtain energy and keep homeostasis. Hence, autophagy is believed to represent a radioprotective mechanism. This radioprotective mechanism is largely utilized by cancer cells during radiotherapy leading to radioresistance. Inhibition of the cytoprotective autophagy seems to be a reasonable approach to sensitize cancer cells. In this respect, several inhibitors of autophagy have been synthesized, but radiosensitizing mechanism has not been fully understood yet.

In order to characterize the radiosensitizing mechanism, we employed SILAC approach based on quantitative phosphoproteomics. Non-small cell lung cancer cells H1299 (representative cancer treated by radiotherapy) were selected as a model cell line in order to study the effect of Lys05, a newly synthesized inhibitor of autophagy. The experimental workflow was based on shotgun proteomics with subsequent enrichment for phosphopeptides using TiO_2 microspheres followed by final identification and quantification of proteins/phosphosites using LC-MS/MS. In total, more than 1,100 proteins were identified with more than 6,000 phosphosites. Proteins that were significantly modified were mostly localized in nucleus with role in regulation of macromolecule metabolic and biosynthetic processes, as well as in cellular response to stress. In addition, regulation of mTOR pathway (the crucial signaling associated with activation of autophagy) was observed suggesting one of the possible mechanisms involved in lung cancer cells radiosensitized by Lys05. Future work will be focused on description of biological processes and further validation.

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P-022:
Ion-induced ionization and dissociation of simple organic molecules by selected keV projectile ions

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The keV ion-induced processes in isolated gas-phase atoms and molecules have been a subject of numerous studies in the last few decades. These studies were motivated by an effort to understand molecular mechanisms of radiation damage of biological systems [1], as well as other high-energy ion/molecule processes such as star wind interactions with planetary atmospheres, interstellar clouds and other objects in space [2, 3]. The variety of ions used in these studies was, however, rather limited. Especially in terms of their chemical nature, which covered mostly small atomic cations (H^+, He^+, He^{2+}, O^+, N^+, etc.) and only a very few simple molecular cations (N_2^+, O_2^+, C_{60}^+, etc.). Moreover, only large scale facilities, like accelerators, have been used for such experiments so far, making these experiments available only to a small number of scientists.

In order to broaden the range of these studies, we measure 5 – 10 keV projectile ion collisions with neutral molecular targets. For that matter, an EI/CI sector type mass spectrometer was modified. The modifications include collision chamber and quadrupole mass analyser which is to analyse secondary ions stemming from the collisions of projectiles with neutrals. Pressure inside the collision chamber, as well as projectile ion current, are measured absolutely so that we are able to determine collision cross sections for given processes from the secondary ion count rates. The amount of internal energy deposited in secondary ions, as reflected by the extent of their fragmentation, is determined from the acquired mass spectra. Some general trends and selected specific results will be presented.

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LITERATURA:

1. *Gomez-Tejedor G. G., Fuss M. C., Radiation damage in biomolecular systems (Springer, New York, 2012).*
2. *Postma J. et al.: Astrophys. J. 708, 435-444 (2010).*
3. *Vuitton V. et al.: Icarus 191, 722-742 (2007).*

PODĚKOVÁNÍ:

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P-023:

Development, optimization and validation of an analytical method for determination of the neurotoxin β-N-methylamino-L-alanine in water using LC/MS

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This work deals with the development, optimization and validation of an analytical method for determination of the neurotoxin β-N-methylamino-L-alanine in water using LC/MS. The β-N-methylamino-L-alanine is a neurotoxic amino acid produced mostly by various ubiquitous planktonic organisms [1]. It has been demonstrated that this neurotoxin causes the death of motor neurons already at concentration of 30 μmol/L [2]. The neurotoxin is associated with untreatable neurodegenerative diseases such as amyotrophic lateral sclerosis [3]. Analyses were performed on liquid chromatograph coupled with tandem mass spectrometry detector. Hydrophilic stationary phase was based on sulfobetaine and mobile phase was a mixture of acetonitrile and deionized water acidified with 0.1 % of formic acid. Firstly, the development and optimization of the analytical method was performed. Secondly, the analytical method was successfully validated with linear range 0.1 – 100 μg/L. Precision of the method ranged from 92 to 112 % and accuracy ranged from 0.3 to 5.8 %. The instrumental detection limit was 10 ng/L and the instrumental quantification limit was 30 ng/L. Then, the validated method was applied to spike samples to assess whether the method provides results with sufficient accuracy and precision with regard to possible matrix effects and sample preparation procedure. Precision of the method ranged from 76 to 116 % and accuracy ranged from 1.4 to 1.5 %. The method detection limit was 82 ng/L and the method quantification limit was 270 ng/L. Finally, the method was applied to the analysis of real samples which were obtained from Culture Collection of Autotrophic Organisms in Třeboň. In three of six samples, the trace levels of a neurotoxin were determined. In other samples, no BMAA was found.

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LITERATURA:

1. *Pablo J. et. al.: Acta Neurol. Scand. 120(4), 216-225 (2009).*
2. *Cox P. A. et. al.: Proc. Natl. Acad. Sci. U.S.A. 102(14), 5074-5078 (2005).*
3. *Banack S. A. et. al.: Toxins 2(12), 2837-2850 (2010).*

P-024:
Protein composition of experimental chemovacccine against Q fever

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Q fever is a highly infectious, airborne zoonotic disease caused by *Coxiella burnetii* bacterium. *C. burnetii* infection is most often latent in animals, but under certain circumstances display low production, infertility, endometritis, placentitis, abortions, stillbirth, and delivery of weak offspring. Humans are usually infected by contaminated aerosols from domestic animals. Many human infections, however, result in nonspecific or benign constitutional symptoms, establishing a diagnosis of Q fever is often challenging for clinicians [1].

Despite the fact that several publications highlight the value of vaccination is implementing, there is no commercially available human vaccine against Q fever except a formalin-inactivated cellular Q-VAX® licensed for use only in Australia. The Institute of Virology, SAS in Bratislava, has developed a subunit experimental vaccine against Q fever that is commendable for licensing. It is composed of soluble antigens extracted to trichloroacetic acid. This vaccine was successfully administered to hundreds of volunteers in the past, and no significant side effects were observed [2, 3].

The aim of this work was to determine the chemical composition of this extract and characterize immune response to identify factors with protective efficacy. We applied the 2D electrophoresis-based strategy. The proteins from extracts were separated using pI strips in the range 3-10 followed by Western blot analyses. Using mass spectrometry we have analyzed 75 spots, from which 38 were identified as unique proteins, and 12 from them were immunoreactive.

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LITERATURA:

1. *Georgiev M. et al: Euro Surveill 18(8), pii=20407 (2013).*
2. *Kazar J. et al: Bulletin of the work Health organization 60(3), 389-394 (1982).*
3. *Kovacova E. et al: Acta virologica 42, 365-368 (1998).*

PODĚKOVÁNÍ:

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P-025:

Separation and identification of lipids in the photosynthetic cousins of Apicomplexa *Chromera velia* and *Vitrella brassicaformis*

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The alveolate algae *Chromera velia* and *Vitrella brassicaformis* (chromerids) are the closest known phototrophic relatives to apicomplexan parasites. Apicomplexans are responsible for fatal diseases of humans and animals and severe economic losses. Availability of the genome sequences of chromerids together with easy and rapid culturing of *C. velia* makes this alga a suitable model for investigating elementary biochemical principals potentially important for the apicomplexan pathogenicity. Such knowledge allows us to better understand processes during the evolutionary transition from a phototrophy to the parasitism in Apicomplexa. We explored a complete lipidome of both algae by means of high-performance liquid chromatography coupled with mass spectrometry (HPLC-MS) or gas chromatography and flame ionization detection (GC-FID). Single HPLC-MS analysis in both ionization modes was sufficient for the separation and semiquantification of lipids in chromerid algae. We detected more than 250 analytes belonging to five structural lipid classes, two lipid classes of precursors and intermediates, and triacylglycerols as storage lipids. Identification of suggested structures was confirmed by high-resolution mass spectrometry (HRMS) with an Orbitrap mass analyzer. An outstandingly high accumulation of storage triacylglycerols was found in both species. All the investigated aspects make *C. velia* a prospective organism for further applications in biotechnology.

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P-026:
Quantification of karrikins in smoke water using UHPLC-MS/MS

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Karrikins (KARs) are a family of butenolide lactones, that were identified in smoke water (SW) from burning vegetation (Flematti et al., 2004, Van Staden et al., 2004). SW contains a mixture of different substances from which KAR1 (3-methyl-2H-furo[2,3-c]pyran-2-one) is the most active and abundant (Flematti et al., 2015). In germination bioassay KARs showed activity at concentration below 10-9M (Light et al., 2009, Nelson et al., 2012). In nature they play a very important role in restoration and creation of habitat for new vegetation after wildfires (Light and Van Staden, 2016). However, to our knowledge, no method suitable for monitoring karrikinins in biological matrices have been developed and published. Therefore, we employed a new analytical approach for quantification of KARs using UHPLC-MS/MS. Due to the separation by reverse phase and quantification by multiple reaction monitoring we developed a fast, specific and sensitive method which will give us the possibility to study the effect of these new interesting class of biostimulants in more detail. Results showing differences in levels of KARs in SW of different origin will be discussed.

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P-027:
Comparison of GC-VUV, GC-FID, and GCxGC-TOF-MS for characterization of weathered diesel fuels

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The potential environmental impact of diesel fuel spills is significant considering its vast consumption. Thus, providing definitive information for litigation regarding characterization and determination of the origins or sources of the fuel spill contamination in the simplest fashion has been a significant need among the environmental forensics community. Diesel fuel contains complex group of components like paraffins, naphthenes, aromatics, and other hydrocarbons ranging from C10-22 with a large amount of isomers which are very hard to be separated and determined by one dimensional GC. Comprehensive two-dimensional GC (GCxGC) is a better technique for speciating complex mixtures, but it requires significant experience and more complicated hardware. Here we evaluate the use of GC – vacuum ultraviolet spectroscopy (GC-VUV) as a potential alternative to GC with flame ionization detection (GC-FID) and GCxGC with mass spectrometric detection (GCxGC-TOF-MS) for diesel fuel and weathered diesel fuel analysis. Diesel fuel, 25% weathered diesel fuel, 50% weathered diesel fuel, and 75% weathered diesel fuel have been analyzed by GC-VUV, and the results have been compared with GC-FID and GCxGC-TOF-MS. Different classes of compounds and biomarkers have been successfully separated and identified by GC-VUV. Deconvolution for different aromatic species have also been achieved. Meanwhile, isoprenoid biomarkers like pristane and phytane fuel have been determined. GC-VUV shows closer biomarker ratio results with GCxGC-TOF-MS compared to GC-FID. The comparison showed that GC-VUV provides a complementary means for solving challenging qualitative problems in diesel fuel analysis.

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PODĚKOVÁNÍ:

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P-028:

Plant hormonomics: A multiple phytohormone profiling by targeted metabolomics approach

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Plant hormones are highly bioactive signalling molecules, which are acting as chemical messengers involved in physiological processes such as plant growth and development including flowering, seed germination, senescence and various stress responses. The occurrence as well as the levels of these compounds strongly depend on plant organ, plant age, developmental stage and environmental conditions. As characteristic for the substances of hormonal nature, they are typically present in plant tissue only in minute concentrations. Thus, their direct quantification in very complex plant extract poses a difficult analytical task.

Plant hormones as extremely large family of diverse compounds could be divided into several structurally different groups such as purine and indole derivatives, plant steroids, lipid-based substances and terpenoid carboxylic acids. In this study, we present a new method based on ultra-high liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) for targeted profiling of more than 100 analytes, members of the main classes of plant hormones like cytokinins, auxins, brassinosteroids, gibberellins, jasmonates and abscisic acid. This plant-based metabolomic approach involves primary metabolites of bioactive hormones as well as their biosynthetic precursors playing indispensable role in intricate signalling network leading to the regulation of various biological processes in plants. We believe that this generalized analytical screening method can be very useful tool for phytohormonal studies dealing with their inter- and intra-cellular communications in plants.

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P-029:

Liquid chromatography with surface-assisted laser desorption/ionization mass spectrometry with silver nanoparticles in lipid analysis

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The presented work aims at system optimization combining reversed phase liquid chromatography (RP-HPLC) with on-line and off-line mass spectrometry detection for lipids determination. On-line detection was facilitated by atmospheric pressure chemical ionization mass spectrometry (APCI MS) and off-line detection by surface-assisted laser desorption/ionization mass spectrometry (SALDI MS) using suspension of silver nanoparticles as matrix.

A simple HPLC method with online UV and APCI MS detection was proposed for analysis of cholesteryl ester oleate (CHE). After separation, a splitter combined with a laboratory-built spotter was used for eluent deposition on a target over a layer of silver nanoparticles for SALDI experiments. Low SALDI detection limit for CHE (7 ng/ml) allowed determination of CHE in a human plasma sample.

Mass spectrometry with APCI and SALDI ionization were also applied for determination of glycerolipids triolein (TO), tripetroselinin (TP) and tristearin (TS). These analytes were characterized based on comparison of identified fragment ions from spectra obtained by those two techniques. Characteristic fragment ions predicting the double bond position in fatty acid chain were observed in SALDI spectra of TP and TO.

Combination of MS techniques with different ionization mechanisms may allow obtaining complementary information for better characterization of studied lipids in real samples.

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LITERATURA:

1. *Lísa, M. et al.: J. Chromatogr. A, 1198, 115-130 (2008).*
2. *Vrbková, B., et al.: J.Chromatogr. A, 1358, 102–109, (2014).*

PODĚKOVÁNÍ:

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P-030:
Detection of CTX-M-15, a clinically relevant β-lactamase, using MALDI-TOF MS

Jonathan Thacker [1], Brad Pierce [1], Kevin Schug [1 *]

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Infections caused by antibiotic-resistant bacteria are estimated to result in about 25,000 deaths and over €1.5 billion in economic losses each year in the European Union [1]. The emergence and spread of genes expressing β-lactamases, enzymes capable of degrading β-lactam antibiotics, is particularly concerning. Combating antibiotic resistance will require, among many complementary efforts, the ability to detect and monitor the rise and spread of genes and/or proteins conferring antibiotic resistance.

We have successfully detected an intact clinically relevant β-lactamase, CTX-M-15, in an expression system consisting of E. coli strain BL21 (DE3) transformed by an IPTG-inducible T7 expression vector, pF1K, harboring the gene blaCTX-M-15. We have also detected the tryptic peptides of CTX-M-15 from an in-gel digestion using MALDI-TOF MS resulting in a sequence coverage of 46%. We believe that this may be an important first step towards detecting and characterizing β-lactamases in clinical isolates.

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LITERATURA:

1. *European Centre for Disease Prevention and Control, European Medicines Agency. (2009) The bacterial challenge—time to react. http://www.ecdc.europa.eu/en/publications/Publications/0909_TER_The_Bacterial_Challenge_Time_to_React.pdf*

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P-031:
Beta-secretase kinetics detected by MALDI TOF MS

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Alzheimer's disease is a serious neurodegenerative illness affecting around 44 million people worldwide. The crucial role in disease development possibly plays the β-site APP-cleaving enzyme (β-secretase, BACE). Its overexpression leads to the production of αβ peptide (αβ42 rather than αβ40 isoform) and to the accumulation of toxic amyloid plaques in brain [1].

β-secretase activity can be measured by a commercial fluorescence resonance energy transfer (FRET) assay with fluorescently marked substrates, however, this approach is relatively insensitive and requires high concentrations of the enzyme. Alternative option is the detection of reaction products by mass spectrometry. Two distinctive techniques were established for this purpose, the first involving the capillary electrophoresis separation and electrospray mass spectrometry detection (CE-ESI MS). The advantages of this method are both sensitivity and preciseness, yet, it includes the time consuming separation step [2].

The separation may be completely omitted if using matrix-assisted laser desorption/ionization (MALDI), which employs UV-laser to desorb and generate ion molecules from the sample surface [3]. While MALDI is not perceived as quantitative in general, we showed that with suitable internal standard, it may represent easy and accurate method useful in enzymatic research. The Km for selected substrate and IC50 values for β-secretase inhibitor were in a good agreement with results obtained by CE-ESI MS.

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LITERATURA:

1. *Vassar R. et al.: Neurosci 29, 12787-12794 (2009).*
2. *Schejbal J. et al.: J. Chromatogr. A 1478, 235-241 (2017).*
3. *Karas M. et al.: Int. J. Mass Spectrom Ion Processes 78, 53-68 (1987).*

PODĚKOVÁNÍ:

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P-032:
Porovnání hmotnostně spektrometrických přístupů v proteomickém profilování léčiv

Tomáš Oždian [1 *], Dušan Holub [1], Gabriela Rylová [1], Jana Václavková [1], Marián Hajdúch [1], Petr Džubák [1]

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Hmotnostní spektrometrie je v současnosti jedním z hlavních přístupů pro identifikaci proteinů. K tomuto účelu se využívá několik typů přístrojů s rozdílnou ionizací a detekcí. Pro účely porovnání hmotnostních spektrometrů byla SILAC značená linie CCRF-CEM ošetřena třemi platinovými léčivy a následně byl po frakcionaci a digesci analyzován celobuněčný proteinový lyzát. Vzorky byly paralelně měřeny na třech hmotnostních spektrometrech s různými analyzátory – iontovou pastí (ESI-IT), analyzátorem doby letu (MALDI-TOF) a orbitální iontovou pastí (nESI-Orbitrap). Každý spektrometr byl spojen s vysokoúčinnou kapalinovou chromatografií a byl nezávisle optimalizován pro nejvyšší výkon. Data byla nezávisle analyzována, přičemž průměrný počet proteinů identifikovaných ESI-IT byl 660 ± 124 s 66% překryvem ve všech třech replikátech, u MALDI-TOF 355 ± 68 proteinů s 41% překryvem a u nESI-Orbitrap 3430 ± 306 proteinů s 76% překryvem. Kvantifikační přesnost vyjádřená jako R2 byla u ESI-IT 0,454 ± 0,047, u MALDI-TOF 0,524 ± 0,134 a u nESI-Orbitrap 0,692 ± 0,063. Při vzájemném porovnání se proteiny identifikované ESI-IT a MALDI-TOF lišily minimálně a byly beze zbytku identifikovány také pomocí nESI-Orbitrap.

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PODĚKOVÁNÍ:

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P-033:
Identifikace a kvantifikace těkavých látek v dechu a potu pro instrumentální charakterizaci lidského pachu

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Lidský metabolismus generuje řadu těkavých organických látek, které částečně opouštějí organismus v podobě par obsažených v dechu a některé mohou být vylučovány kůží ať již přímo nebo jako složka potu. Podobně bakterie vyskytující se v ústní dutině, dýchacích cestách, trávicím traktu a na pokožce produkují další těkavé organické látky, které přispívají k celkovému složení plynných látek někdy nazývanému jako „volatolom“. Cílem této studie bylo charakterizovat individuální složení těkavých látek v dechu a potu jednotlivců a ověřit možnost jeho využití pro identifikaci osob. Pro určení významných látek byla použita metoda SPME-GC/MS a pro jejich rychlou kvantifikaci SIFT-MS.

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LITERATURA:

1. *Martinez-Lozano Sinues P, et al.: PLoS ONE 8(4), e59909, (2013).*
2. *Španěl P. et al.: Mass Spectrom. Rev. 30, 236-267, (2011).*
3. *Curran A.M. et al.: J. Chromatogr. B 846, 86-97, (2007).*

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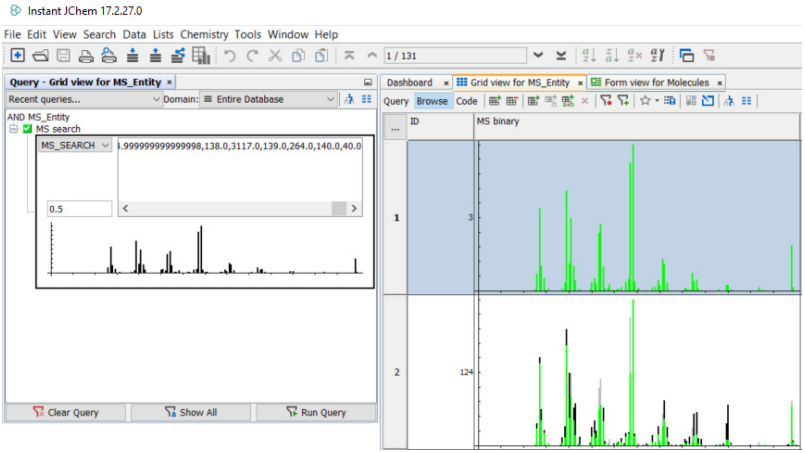

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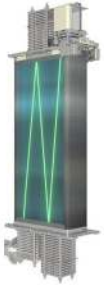

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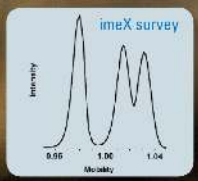

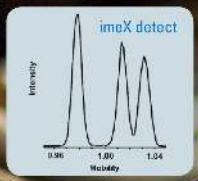

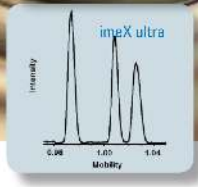

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