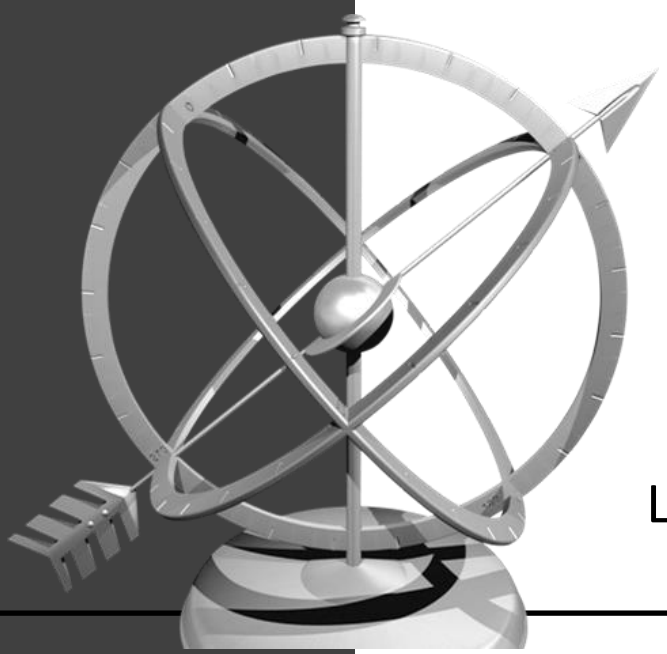
14th European Fourier Transform Mass Spectrometry Workshop & 3rd EFTMS School

Book of Abstracts



EFTMS 2022

Lisbon, 11 - 14 July

Book of Abstracts

14th European Fourier Transform Mass Spectrometry Workshop

& 3rd EFTMS School

Lisbon, 11 - 14 July 2022

http://eftms2022.campus.ciencias.ulisboa.pt

Editors

Carlos Cordeiro, Marta Sousa Silva *&* **António Ferreira**

Faculdade de Ciências da Universidade de Lisboa

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Preface

The EFTMS Workshop started in 1991 in Germany and takes place every two years. It aims to promote interaction and exchange between academy and industry researchers working in the field of Fourier transform mass spectrometry. Together with the workshop, the 3rd EFTMS School will follow its previous successful editions. Started in Italy in 2016, this school is targeted to PhD students, post-docs and any scientist who want to keep up with the most relevant developments in this field.

Welcome to Lisboa and to the 14th European Fourier Transform Mass Spectrometry Workshop & 3rd EFTMS School!

The Chair of the EFTMS 2022,

Carlos Cordeiro,

Faculdade de Ciências da Universidade de Lisboa

Laboratório de FT-ICR e Espectrometria de Massa Estrutural

Programme

11th July 2022 - SCHOOL

12:30 - 13:30	Registration + welcome cocktail
13:30 - 13:40	Opening school Carlos Cordeiro
13:45 - 14:30	Fourier Transform Ion Cyclotron Resonance Mass Spectrometry: Fundamental Concepts Peter O'Connor
14:30 - 15:15	2D FT-MS Christian Rolando
15:15 - 16:00	*Discussion over coffee*
16:00 - 16:45	Fundamental concepts of Orbitrap Alexander Makarov
16:45 - 17:30	FTMS proteomics methods for post-translational modifications Roman Zubarev
17:30 - 18:15	MRMS in the new world of metabolomics Carlos Cordeiro

12th July 2022 - WORKSHOP

09:30 - 10:00	Registration
10:00 - 10:15	Opening workshop Carlos Cordeiro & Luis Carriço
Session 1: FTMS fundamentals and instrumentation	**Chair: Alexander Makarov**
10:15 - 11:00	Expanding Capabilities of Orbitrap Instrumentation Alexander Makarov
11:00 - 11:20	*Coffee break*
11:20 - 12:05	FT Mass Spectrometer Based on Multielectrode Harmonized Kingdon trap Evgeny Nikolaev
12:05 - 12:50	Combining Ultraviolet Photodissociation and 2-Dimensional Mass Spectrometry Peter O'Connor
12:50 - 14:00	*Lunch & posters*
14:00 - 14:40	FT Mass spectra simulation: Fundamentals and applications Yury Tsybin

14:40 - 15:20	FTMS in Cultural Heritage Christian Rolando
15:20 - 15:50	Vacuum Photoionization on an Orbitrap FTMS Platform: Prototype and Perspectives Christopher Rüger
15:50 - 16:30	Differential Ion Mobility Spectroscopy of Metabolites Chiraz El-Saddik
16:30 - 17:00	*Coffee break*
Selected oral presentations	**Chair: Christopher Rüger**
17:00 - 17:15	Investigation of Asphaltenes and Asphaltene-related Materials with Thermal Analysis coupled to Fourier Transform Ion Cyclotron Resonance Mass Spectrometry Anika Neumann
17:15 - 17:30	Linking Asphaltene characterization by LDI(+) FT-ICR MS with its stability behavior Boniek Gontijo
17:30 - 17:45	Speciation and semi-quantification of nitrogen-containing species in complex mixtures: application to plastic pyrolysis oil Charlotte Mase
17:45 - 18:00	Chemical characterization of wildfire particulate matter emissions by ESI/APPI FT-ICR MS Eric Schneider
18:00 - 18:15	Investigating the insoluble organic matter in primitive chondrites using ultra-high-resolution mass spectrometry Julien Maillard
18:15 - 18:30	Selective characterization of petroporphyrins in shipping fuels and their corresponding emissions using electron-transfer matrix-assisted laser desorption/ionization Fourier transform ion cyclotron resonance mass spectrometry Maxime Sueur
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13th July 2022 - WORKSHOP

Session 2: Protein analysis and Proteomics	**Chair: Francisco Amado**
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10:15 - 11:00	Utilization of Fast Photo-Oxidation of Proteins and Top down Mass Spectrometry for structural characterization of proteins Petr Novak
11:00 - 11:20	*Coffee break*
11:20 - 12:05	Structural characterization of major donkey seminal plasma proteins with high-resolution bottom-up/top-down mass spectrometry Janne Janis
12:05 - 12:50	Current Advances in Deep, Proteome-Wide, MS-based PISA Assay for High Throughput Identification of Drug Targets and Action Mechanisms Massimiliano Gaetani
12:50 - 14:30	*Lunch (supported by Bruker)*
Session 3: MRMS	**Chair: Mike Easterling**
14:30 - 14:50	Comprehensive top-down analysis of proteins using multi-mode fragmentation on ScimaX MRMS Alina Theisen
14:50 - 15:30	The Paracell; optimisation and MRMS developments Christopher Wootton
15:30 - 16:10	New insights in bitumens and lubricants characterization by Fourier transform Mass spectrometry Carlos Afonso
16:10 - 16:30	*Coffee break (supported by Bruker*
Selected oral presentations	**Chair: Maria Elisa Crestoni**
16:30 - 16:45	Structural Characterization of Harwood Xylan with Direct-Infusion ESI FT-ICR Mass Spectrometry Mikko Nikunen
16:45 - 17:00	Noble gas oxide cations in the gas phase - examining Ng+–O energetics (ng = Kr, Xe, Rn) by experiment and theory Sandrina Oliveira
17:00 - 17:15	Molecular characterization of hydrophobic burned soils by ultra-high resolution mass spectrometry Nicasio T. Jiménez-Morillo

17:15 - 17:30	FDS – first instrument independent database for natural organic matter Alexander Zherebker
17:30 - 17:45	PyC2MC: A Python-Based Framework for Processing Multidimensional High-Resolution Mass Spectrometry Data Carlos M. Celis-Cornejo
17:45 - 18:00	Our metal brain: amyloid protein aggregation and metal binding Francesca O. Bellingeri
18:00 - 18:15	Glycoproteomics of glycoengineered simples cells for the identification of bladder cancer molecular targets André M. N. Silva
18:15 - 18:30	Dark Charge Callan Littlejohn

14th July 2022 - WORKSHOP

Session 4: FTMS in real life **Chair: Petr Novak**	
09:30 - 10:15	From ESI analysis to MALDI imaging – studying lipid oxidation on a 7T MALDI FT-ICR instrument Martina Marchetti-Deschmann
10:15 - 11:00	Salivary proteome of patients with Autoimmune Hepatitis (AIH) and Primary Biliary Cholangitis (PBC): scratching problems and solutions Francisco Amado
11:00- 11:30	*Coffee break*
11:30 - 12:10	LC-HRMS Analysis of Marine Biotoxins in Complex Samples José Paulo da Silva
12:10 - 12:50	Cation-π Interactions in Ag+(Benzylamine) Complex Unveiled by IRMPD Spectroscopy and Ion-Molecule Reactions Maria Elisa Crestoni
12:50 - 14:00	Awards by Refeyn & closing Carlos Cordeiro & Margarida Santos-Reis
14:00 - 15:00	*Farewell cocktail, by Refeyn*

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MRMS is the pinnacle of mass spectrometry for both resolving power and mass accuracy. Bruker's scimaX® and solariX series instruments are powered by MRMS (Magnetic Resonance Mass Spectrometry) technology. Compared with common FTMS technologies, these instruments feature unmatched eXtreme Resolution (XR) and mass accuracy. This enables routine Isotopic Fine Structure (IFS) analysis for a broad mass range, resulting in unmatched confidence for compound identification.

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The unrivaled eXtreme Resolution (XR) capabilities of our scimaX® system enables scientists to see vital "hidden" information that is missing in data from other types of mass spectrometers. This advanced technology provides sub-ppm accuracy over a wide mass range, and is a prerequisite for IFS analysis and formula confirmation. The result is unmatched confidence for compound identification, addressing the needs of specialists in a variety of focus areas that all desire efficient and precise solutions for their analytical problems.

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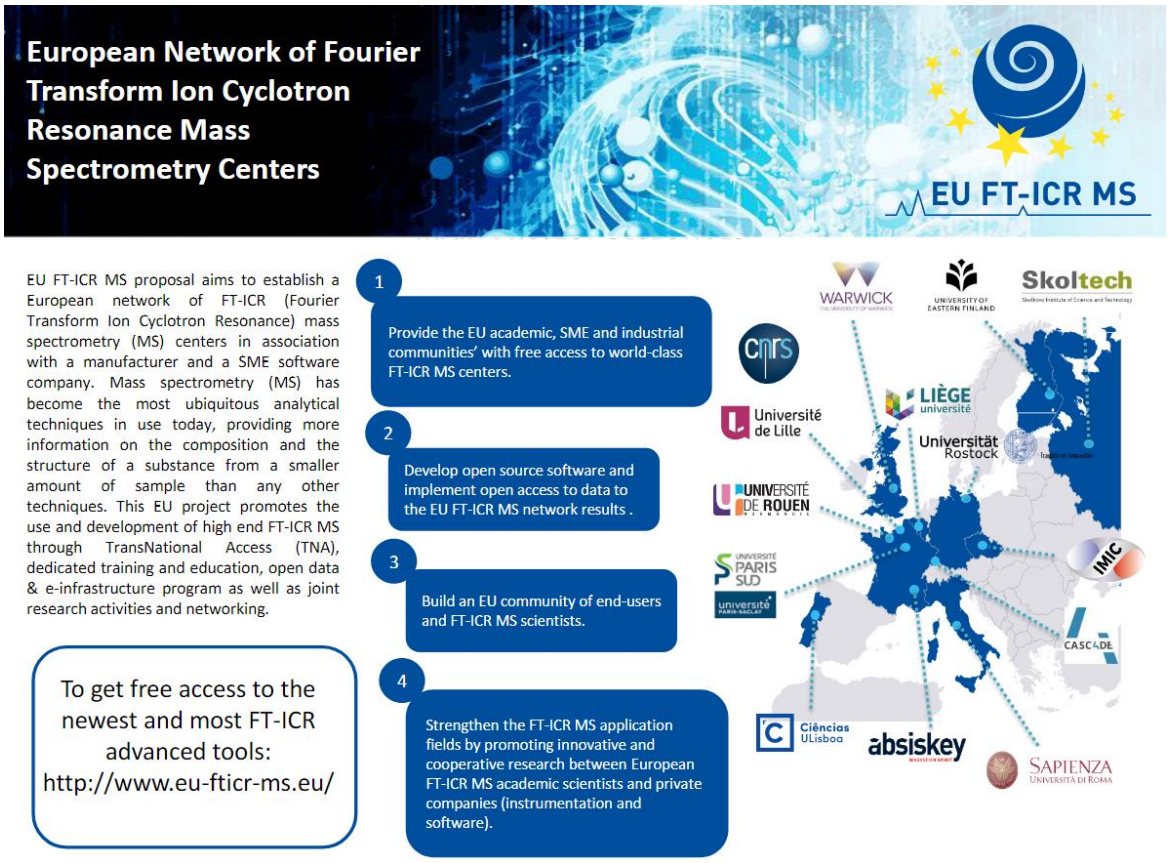

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Keynote Presentations

Expanding capabilities of Orbitrap instrumentation

Alexander Makarov[1,2]

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[2] Utrecht University, Utrecht, Netherlands
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Since its commercial launch 17 years ago, the utility of the Orbitrap analyser has been continuously extended by coupling with additional capabilities such as quantitative analysis, new fragmentation methods, different vacuum and ambient ion sources, imaging and ion mobility. These capabilities are exemplified for four major families of Orbitrap-based instruments, with numerous new modes of operation enabled by parallelization of detection and ion processing, and intricate coordination with different ion-optical devices. New modes of data-independent, targeted and top-down analysis are overviewed, including acquisitions at 40 spectra/second or at 1 million resolution setting.

Major directions of further progress include trap and instrument designs, analytical modalities, acquisition methods as well as signal processing. New Exploris platform implements a number of such innovations, such as a new concept of Orbitrap layout, counter-contamination measures, data-independent acquisition with or without field asymmetric waveform ion mobility analysis (FAIMS), TurboTMT processing using phase-constrained signal deconvolution method, etc. New research opens avenues to elucidation of charge state and collisional cross-section along with the conventional mass-to-charge measurement.

Special attention is devoted to analysis of proteins and protein complexes that poses unique challenges to mass spectrometry and drives the deep re-thinking of principles earlier validated on small molecules and peptides. An example of such re-thinking is the shift towards detection of individual ions that enables both charge detection and boost of mass resolution. Such detection allows following the motion of massive ions of intact viruses in the trap over prolonged periods of time, providing insights into their evolution.

FT mass spectrometer based on multielectrode harmonized Kingdon trap

Oleg Kharybin, Gleb Vladimirov, Sergey Gorbatov, Alexander Semenov, Anton Lioznov, Petr Borisovets, **Evgeny (Eugene) Nikolaev**

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By generalization of the idea of Yury Golikov group [1] and Claus Koester [2] we have made Fourier Transform mass spectrometer on the bases of harmonized Kingdon trap with multiple internal electrodes, described by us in [3]. Here we are showing the results obtained with this mass spectrometer operating in orbitrap and FT ICR like modes with trapping ions by pulsed and continues accumulation, DC and RF excitation of their motion and detection of induced voltage. Electrode geometries in these traps were obtained by composing electric potential distribution from quadrupolar potentials and sum of logarithmic potentials and choosing equipotential surfaces for the trap electrodes on the bases of practical reasons (trap dimensions, ion frequencies and maximum allowed voltages on the internal electrodes. The geometry of the trap electrodes was obtained by solving the Laplace equation. The four internal electrodes of the Kingdon Trap, with merged electrodes, were fabricated by regular high-precision machine work. Homemade electronic modules were used to ionize, control the capturing voltage, detect an induced signal, and switch external electrodes between excitation and detection modes. The maximum resolving power (around 300K) has been obtained in case of thermoemission and electron impact.

Thermal ionization methods EI, ESI, LDI, and MALDI are implemented on the one or three-chamber installation. Potential applications for space research and biomedical analyses will be discussed.

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Combining Ultraviolet Photodissociation and 2-Dimensional Mass Spectrometry

Alina Theisen[1]; Christopher A Wootton[2]; Anisha Haris[3]; Bryan P. Marzullo[3]; Mark P. Barrow[3]; **Peter B. O'connor**[3]

[1]Bruker Daltonics GmbH & Co. KG, Bremen, Germany; [2]Bruker Daltonics GmbH & Co.KG, Bremen, Germany; [3]University of Warwick, Coventry, United Kingdom

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Two-dimensional mass spectrometry (2DMS) is an MS/MS technique, pioneered on FTICR mass spectrometers, which allows fragmentation of a complex mixture of precursor ions while retaining the information of which fragment comes from which precursor. 2DMS works by modulation of all ions spatially through a fragmentation zone, with modulation frequencies programmed as a function of m/z. The Fourier transform can then extract those modulation frequencies, linking each fragment to its precursor. Key to 2DMS is setting up a defined fragmentation zone, which is readily done with ultraviolet photodissociation, and has recently been done on 12T and 15T FTICR mass spectrometers with two different ICR cell geometries at the university of Warwick. The instrumentation, results, and performance parameters will be presented.

A 193 nm Coherent Existar laser and a 213 nm Litron Nd:YAG frequency quintupled laser were used for ultraviolet photodissociation on a 12T and a 15T FTICR mass spectrometer. The 12T system uses the Infinity cell while the 15T uses the newer dynamically harmoniced ICR cell. In each case, the laser is far-field aligned with a red photodiode laser beam, and then aligned through a BaF2 laser window and through the hollow cathode electron gun to hit the trapped ions. The system uses a dichroic mirror to combine the IRMPD laser beam and the UVPD laser beam which potentially allows combined IRMPD/UVPD experiments as well.

A range of different samples has been analysed using ultraviolet photodissociation (UVPD) on the FTICR mass spectrometer including vitamin D3 isomers, a mixture of agrichemical small molecules, tryptic peptides from a range of different proteins (mABs, spike, scorpion venom proteins, and yeast whole cell lysate) and peptides including phosphopeptides and glycopeptides, and we expect to have further data in the coming months to present. As UVPD generates fragment-rich tandem mass spectra, the resulting 3D surfaces generated by a 2D mass spectrometry experiment are exceptionally rich in peaks allowing for remarkable peak density, particularly in complex mixtures. The instrumentation setup will be discussed, and the data shown for a range of sample types. Furthermore, the analytical performance parameters of UVPD/2DMS will be studied. The radial profile of the fragmentation zone will be plotted out as we've previously done with ECD and IRMPD fragmentation on both the Infinity cell and the dynamically harmonised cell, which will allow us to profile the beam shape as it overlaps with the ion cloud. Since the dynamically harmonised cell reportedly properly positions the ions in the center of the cell, we expect a sharper beam profile than previously observed with IRMPD on the Infinity cell. Overall, UVPD works very well with 2DMS on an FTICR mass spectrometer to the point that the experiment is close to routine at the Warwick Ion Cyclotron Resonance Laboratory.

FT mass spectra simulation: fundamentals and applications

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The analytical characteristics of FT mass spectra depend on the underlying properties of data generation (ion oscillation frequencies, frequency-m/z conversion principles, etc.), acquisition (transient length, signal digitization, etc.), and processing (FT parameters, etc.). Disregarding these properties upon accurate analysis of FT mass spectra may result in the erroneous interpretation of the data and/or false (inaccurate) statements. Due to the FTMS specifics, such as characteristic isotopic beat patterns, the interpretation of (large) protein mass spectra is particularly prone to errors. To overcome these limitations, we developed the FTMS Simulator – a software tool to accurately simulate FTMS isotopic envelopes and mass spectra [1]. We recently upgraded the FTMS Simulator to enable accurate simulation of mass spectra from any size molecules, including monoclonal antibodies (mAbs) and viruses, Figure [2].

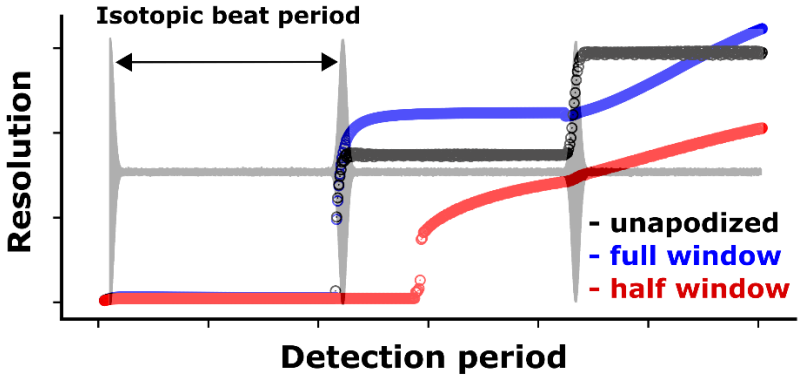

Figure: Isotopic beats and apodization functions influence on resolution in mAbs mass spectra [2].

The access to the accurately simulated FTMS data helps to routinely verify the hypotheses and interpretations. Furthermore, the FTMS Simulator is useful for teaching about the FT processing options – apodization, zero fills, phase artifacts, time-domain transient decay, frequency-m/z conversion, and others. These capabilities may address today's FTMS field needs in the education of the FTMS end-users.

Finally, the accurately simulated data can be employed in data processing workflows for feature extraction in analyzing small and large molecules. For the latter, we will demonstrate a particular application of the thus simulated data in a targeted deconvolution workflow for the intact mass analysis of proteins, focusing on the mAb biotherapeutics.

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FTMS in Cultural Heritage

Christian Rolando

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This talk will be a journey in the field of Cultural heritage by Fourier Transform Mass Spectrometers Orbitrap and FTICR. A first example will show the identification of fish proteins trapped in the clay of Roman amphora which contained garum, a highly valuable sauce made of fermented fish by proteomics performed by nanoLC hyphenated to nanoESI-Q-Exactive Orbitrap. We will describe how to collect data to build up a database for non-sequenced species and how to work on highly processed samples. In a second example we will compare results obtained the results on the identification of Pleistocene bone fragments and tools not recognizable from their osteomorphological by proteomics performed either by ultrahigh resolution MALDI FT-ICR or by nanoLC nanoESI-Q-Exactive Orbitrap. We will show that ultrahigh resolution MALDI FT-ICR allows the high throughput identification of thousands of bones collected in excavations. In a third example we will present integrated and straightforward new analytical protocol that identifies plant gums from various sample sources including cultural heritage. Our approach is based on the identification of saccharidic fingerprints using mass spectrometry following controlled enzymatic hydrolysis. The results obtained by MALDI-TOF, ultrahigh resolution MALDI FT-ICR and nanoLC nanoESI-Q-Exactive Orbitrap will be compared. In a fourth example we will describe a mild depolymerization of oil paints and the identification of their crosslinks by MALDI FT-ICR LC-Orbitrap allowing the identification of the siccative oil used by the artist. In a last example we will present the application to the just published to depolymerize and analyze insoluble polydienes for elucidating the structure of amber.

Vacuum Photoionization on an Orbitrap FTMS Platform: Prototype and Perspectives

Christopher P. Rüger[1,2], Paul Kösling[1,2], Julian Schade[1,2], Sven Ehlert[3], Yury Tsybin[4], Anton Kozhinov[4], Konstantin O. Nagornov[4], Martin Rigler[5], Andreas Walte[3], Ralf Zimmermann[1,2,6]

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Gas-phase, pulsed-laser photoionization methods are versatile, soft ionization approaches. We recently modified an Exactive™ Orbitrap mass spectrometer allowing direct photoionization inside the C-trap (*PhotOrbi*) [1]. Resonance-enhanced multiphoton ionization (REMPI) allows for sensitive and selective description of the aromatic profile, whereas the high resolution and high mass accuracy allowed a molecular-level description. The aromatic profile of complex mixtures is of high priority in various research fields due to the environmental and health aspects of polycyclic aromatic hydrocarbons (PAHs).

Vacuum REMPI was conducted using either a frequency-quadrupled Nd:YAG laser (266 nm) or a Krypton-Fluoride excimer laser (248 nm). The system can be easily coupled to different sample introduction techniques, and the specialized data acquisition by Spectroswiss SARL enabled us to record transients with freely adjustable lengths of up to 2 s.

We explored the capabilities of the *PhotOrbi* for various complex mixtures and applications areas:

1) Direct gas chromatographic hyphenation, *e.g.*, for the description of bio-oils,
2) Analysis of carbonaceous aerosols, heavy petroleum residues and asphaltenes by hyphenation of a thermal desorption and pyrolysis approach,
3) Direct field-usage of the prototype measuring primary emissions of a research ship diesel engine.

Remarkably, despite the harsh conditions during the field campaign, the characteristics of the Orbitrap mass spectrometer could be preserved, achieving ppm mass accuracy and >100,000 resolving power across the targeted mass range. Compared to commonly deployed time-of-flight instrumentation, the molecular complexity could be directly tackled, and the mutagenic and carcinogenic PAH profile was accessed, even for highly dynamic processes.

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Differential Ion Mobility Spectroscopy of Metabolites

Chiraz El-Saddik

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Our objective was to assess the performance of protocols based on differential mobility spectrometry (DMS) hyphenated to MS/MS for targeted metabolomics. LC-MS/MS protocols for biomarker quantification used for clinical diagnosis are particularly robust, but there is a need for alternative and/or complementary methods that are more specific and rapid, and that could resolve specific isobaric or isomeric metabolites, and address multiple classes of metabolites simultaneously. Proteinogenic amino acids and related compounds, which are metabolites which can act as sensitive biomarkers of metabolic diseases (MDs), were among our targets, as well as organic acids.

A home-built device has been specifically designed for this purpose, which could be mounted on two of our MS/MS instruments, including a 7 tesla FT-ICR MS/MS. We were also interested in fundamental aspects of separation and identification of isomeric species using DMS. For this purpose, infrared multiple photon dissociation (IRMPD) was used in DMS-MS/MS(IRMPD) sequences in order to identify DMS peaks and even resolved overlapping peaks. In the latter case, addition of polar molecules in the carrier gas leading to modifier-assisted DMS was found as an alternative for resolving DMS peaks, which are characterized by a compensation voltage (CV) applied to one of the two DMS electrodes.

An important added-value of this work was to show that CV value associated with one metabolite is very stable and could thus be used as an identifier. Using a dedicated R-suite of programs, quantitative data analysis and statistics could be rapidly performed. Quantification results for a set of metabolites using DMS-MS were found to be in excellent agreement with those obtained at the hospital using LC-MS/MS. We even showed that faster protocols based on few selected DMS-MS analyses at specific fixed CV values are also reliable, and could be used, for example, for emergency diagnosis of MSUD.

Proteomics-compatible Fourier Transform Isotopic Ratio Mass Spectrometry of Polypeptides

Hassan Gharibi, Alexey L. Chernobrovkin, Amir Ata Saei, Xuepei Zhang, Massimiliano Gaetani, Alexander A. Makarov, **Roman Zubarev**

Division of Physiological Chemistry I, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm, Sweden, SE 171 77
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Measuring the relative abundances of heavy stable isotopes of the elements C, H, N and O in proteins is of interest in environmental science, archaeology, zoology, medicine, and other fields. The isotopic abundance measurements of the fine structure of the immonium ions with ultrahigh resolution mass spectrometry obtained in gas-phase fragmentation of polypeptides has previously uncovered anomalous deuterium enrichment in (hydroxy)proline of bone collagen in marine mammals [1]. Here we provide a detailed description and validation of this approach and demonstrate per mil-range precision of isotopic ratio measurements in aliphatic residues from proteins and cell lysates. The analysis consists of proteomics-type experiment demanding sub-microgram amounts of protein sample and providing concomitantly protein sequence data allowing one to verify sample purity and establish its identity. A novel software tool Protein Amino Acid-resolved Isotopic Ratio Mass Spectrometry (PAIR-MS) is designed for extracting isotopic ratio data from the .raw data files acquired on an Orbitrap mass spectrometer.

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Utilization of Fast Photo-Oxidation of proteins and top down mass spectrometry for structural characterization of proteins

Marek Polak, Ghazaleh Yassaghi, Petr Halada, Daniel Kavan, Jan Fiala, Dmitry Loginov, Zdenek Kukacka, Michael Volny, **Petr Novak**

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Protein footprinting coupled to mass spectrometry is commonly applied for protein structural studies, providing information on protein conformations and dynamics. Traditional mass spectrometry approaches for structural elucidation include hydrogen deuterium exchange, chemical cross-linking, ion mobility and covalent labeling. Among these, hydroxyl radicals are a perspective probe for the fast protein footprinting as introduced two decades ago. There are different methods to generate them including Fenton reaction, radiolysis of water and fast photochemical oxidation of proteins (FPOP); Bottom up mass spectrometry is the dominant method to identify modified amino acids and determine the solvent accessible area of proteins. Here, we present utilization of the Top-down sequencing for localization of modified residues within the protein structure.

For protein footprinting experiment, an excimer laser (248nm KrF) was used to generate hydroxyl radicals in a quench flow set-up. The extent of the hydroxyl radical incorporation was checked by high-resolution mass spectrometry (solariX XR 15T, Bruker Daltonics). When a significant oxidation of proteins (aMyo and hMyo, apoFOXO4 and holoFOXO4) was observed, intact, singly and doubly modified protein ions were isolated in quadrupole and the ions of interest were fragmented by a broad repertoire of techniques. CID and ETD were performed in the hexapole, while ECD and IRMPD in the ICR cell. Ms2links algorithm was used for annotation, and the lab-built software to calculate the extent of modification. Detail quantification of modifications obtained from fragment spectra allowed determining which amino acids are more exposed to the solvent when the heme (myoglobin) or DNA (FOXO4) is removed (Fig.1). [1]

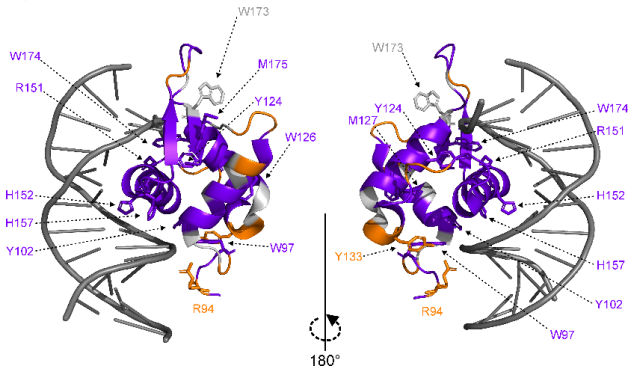

Figure 1. X-ray structural model of FOXO4-DBD•DAF16 (PDB 3L2C)22 with the highlighted differently oxidized regions detected by top-down approach. The individual residues detected in bottom-up approach were highlighted into these region using a stick represen-tation. Purple – regions/residues detected as more modified in apo form, orange – regions/residues detected as more modified in holo form.

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Structural characterization of major donkey seminal plasma proteins with high-resolution bottom-up/top-down mass spectrometry

Janne Jänis[1], Mikko Laitaoja[1], Sk Alim[2], Sudheer K. Cheppali[2], Thirumala Rao Talluri[3], and Musti J. Swamy[2]

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Capacitation is an important biochemical process in which motile sperm cells (spermatozoa) become competent for fertilization during their journey through the female reproductive tract. In mammals, several different proteins present in seminal plasma play a crucial role in the sperm capacitation, collectively known as binder of sperm proteins (BSPs). All these proteins have a common structure comprising of an N-terminal flanking region followed by two or four tandemly repeating fibronectin type-II (FnII) domains. In addition to phospholipid binding, mammalian BSP proteins can also act as small heat shock proteins, exhibiting chaperone-like activity. We have recently isolated and purified a new seminal plasma protein from donkey (*Equus hemionus*), abbreviated as DSP-1.

An extensive biochemical and biophysical characterization of this protein shows that it is highly homologous to the other mammalian seminal plasma proteins.

A detailed structural characterization of DSP-1 with high-resolution bottom-up/top-down mass spectrometry revealed that the protein is heterogeneously glycosylated, carrying several complex-type O-glycans in the N-terminal region, with the distal sialic acid (N-acetyl neuraminic acid) residues being heavily acetylated.

Both bottom-up (LC–FT-ICR MS/MS) and top-down (TIMS-QTOF MS/MS) mass spectrometry methods were needed for the full sequencing of DSP-1. A further characterization of the other two minor donkey seminal plasma proteins DSP-2 and DSP-3 is in progress.

Current Advances in Deep, Proteome-Wide, MS-based PISA Assay for High Throughput Identification of Drug Targets and Action Mechanisms

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Chemical Proteomics methods explore drug-target-phenotype relationship from different angles, with relative limitations. For example, drug-specific abundance changes regulated by cells in late apoptosis can reveal drug targets and mechanistic proteins. In thermal proteome profiling, the targets are found by the difference of protein thermal stability of drug-treated versus vehicle-treated samples, using "melting temperatures" derived by curve fitting of samples incubated at different temperatures points. In TPP, such thermodynamic interpretation is questionable, plus sample amounts and analysis costs are high, complicating scalability, the use of more than two biological replicates per condition and data analysis of multibatch of TMT.

We developed the MS-based proteome integral solubility alteration (PISA) assay, which provides no thermodynamic interpretation but instead a deep, proteome-wide and high-throughput target identification, with current advances enabling ~10 thousand proteins to be identified and quantified in mammalian cells, up to 18 biological samples (cells or lysates) per TMT batch. Current protocols are also optimized for primary cells and bacteria. PISA provides maximized throughput, sustainability, proteome coverage, statistical power of analysis, confidence in results, and up to five molecules analyzed per TMT batch. Proteins interacting with the molecule of interest (targets and off-targets), activated mechanistic factors or proteins modified during the treatment show reproducible soluble amount changes between drug treatments and controls, after exposure to a gradient of solubility perturbing agent. For fuller target deconvolution, PISA is integrated with the orthogonal expression proteomics ("PISA-Express"). A third analysis dimension is RedOx proteomics, soon added to the above in one TMT batch ("PISA REX").

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Comprehensive top-down analysis of proteins using multi-mode fragmentation on ScimaX MRMS

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Top-down analysis of protein sequence and modifications requires efficient gas-phase fragmentation, which may be obtained by a multi-mode approach in which multiple MS/MS methods are employed. As CID becomes less efficient for larger analytes, approaches with electron-based methods such as ETD, ECD and EID available on scimaX MRMS are highly beneficial and produce complementary data. Experiments were performed on a 7T scimaX MRMS system equipped with a ParaCell 2xR detector using the standard Apollo II ESI source. 3 s and 1.5 s transients were acquired in 2-omega detection mode resulting in a mass resolution of 900k and 450k at m/z 400, respectively. Model proteins ubiquitin and carbonic anhydrase were subjected to CID, ETD, ECD and EID. MS/MS parameters were optimised for each protein and charge state and fragments were assigned with a 2 ppm mass error tolerance.

Using all methods, full cleavage coverage was achieved for ubiquitin and a cleavage coverage of 93% was achieved for the 29 kDa protein carbonic anhydrase. ECD consistently gave the highest cleavage coverage, and this could be enhanced even further by the complementary data of EID or CID which are able to cleave N-terminal proline bonds. Confident fragment assignment, especially in highly complex ECD spectra, was facilitated by the ultra-high mass resolution and mass accuracy of the MRMS platform.

The Paracell: optimisation and MRMS developments

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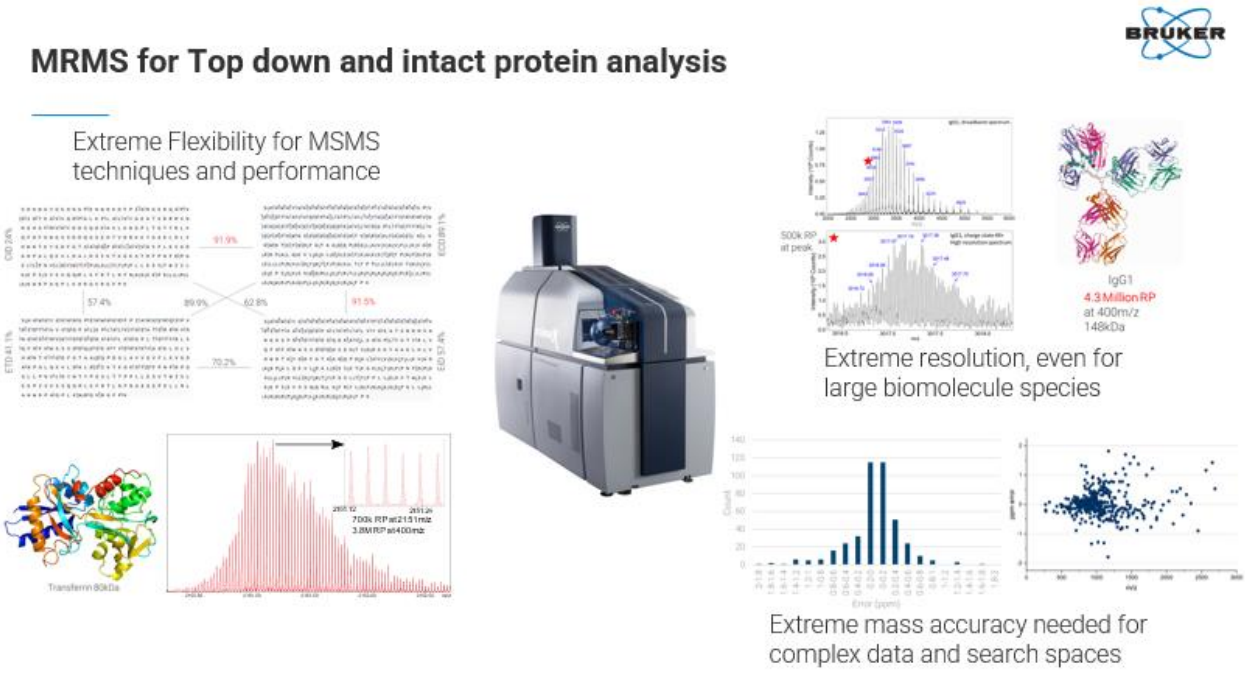
The Bruker SolariX and ScimaX MRMS instruments offer not only the highest resolving power and mass accuracy performance of any MS type, but also the largest array of fragmentation techniques available, including, but not limited to; CID, ISD, ECD, ETD, EID, EDD, EED, nETD, nECD, SORI-CID, and MALDI-ISD. Herein we showcase an array of dissociation techniques and how, when coupled with its ultra-high performance, they can be used to study a range of biomolecules.

The performance required to resolve targets of interest and identify proteoforms scales with size and complexity. A range of proteins are shown at ultra-high resolving power, values at 400m/z; BSA (66kDa) 5.7M, IgG1 (147kDa) 4.3M, ADH tetramer (147kDa) 6.8M, Yeast enolase dimer (93kDa) 8.5M resolving power. All achieved in magnitude mode on a standard SolariX/ScimaX MRMS 7T instrument using the Paracell. AMP phasing can readily increase these values by accessing absorption mode FTMS.

MS/MS of model proteins shows the advantages and complementarity of dissociation techniques. Front end CID combined with ETD data show >80% cleavage coverage of some model proteins. Whereas ECD MS/MS is able to show up to 100% coverage of cleavable residues. EID produced a/x, b/y, and c/z fragments, achieving high cleavage coverage and showed clear cleavage of proline residues via a and x fragmentation channels – providing UVPD-like fragments using the ECD cathode equipped as standard on the MRMS. Broadband ultra-high resolution of >1M resolving power allows resolution of complex top-down spectra and when coupled to low-sub-ppm accuracy of the MRMS allows unambiguous assignments.



New insights in bitumens and lubricants characterization by Fourier transform Mass spectrometry

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Heavy oil products are obtained from the vacuum distillation of crude oil. They correspond to extremely complex matrices that can be used as lubricating base oils or as bitumen for asphalt pavements. To support the gradual energy transition which aimed to limit the use of fossil fuels, a great deal of research is carried out to improve the sustainability of these various products, in particular to reduce their carbon footprint. This includes the addition of additives to improve the performance and durability of a given product, or the recycling of waste products to reduce costs, but also to contribute to resource conservation. The aim of this work was to develop new analytical methods and new data processing approaches for the molecular characterization of petroleum formulated products. To characterize such matrices at the molecular level, Fourier transform mass spectrometry was used. This includes the FTICR and Orbitrap mass spectrometers, which have the best performance on the market in terms of mass accuracy and resolving power. These properties allow to separate the different signals present in a complex matrix and to assign a unique molecular formula to each m/z value. This work allowed to set up simple analytical methods in direct introduction (DIP-APCI), and in direct infusion (ESI, APPI, APCI) for the molecular characterization and the study of the aging of lubricants and bitumens. The use of molecular maps (DBE vs C#, Kendrick representations) coupled or not to multivariate statistical approaches allowed to characterize the additives and the oxidation markers of the various products studied.

From ESI analysis to MALDI imaging — studying lipid oxidation on a 7T MALDI FT-ICR instrument

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Lipids are essential molecules for cellular signaling, metabolism, and key components of our cells. At the skin level, exposure to solar radiation, high oxygen levels, and environmental pollutants leads to premature skin aging, tissue inflammation, and photocarcinogenesis. This oxidative stress activates cutaneous lipoxygenases or causes nonenzymatic lipid peroxidation, thus forming oxidized lipids (oxLipS). We present a systematic investigation of lipids and their non-enzymtic oxidation products generated in a controlled environment after UV exposure. Untargeted OxLipS analysis was performed on a dual-source ESI/MALDI 7T scimaX FTICR MRMS (Bruker). ESI was used for species identification, while the MALDI Imaging platform (MSI) complemented the analysis with spatial localization in tissue. A lipid standard solution (LipS), PAPC and not UV affected references DPPC and DNPC in chloroform were exposed to solar-like conditions (UVA radiation). Direct infusion ESI experiments of the LipS generated crucial information on oxLipS species produced by ROS formed in solution. Accurate, high-resolution MS data allowed identification of known and newly formed species via database search (LipidMAPS).

Skin biopsies were mounted on ITO slides for MALDI MSI. Different sample preparation methods after tissue washing have been investigated, and instrument parameters adapted for optimized FT ICR imaging [1,2]. Selected *m/z* values were compared between ESI and MALDI, thus aiding the translation of *ex vivo* experiments to *in vivo* MALDI MSI results. Several oxLipS were localized, visualized, and identified in skin section samples.

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Salivary proteome of patients with Autoimmune Hepatitis (AIH) and Primary Biliary Cholangitis (PBC): scratching problems and solutions

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Autoimmune Hepatitis (AIH) is a non-resolving inflammation of the liver characterized by interface hepatitis, hypergammaglobulinemia and autoantibodies, while Primary Biliary Cholangitis (PBC) is a complex autoimmune chronic liver disease characterized by biliary injury and cholestasis.

We perform the characterization of the salivary proteome from patients affected by AIH and PBC in order to explore the potential clinical use of saliva proteins and identify qualitative/quantitative variations in protein profile useful for diagnostic and prognostic purposes and solve the differential diagnosis, since there is an overlap syndrome.

Results obtained with the use of various methodologies and bioinformatics tools and achieved conclusions will be discussed.

LC-HRMS Analysis of Marine Biotoxins in Complex Samples

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The analysis of marine biotoxins in food is still a challenging task due to the complexity and diversity of matrices. LC-MS/MS provides the required sensitivity and specificity by combining unambiguous identification with the high selectivity of tandem MS. However, information on compounds other than the targeted toxins is lost. Furthermore, LC-MS methods are prone to matrix effects (ME), which show large sample-to-sample variability, even between samples of the same species and geographic origin. ME need to be evaluate and controlled continuously. Here we discuss a quantitation method for marine biotoxins in complex samples using high resolution and accuracy full scan profiles (LC-HRMS) obtained with an Orbitrap instrument, followed by the extraction of accurate mass ion chromatograms (AM-XIC).[1,2]

The chosen toxins are the hydrophilic tetrodotoxin (TTX) and the lipophilic okadaic acid (OA), dinophysistoxins DTX-1 and DTX-2, pectenotoxin (PTX-2), yessotoxin (YTX) and azaspiracid-1 (AZA-1). Matrices are mussels *Mytilus galloprovincialis* and marine snail *Charonia lampas*. ME varies with age/size, tissue and processing (fresh or thermal processed/pasteurized). ME on lipophilic toxins ranged from 20 to 120% while for TTX ranged from 90 to 380%. ME were corrected by following a standard addition method. The LC-HRMS profiles allowed the detection of non-targeted toxins in the analyzed samples.

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Cation-π Interactions in Ag^+(Benzylamine) Complex Unveiled by IRMPD Spectroscopy and Ion-Molecule Reactions

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Phenylalkylamines are bifunctional ligands able to establish bimodal interactions based on the π-electron rich aromatic ring and the amino functional group. Being the building block of numerous neurotransmitters and psychoactive molecules, the study of their structural characteristic may offer insight into their recognition mechanism.[1] In particular, the influence of protonation on their conformational arrangement and propensity to intramolecular cation-π interactions has been thoroughly investigated by IR multiple photon dissociation (IRMPD) spectroscopy and quantum chemical calculations.[2] In this study, the binding motifs in the interaction of the parent phenylalkylamine, namely benzylamine (BA), with Ag^+ ion have been explored. The selected electrophile is expected to interact efficiently with the aromatic portion of aromatic amino acids and the role of cation-N and cation-π interactions can thus be established. The IRMPD spectrum of the Ag^+(benzylamine) complex has been recorded in the fingerprint range (800-1800 cm^{-1}) and evaluated by comparison with calculated IR absorption spectra for the plausible candidate complexes. The structure that best reproduces the IRMPD spectrum corresponds to the most stable geometry and is characterized by a chelate-like, folded geometry presenting both Ag^+-π and Ag^+-N interactions. The gas-phase reactivity of the Ag^+(benzylamine) complex with a neutral ligand (L) possessing either an amino/aza functionality or an aryl group confirms the remarkable affinity of Ag+ for π-donors and suggests an increased silver coordination in the product adduct ion Ag^+(benzylamine)(L).[3]

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Short Oral Communications

Investigation of asphaltenes and asphaltene-related materials with thermal analysis coupled to Fourier Transform Ion Cyclotron Resonance mass spectrometry

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Asphaltenes are known to be the most complex fraction of petroleum, strongly related to problems during production, storage, and refining [1]. In recent years, direct infusion high resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) provided deep insight into the molecular structure of asphaltenes, especially concerning the presence of island- and archipelago-type molecular architecture [2-4]. Thermal gravimetry (TG) coupled to FT-ICR MS adds with a temperature-resolved dimension a complementary view to the analysis of asphaltenes. With this technique, remaining occluded material on the asphaltenes could be released and investigated. Furthermore, typical pyrolysis behavior and pyrolysis products could be identified for island- and archipelago-type asphaltenes as well as for their solubility fractions [5].

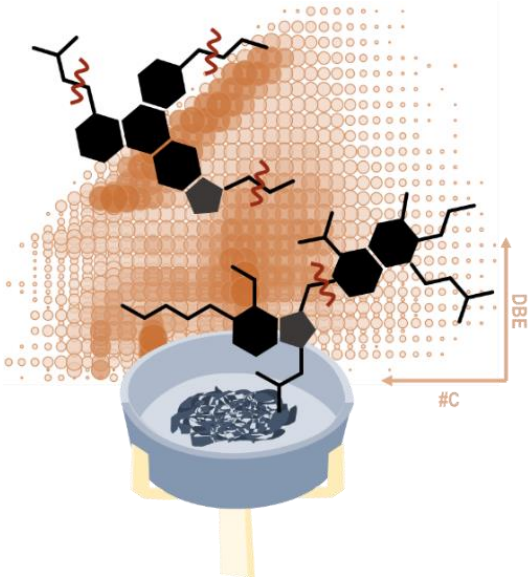

Figure 1: Asphaltenes show different pyrolysis products according to their molecular architecture.

As asphaltenes currently remain mainly as undervalued side products from petroleum refineries, intensified attempts are made for upcycling. In this respect, because of their high carbon content, the production of carbon fibers is a promising approach. Preliminary data presented here suggest TG-FT-ICR MS as a valuable tool concerning the chemical characterization of different process steps of asphaltene fiber production and helps for process optimization.

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Linking asphaltene characterization by LDI(+) FT-ICR MS with its stability behavior

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Analyzing the most unstable fractions is fundamental to understanding the mechanisms involved in asphaltenes precipitation [1]. For this, Fourier transform ion cyclotron resonance mass spectrometers (FT-ICR MS) play a significant role due to their capability to provide information at the molecular level of complex samples. However, asphaltenes deposition in capillaries of the typical ionization sources as ESI, APCI, and APPI during ionization hinders the analysis of unstable fractions. Our previous work [2] demonstrated laser desorption ionization (LDI) as an alternative to FT-ICR MS analysis of unstable asphaltene fractions without capillary obstructions. Herein, we present a new fractionation methodology employing soxhlet extraction to separate asphaltenes with the most significant aggregation tendencies. Asphaltene sub-fractions were obtained by the following acetone, acetonitrile (ACN), heptane (Hep), heptane/toluene (1:1, v/v), and toluene (Tol). The sample's stability was evaluated by the TurbiscanLab analysis described elsewhere [2]. The whole asphaltene samples and their sub-fractions were characterized by LDI (+) FT-ICR MS. The fractionation methodology developed in this study effectively separated fractions with high ionization efficiencies. Therefore, the method proved to extend the characterization of asphaltenes samples, allowing access to information on species of complex ionization. In addition, the fractionation method effectively separated the species that presented a more significant aggregation trend. Higher O/C and S/C ratios were observed in the more unstable asphaltenes subfractions. CID experiments verified the predominance of archipelago-type asphaltenes in the most unstable samples. Therefore, the results indicate a correlation between high aggregation tendencies and archipelago-type asphaltenes' predominance.

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Speciation and semi-quantification of Nitrogen-containing species in complex mixtures: application to plastic pyrolysis oil

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Plastic pyrolysis oil is of particular interest for the waste management in the current context of circular economy. Due to their uncontrolled origin, these oils may contain significant amount of unwanted compounds such as nitrogen-containing species. These compounds are known to be catalyst poisons during refining processes. Therefore, the removal of these species is crucial, and for this purpose, their characterization from a structural and quantification point of view is essential. This study presents a method to specify and quantify nitrogen-containing classes in a plastic pyrolysis oil by direct infusion mass spectrometry. Two steps were used, structural characterization in order to select suitable standards followed by semi-quantification. Structural speciation of nitrogen-containing compounds was first obtained by electrospray ionization Fourier transform mass spectrometry followed by tandem mass spectrometry using high-resolution mass isolation and infrared multiphoton dissociation fragmentation. A semi-quantification is then performed by standard addition method, very appropriate for such complex matrices. Aromatic cores such as quinoline and quinoxaline were evidenced for both N1 and N2 classes allowing to propose 2-methylquinoxaline and 2-butylquinoline as standards for the semi-quantification of N2 and N1-containing compounds respectively. The amount of nitrogen detected from the sum of individual species was consistent with the bulk analysis. The reported methodology can be applied to numerous other families of compounds in various other complex matrices.

Chemical characterization of wildfire particulate matter emissions by ESI/APPI FT-ICR MS

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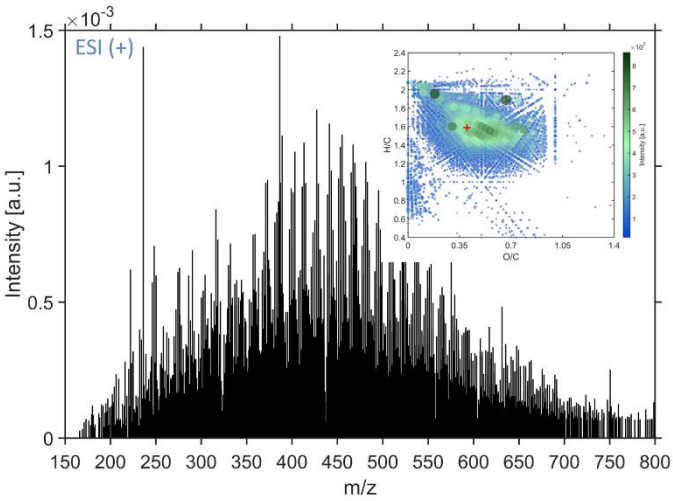

Figure 2. ESI(+) FT-ICR MS mass spectrum and Van-Krevelen fingerprint plot of arctic wildfire PM extract.

Chemical characterization is crucial for understanding both the environmental and health impact of particulate matter (PM). For example, biomass burning, e.g., wildfires, and its products from atmospheric ageing are known for increased oxygen content, functionalization, and polarity, which may cause decomposition in thermal desorption-based techniques like GC-MS. Therefore, electrospray ionization (ESI) and atmospheric pressure photoionization (APPI) are applied for the soft ionization of polar, nonpolar, and non-volatile organic compounds.[1] Furthermore, the observation of the same sample from three perspectives is enabled by different selectivity of ESI in positive and negative ionization mode as well as APPI. Combined with the abilities of Fourier-Transform ion cyclotron resonance mass spectrometry (FT-ICR MS), many sum formulae can be assigned and applied to calculate sum parameters and metrics for the chemical and structural characterization of oxidized organic aerosols.[2] A unique sample set, originating from wildfires in the subarctic regions of Siberia, was analyzed ESI/APPI FT-ICR MS. This special event in August 2021 showed a wildfire plume, being at first transported from east to west for several days, followed by a switch in wind direction transporting the airmasses directly north into the arctic at the Yamal peninsula. PMs were analyzed from two separate locations that were heavily impacted by the same wildfire plumes. The generated data reveals highly complex mixtures of oxidized organic compounds with several heteroatoms, giving insights into the impact of atmospheric aging on wildfire aerosol emissions as well as the overall composition of this PM and its impact on the sensitive arctic ecosystem.

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Investigating the insoluble organic matter in primitive chondrites using ultra-high-resolution mass spectrometry

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Primitive carbonaceous chondrites exhibit an unparalleled diversity in terms of their organic content, in addition to a variable degree of hydrothermal alteration. Whether this diversity results from the circulation of fluids or from a multiplicity of precursors remains an open question of prime interest to understand the formation of carbonaceous asteroids. We applied laser desorption ionization - Fourier transform ion cyclotron resonance mass spectrometry on the macromolecular carbon of recent CM carbonaceous chondrite falls, as well as Orgueil (CI) and Tarda (C2). We intended to probe the diversity of molecular fragments released under low power laser beam. The abundance of the chemical families is correlated to the extent of aqueous alteration, which promotes a structural aromatisation. The weakly altered Paris has retained the largest chemical heterogeneity, whilst it is lost in more altered chondrites. Orgueil and Tarda IOM share similarities; this is consistent with Tarda and Orgueil originating from the outer belt region. Applied to returned asteroidal samples, FTCIR-MS may help unravelling the origin and evolution of organic compounds during the early stages of the solar system.

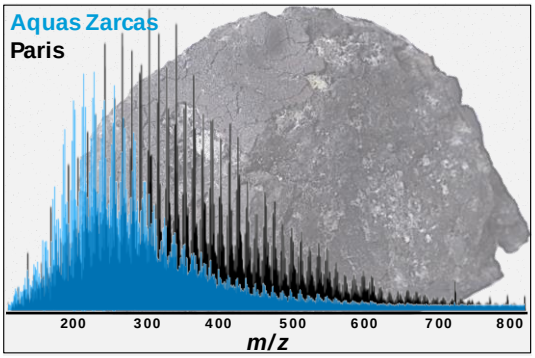

Illustration of resulting mass spectra obtained for the IOM of Aguas Zarcas and Paris chondrites.

Selective characterization of petroporphyrins in shipping fuels and their corresponding emissions using electron-transfer matrix-assisted laser desorption/ionization Fourier transform ion cyclotron resonance mass spectrometry

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In the context of the global transportation of goods, shipping emissions account for a significant proportion of air pollution. Indeed, a focus has been made over the recent years on this primary emission source, leading to several regulations with respect to the chemical composition of shipping fuels. However, these regulations mainly concern the fuel sulfur content (FSC) and do not consider other compound classes such as polycyclic aromatic hydrocarbons (PAHs) or metal-containing aromatics, i.e., petroporphyrins, known to be present in bunker fuels. Petroporphyrins are tetrapyrrole-based metal complexes derived from the transformation of chlorophylls through geological time scales. In contrast to PAHs, their fate in the combustion process and effects on environmental health are widely unknown. We present electron-transfer ionization in matrix-assisted laser desorption/ionization FTICR-MS for the characterization of vanadyl and nickel porphyrins in shipping feed fuels and primary particulate matter emissions. For the first time, these petroporphyrins could successfully be described in the heavy fuel oil feeds but also in the particles emitted by the combustion of the respective fuel on a molecular level. Three main alkylated series of porphyrins were observed, these series can be qualified by their double bond equivalent and correspond to various core structures. Our results highlight the molecular fate of the petroporphyrins through combustion and show that a significant amount of petroporphyrins is released unburned or partially dealkylated to the atmosphere. Furthermore, our results suggest that higher amount of petroporphyrins might be released in harbors than in open sea, due to a less efficient combustion.

Structural Characterization of Harwood Xylan with Direct-Infusion ESI FT-ICR Mass Spectrometry

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Profiling the structure and composition of plant-based biopolymer feedstocks has become an interest during the past decade. Xylan is a complex polysaccharide, consisting of 1,4-β-linked xylose residues with various substituents, mainly arabinose and glucuronic acid as well as acetyl groups. Hardwood and softwood xylans differ considerably by the degree of polymerization and substituents. Xylan is one of the major hemicelluloses in nature. A challenge in the characterization of these polysaccharides is mainly caused by heterogeneity in the structure of polymer as well as poor solubility in most of the common solvents.

In this study we screened different solvent systems in order the enhance the measurability of native hardwood xylan with ESI FT-ICR MS. The hardwood xylan samples were measured in different solvent systems (i.e., H_2O/NH_4OH, H_2O/HCOOH, MeOH, DCM, DMSO, and $CHCl_3$) by high-field FT-ICR mass spectrometry (12-T Bruker Solarix XR), using direct-infusion positive- and negative-ion electrospray ionization (ESI).

While (+)ESI targeted mainly neutral mono- and oligosaccharides in the sample, (–)ESI mainly ionized acidic glucuronic acid-substituted ones. The best solvent system for negative-ion ESI was H_2O/NH_4OH, while H_2O/HCOOH was the most efficient for positive-ion ESI. The weight-average molecular weights of the xylan samples were considerably lower than that measured with size exclusion chromatography. It is not yet clear if this is due to the sample solubility of the ionization efficiency. For further studies, a chemical modification, e.g. permethylation, will be tested in order to overcome challenges in solubility of the sample matrix.

Noble gas oxide cations in the gas phase – examining Ng^+–O energetics (Ng = Kr, Xe, Rn) by experiment and theory

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Speranza and co-workers previously prepared NgO^+ ions (Ng = Kr [1], Xe [2]) using chemical ionization (CI) and Fourier transform ion cyclotron resonance mass spectrometry (FTICR/MS). Experiments were complemented by density functional theory (DFT) computations and the authors made combined Ng^+–O dissociation energy estimates of 57 and 51 kcal/mol for Ng = Kr and Xe, respectively [2,3].

In this work, we examined reactions of Ng^+ ions (Ng = Kr, Xe) with neutral oxidants O_3 and N_2O by FTICR/MS, employing electron ionization (EI) in the ICR cell under low pressure. The experimental O-dissociation energies of O_3 and N_2O of 25.5 and 39.9 kcal/mol, respectively [3], indicate that both should lead to NgO^+ ions if the estimates of Speranza and co-workers are correct. However, our results showed that only O_3 leads to XeO^+ (Fig. 1, center), while N_2O is unreactive. For Kr^+, only electron transfer was observed, indicating that these exergonic reactions (endergonic for Xe^+) dominate [3]. We assessed the energetics of Ng^+–O bonds using *ab initio* computations at the BCCD(T) level, which provided a Xe^+–O dissociation energy of 37.7 kcal/mol, in accord with non-observation of reaction between Xe^+ and N_2O. Computed Ng^+–O dissociation energies for Ng = Kr, Xe, Rn show a decreasing trend down Group 18 (Fig.1, right) but indicate that it should still be possible to form RnO^+ from the reaction of Rn^+ with O_3.

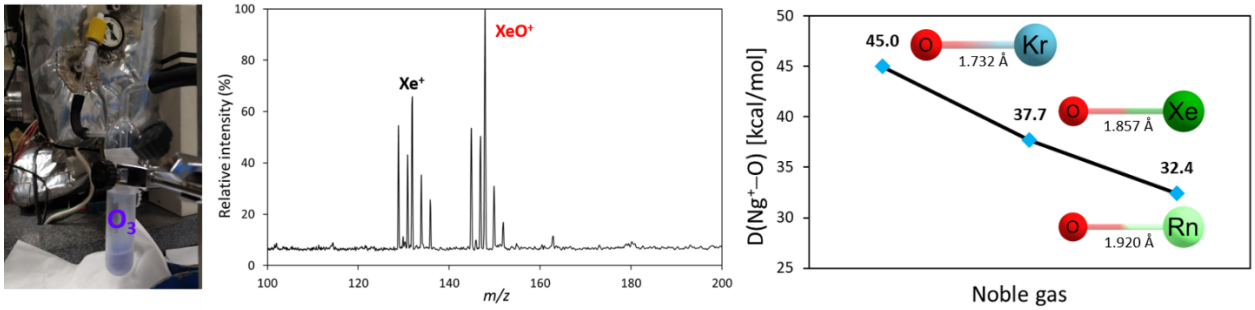

Fig. 1. Mass spectrum of a 0.2 s reaction of Xe^+ with O_3 at a pressure of ca. 7 x10^{-8} Torr (center). Graphical display of the Ng^+–O dissociation energies for Ng = Kr, Xe, Rn, obtained computationally at the BCCD(T) level (right).

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Molecular characterization of hydrophobic burned soils by ultra-high resolution mass spectrometry

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Soil water repellency (SWR) is attributed to the accumulation of hydrophobic organic compounds, mainly lipids. Nonetheless, lipid extraction not always suppress SWR and unextractable soil constituents may be related to residual SWR. Burnt (B) and unburnt (UB) soils from the Doñana National Park (Huelva), under two vegetation canopies (cork oak and heather) and soil fractions, coarse (2–1 mm) and fine (< 0.05 mm), were studied. The molecular composition of soil organic matter (SOM) was studied by Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR/MS) and partial least squares regression (PLS) to predict SWR based on the abundance of the 1221 common compounds found. An omic approach using various indices (factor loadings of PLS models, Pearson's R-squared coefficients, and subtraction of average values of compound abundances in groups of samples with extreme SWR) was applied to identify compounds which could be used as proxies for SWR (Fig 1A-C). In the case of B soils, SWR was related (P < 0.05) to aromatic and condensed compounds, while in the UB soils it mainly relied on aromatics and lignin compounds. In the fine fractions, lipid compounds were the moieties associated with SWR and no correlation was found in the coarse fractions. In conclusion, hydrophobicity was related to lipids as expected, but also to lignin and aromatic components. The combination of FT-ICR/MS with the omic, graphical statistical approach was effective in finding molecular SWR predictors.

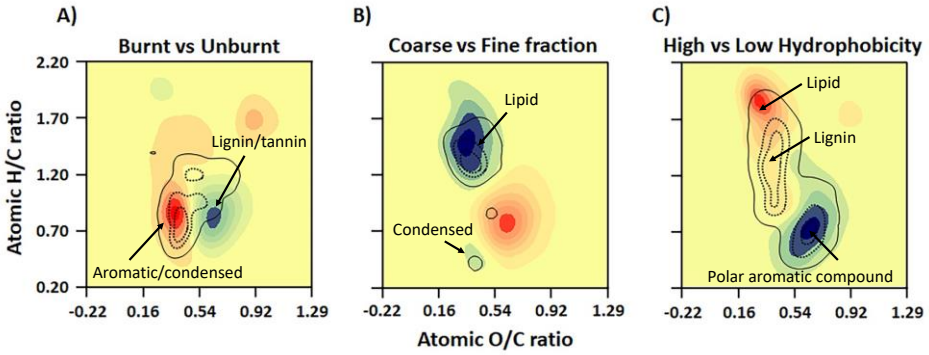

Figure 1. van Krevelen subtraction density plots illustrating the difference between the molecular composition of the SOM in scenarios influenced by different factors: A) Burnt (red) vs Unburned (blue) samples, B) Coarse (red) vs Fine (blue) fractions, and C) High (red) vs Low (blue) hydrophobicity. Compounds significantly correlated with the SWR were represented by a contour plot superimposed on the subtraction plot (the most external contour indicates p > 90%, and the internal contour indicates the p > 95%).

ACKNOWLEDGMENT
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FDS – First instrument independent database for natural organic matter

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Natural organic matter (NOM) is an important part of the organic carbon pool, cycling through different environments. Fourier transform mass spectrometry (FTMS) reaches molecular formula level resolution and reveals the extreme complexity of NOM. FTMS application in environmental studies grows exponentially: for the last twenty years, thousands of works have been published. The next logical step is the development of a molecular library for NOM based on this data. However, direct comparison of FTMS data acquired with different instrumentation has shown drastic inconsistency even for the same samples [1,2]. To overcome this problem, we proposed a method that classifies FTMS data based on the formulae differences (FDs) - differences in the number of elements between each pair of molecular formulae within the NOM formulae list. Analyzing data from the interlaboratory studies we found important FDs ((FD features) characteristic to the fourteen samples of various origin. These features turned out to be independent of the FTMS instrumentation. The features were formed by calculation of a new metric - FD Chains Expected Length (FDCEL) which considers both abundance and homology within spectra. The advantage of the FDCEL measure as it is independent of the exact peaks position eliminating the need to consider the alignment of spectra, which are often mismatched especially in the case of interlaboratory studies. Employment of the FDCEL measure enabled to create the first prototype for a uniform NOM database based on FTMS data. The database and all the functionality is presented as the web application hosted at https://nommass.com.

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PyC2MC: A Python-Based framework for processing multidimensional high-resolution mass spectrometry data

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Using sophisticated separation techniques coupled with mass spectrometry, makes it possible to distinguish species that could otherwise remain hidden[1]. The main challenge comes with the difficulty in processing the large amount of data resulting from these multidimensional analyses. Thus, to approach this objection, a Python-based framework was developed called PyC2MC, which stands for Python for Complex Matrices Molecular Characterization. This framework allows robust feature detection in multidimensional mass spectrometry data using a heuristic K-means Clustering methodology[2] and an iterative method as suggested by Tautenhahn et al.[3], the molecular formula attribution, and 2-dimensional mass spectra recalibration. Complex data sets of asphaltene samples obtained by gel permeation chromatography coupled with 21 Tesla Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (GPC 21T-FT-ICR MS) were processed using PyC2MC (see Fig 1.). Compared to the iterative method[3], which, due to the presence of nested loops, takes a total time of 14 min to regroup the data, the K-means[2] takes about 5 min accomplishing to detect the features of interest, being significantly more time- and resource-efficient.

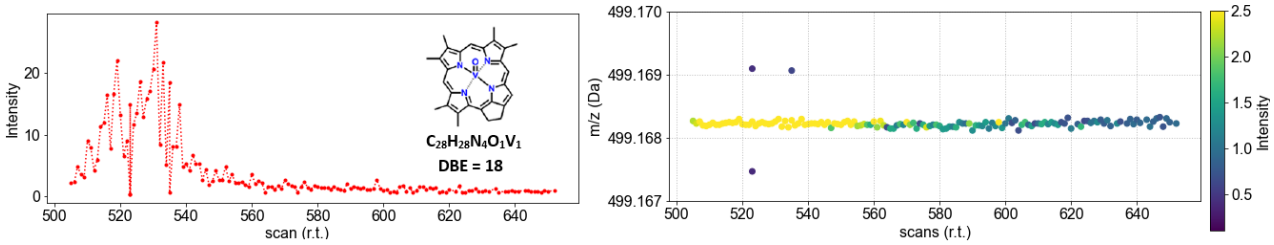

Figure 1. Feature detection and molecular formula assignment of one pattern at $\mu_{m/z}$ = 499.168232 Da.

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Our metal brain: amyloid protein aggregation and metal binding

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Type-2 diabetes is a risk factor in cognitive decline and neurodegeneration. Visible physical symptoms often occur after the disease has been allowed to progress undetected for some time, with treatment limited to symptom management. Research into disease biomarkers has revealed that amyloid protein aggregation and fibril formation within the body as reliable indicators of disease progression: in the case of Parkinson's, the a-Synuclein protein experiences misfolding leading to aggregation, and similar behaviour is observed in type 2 diabetes with aggregation of the human islet amyloid polypeptide.

As of yet, research to understand the trigger for this process has not proved fruitful, with several different theories emerging yet remaining unconfirmed. The aggregation process varies depending on each protein and its environment making it a challenge to categorise and pinpoint exact mechanisms, particularly in the preliminary stages of aggregation. These early-stage oligomers, such as dimers, trimers, and tetramers, could provide vital insights into structural shifts and mechanism initiation. Alzheimer's brain tissue studies revealed the presence of amyloid protein aggregates, but also elevated levels of iron, indicating metals are somehow implicated in the aggregation process.

To probe these structures in more detail, FT-MS was used in combination with CID, ECD, UVPD and IRMPD fragmentation on the a-Synuclein and hIAPP proteins to gain early-stage structural information. This combination of fragmentation techniques generates the most detailed sequence coverage and insight into how the protein strands are linked. In parallel, hIAPP was co-incubated at body temperature with a series of physiologically relevant metals and sampled at regular intervals. Quantification measurements were performed with TimsTOF-MS throughout the duration of incubation and compared in order to track unique metal impact on aggregation and monitor oligomer conversion.

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Glycoproteomics of glycoengineered simples cells for the identification of bladder cancer molecular targets

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Bladder cancer (BC) is a global disease, facing limited therapeutic options [1]. Glycosylation of cancer cell surface presents a promising source of neoantigens [2, 3]. Therefore, the cancer glycoproteome may provide the bi-specificity necessary for targeted therapeutics, taking advantage of both glycan and protein expression specificity [4]. However, the heterogeneity of glycan structures poses a challenge for proteome identification by mass spectrometry [5, 6].
Here in we describe a strategy to interrogate the glycoproteome of a glycoengineered BC cell line, reducing the complexity of O-glycosylation to the simplest and cancer-associated O-glycan, Tn. Glycoproteomic analysis based on enrichment for plasma membrane proteins expressing this glycan resulted in the identification of more than 5,700 proteins. This list of proteins was then used to generate a customized database for subsequent O-glycoprotein validation following lectin affinity chromatography for Tn-glycopeptides enrichment. The O-pair search algorithm, inbuilt in MetaMorpheus [7], was used for confirmation of protein *O*-glycosylation. Over 170 glycoproteins carrying the Tn antigen were identified with confidence and ranked according to their potential for clinical applications, namely targeted therapeutics, using an in-house developed algorithm [8]. Validation in patient samples is now required, envisaging clinical translation.

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Dark charge

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Ion-Ion interactions; otherwise known as space charge; are the Achilles heel of any MS technique, space charge can pose significant difficulties in any instrument, however the impact on Ion-trap based instruments is particularly significant. These interactions between charged particles can be limited through experimental practice and instrument design, however, is often difficult to completely remove. Space Charge in FTICR has been shown to induce significant mass errors.[1] Calibration methods exist to partially account for these effects however they rely on quantification of peaks above the noise floor to correct for space-charge induced frequency shifts.[2] Ion signals that fall below the noise level are herein referred as "Dark Charge", in reference to the astronomical phenomenon of "Dark Matter" which is only observed by its effect on other observed masses.

Although ultra-high resolving power and sub-ppm mass accuracy of FTICR-MS has allowed analysis of complex samples such as those present in the fields of proteomics and petroleomics. "Dark Charge" will interfere with the calibration of any spectrum where present, due to interactions of these hidden charges with signal-producing ions.

The effects of "Dark Charge" on calibrations are probed in this work. Using the 12T and 15T Solarix FT-ICR the effects of dark charge were investigated using various proteomics-based samples. The effect of common isolation methods on this phenomenon was also probed using both quadrupole and TIMS based methods. Herein we use these isolation tools to quantify the presence and magnitude of these dark-charge effects and develop a new methodology to correct for them.

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FT-ICR MS for Detection and Functional Group Elucidation of Ozonation Byproducts in Effluent Organic Matter with Isotopically Labeled Ozone

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Ozonation is a chemical oxidation method for the treatment of wastewater to enhance removal of contaminants.[1] Compared to known contaminants, effluent organic matter (EfOM) is not well studied in terms of ozonation byproducts (OBPs), even though EfOM is a major ozone consumer.[2]

Ultra-high resolution mass spectrometry (e.g. FT-ICR MS) is the primary method for non-target analysis of EfOM, but OBP detection is still limited.[3] With stable isotopically labeled heavy oxygen, heavy ozone can now be used to produce isotopically labeled OBPs. This mass label can unambiguously identify OBPs formed from EfOM and improve detection.

Using the ratio between ^{16}O and ^{18}O OBPs generated during the experiment, direct oxygen transfer reaction products (direct OBPs) can be identified in EfOM. Fragmentation data of these direct OBPs generated by FT-ICR MS/MS can be screened for indicative neutral losses with ^{18}O, yeilding functional group information. Using this technique the original functional group can be infered based on the well understood reaction mechanisms of ozone. Heavy oxygen formula assignments present a unique data processing challenge addressed in this work.

With this approach, 968 molecular formulas were detected as OBPs with ^{18}O in EfOM. Nearly one third of these OBPs would only be detected as non-reactive without ^{18}O. Of the labeled OBPs detected, 85 are classified as a direct OBP. Three direct OBPs were fragmented with FT-ICR MS/MS and sulfur containing functional groups with ^{18}O were detected via indicative neutral loss. This implies the presence of reduced sulfur functional groups in the original EfOM parents.

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Poster Communications

P1

Protonated forms of Naringenin and Naringenin Chalcone elucidated by IRMPD spectroscopy, IMS, CID-MS, and computational approaches

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Naringenin (Nar) and its structural isomer, Naringenin Chalcone (ChNar) are two natural phytophenols with beneficial health effects belonging to the Flavonoids family. Due to the wide range of biological activities of Flavonoids and to the existence of numerous natural isomers, many efforts have aimed to differentiate and characterize the flavonoid components by tandem mass spectrometry[1]. In particular, collision-induced dissociation (CID) mass spectrometry studies have allowed to successfully characterize several isomeric flavonoids belonging to distinct subgroups, through their different fragmentation patterns. However, it was not possible to apply this powerful technique for the direct discrimination of Nar and ChNar due to their same behavior under CID.

With this contribute, we present our results about the discrimination and structural characterization of the protonated forms of Nar and ChNar. A combined approach has been exploited, based on electrospray ionization coupled to (high resolution) mass spectrometry, CID measurements, IRMPD spectroscopy, theoretical DFT calculations and ion mobility-mass spectrometry (IMS). While IMS and variable collision-energy CID experiments hardly differentiates the two isomers, IRMPD spectroscopy appears to be an efficient method to distinguish naringenin from its related chalcone. In particular, the spectral range between 1400 and 1700 cm^{-1} is highly specific in discriminating between the two protonated isomers. Selected vibrational signatures have allowed to identify the nature of the metabolite present in methanolic extracts of commercial tomatoes and grapefruits by the means of IRMPD. Furthermore, comparisons between experimental IRMPD and calculated IR spectra has clarified the geometries adopted by the two protonated isomers.

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P2

Coinage metal cation adducts with acetonitrile – probing relativistic effects with FTICR/MS experiments and DFT computations

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Bohme and co-workers recently demonstrated that, in a selected-ion flow tube (SIFT) tandem mass spectrometer with an helium bath gas at 0.35 Torr, relativistic effects could be assessed in the binding of neutral molecules to atomic metal cations from measurements of the kinetics of association [1-3]. In an attempt to evaluate relativistic effects within Group 11 using a low-pressure mass spectrometry (MS) technique, namely Fourier transform ion cyclotron resonance mass spectrometry (FTICR/MS), adduct formation with acetonitrile of the three coinage metal cations Cu^+, Ag^+ and Au^+ was studied experimentally and also computationally using density functional theory (DFT).

Experimental reaction kinetics were determined for sequential adduct formation, $M^+ + CH_3CN \rightarrow M(CH_3CN)^+$ and $M(CH_3CN)^+ + CH_3CN \rightarrow M(CH_3CN)_2^+$ (Fig. 1, center), and disclosed rather inefficient reactions for all three metal cations that did not show a clear enhancement from Ag^+ to Au^+. Conversely, the computational studies revealed an increase in dissociation energy of Au^+ compared to Ag^+ and Cu^+ (Fig. 1, right). These results suggest that a broader survey of neutral molecules should be undertaken before reaching any conclusion about the adequacy of low-pressure FTICR/MS to assess relativistic effects from measurements of kinetics of association.

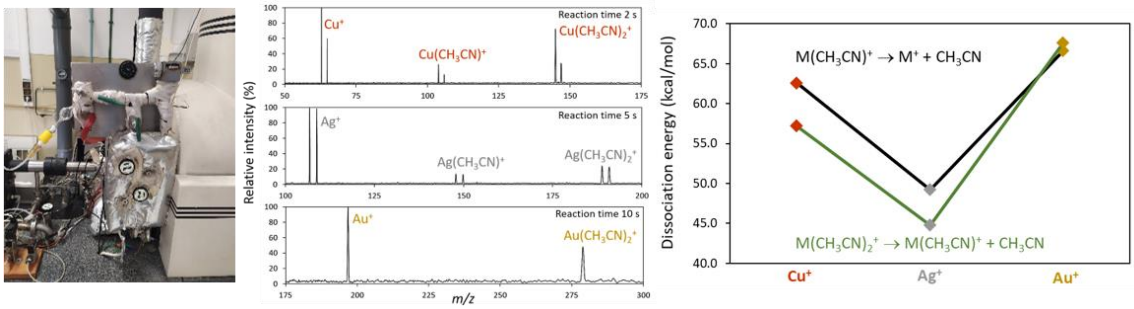

Fig. 1. Mass spectra of the reactions of Cu^+, Ag^+ and Au^+ with CH_3CN at a pressure of ca. 3.0 x10^{-7} Torr (center). Graphical display of the dissociation energies obtained by DFT computations (right).

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P3

Effect of mass analyzer anharmonicity on resolution in a 2D FT MS experiment

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2D Fourier Transform Mass Spectrometry is a method that allows to MS/MS analyses of individual components of the complex mixture in parallel. In this method ensembles of different m/z ions are excited by voltage containing ICR frequencies of these ions. After encoding delay the excitation pulse is applied once more, and ions with opposite phase are deexcited back down to the center of the cell where fragmentation occurs by different physical methods.

Using computer simulation, the influence of the anharmonicity of the FT ICR cell on the resolution was estimated. To do this it was necessary to simulate the movement of ion clouds considering the ion-ion interaction. It was possible by using simulation software that implements Particle-in-Cell approach (PIC-code). To simulate the electric field in space from different types of ICR cells, models of the corresponding electrode configurations were created in the SIMION program. PIC-code has been modified to incorporate the SIMION models. As a result of the simulation, the trajectories of the movement of ion clouds were obtained (coordinates of the position of ions depending on time), which were then processed using Python 3.

Simulation of first stages of 2D FT MS experiment was performed for two different FT ICR cells: regular open cylindrical cell and dynamically harmonized cell (DHC). It was shown that the formation of comets in a non-harmonized trap leads to incomplete deexcitation of ion clouds to the center of the trap, which is expressed in a weaker dependence of the number of fragmented ions on the encoding delay time. This results in a reduction in resolution in vertical (precursor ion mass scale) coordinate.

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P4

FT-ICR-MS uncovers large-scale metabolic differences between phenotypically identical yeasts

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Saccharomyces cerevisiae is a model eukaryote with around 6000 genes, most of which can be deleted without causing changes to the yeast growth phenotype. Nevertheless, mutated cells may differ substantially from their wildtype counterparts at the metabolome level, particularly if the mutations in question happen to be associated with key metabolic pathways. Extreme resolution analytic techniques such as FT-ICR-MS allow us to identify and interpret these differences through an untargeted metabolomics approach.

In this work, we demonstrate an application of this paradigm to five isogenic *S. cerevisiae* strains, including a reference wildtype strain, three mutants with methylglyoxal catabolism-related single gene deletions, and one glycolysis-related single gene deletions mutant. The yeasts were grown in the same conditions, metabolites were extracted, and samples analysed through direct infusion ESI in positive mode FT-ICR-MS. The identified metabolites were annotated with names (human and yeast metabolomic databases) and chemical formulas (predictions based on a set of heuristic rules). We found a wide variety of metabolic differences between them, as shown through multi-variate statistical analysis and other metrics. Furthermore, we explained some of the observed metabolic differences based on our pre-existing knowledge of methylglyoxal catabolism (e.g. a greater degree of similarity was found between mutant strains related to the glutathione-dependent pathway of methylglyoxal catabolism), showing that biologically meaningful conclusion can be drawn from the study of single-gene deletions at the metabolome level.

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P5

Study on the formation of aromatic networks during pyrolysis and hydrothermal carbonisation of kraft lignin with thermal analysis coupled to high-resolution mass spectrometry

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Production of high-value carbon materials, e.g., graphene and graphite, from lignin is a promising but challenging approach. Kraft lignin (KL), as a by-product of the pulp and paper industry, has an increased content of aromatic structures with high number of oxygen atoms. These starting materials tend to form turbostratic carbon structures under pyrolytic conditions. Unfortunately, high temperatures are required for the direct production of such materials. Well-developed controllable aromatic networks reached at low-temperatures from lignin would be beneficial to obtain affordable commercial graphite-like products. Therefore, the catalytic effect of a eutectic salt mixture (KCl/LiCl) and the hydrothermal carbonization (HTC) process on KL has been studied. The HTC process is a novel and green method for mild thermochemical processing of wet biomass to increase the degree of aromaticity. The aromatic network from the biochar samples produced in different pyrolytic conditions was investigated with thermogravimetry coupled to atmospheric pressure chemical ionization Fourier transform ion cyclotron resonance mass spectrometry (APCI-FTICR-MS). The analyses provide more detailed information on molecular rearrangements of these complex organic mixtures. A mixture of different monomeric and oligomeric species could be found. Furthermore, the HTC process reduces the amount of sulphur significantly by a factor of two. Most importantly, it was possible to mimic the eutectic pyrolysis process in the thermal balance at a laboratory-scale and to directly explore the evolved gas mixture on a molecular level. The investigations indicated the catalytic influence of the salt mixture on the production process of the biochars, achieving partially graphitization already at relatively low temperatures.

P6

The assembly of protein complexes: from monomers to tetramers

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Native mass spectrometry emerged in the structural biology field as it has a wide range of applications from inferring the composition of a protein complex to determining the kinetics of assembly and disassembly reactions and studying protein-protein interactions [1]. The study of proteins in their native conformation by native MS is challenging, and a small number of proteins are used as models, to enlighten key mechanisms and processes [2]**.** Yeast alcohol hydrogenase (ADH) is a tetrameric protein (147 kDa) stabilised by two disulphide bonds [3,] and one of the proteins used for this purpose**.**

In this work, we aimed to study the assembly/disassembly reaction of these model proteins, assessing their behaviour when dissociation-inducing compounds were added. ADH was incubated with a reducing agent (TCEP) and after optimization of TCEP concentration, dissociation was induced with 10 mM TCEP and analysed on both a modified Q-ToF and FT-ICR. A kinetic model was optimised and used to estimate the kinetic constants involved in the dissociation reaction. Close analysis of the reaction rates and time-course analysis allied with interpretation of the CSD variations helps unravel intermediate conformational changes. Furthermore, it was possible to shown that the Anfinsen's hypothesis holds true for multimeric proteins: Altogether, these results enlighten the potential of native mass spectrometry to unravel the mechanism of protein complex assembly and disassembly, as well as to estimate the corresponding kinetic parameters.

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P7

Multimodal Fragmentation and, 1- and 2-Dimensional Tandem Mass Spectrometry of Human Placental Lipids

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Lipids are a vastly diverse class of complex biomolecules that serve as principal components of cellular membranes. Lipids provide cellular signal transduction, apoptosis, and gene expression. However, their biophysical architecture can provide an insight into the dissociation mechanism in adverse obstetrics, where the lipid pathophysiology can have tremendous implications on a developing foetus, and consequentially on a new-born and its mother. The aim of this study was to develop a 1- and 2-dimensional tandem mass spectrometry method, with multiple fragmentation techniques with positive nano-electrospray ionisation to accurately identify the structural lipidomic signatures in relation to lipid species, the polar head groups, *sn*-positional fatty acyl chain lengths and double-bond position(s).

In this study, direct infusion of the placental tissue extracts was performed on a Bruker 12T SolariX FT-ICR MS. 1DMS spectral assignments was then performed by matching each precursor mass against a database to identify the various lipid classes based on the individual elemental composition, with sub-ppm mass accuracy. Multimodal fragmentation (CID, IRMPD, UVPD and EID) was then performed for the detailed structural characterisation of the various lipid species. 2DMS on FT-ICR with EID and UVPD enabled multiple lipid species to be identified and characterised without the need for any prior chromatographic separation or quadrupolar isolation. Each lipid precursor species was then grouped by common fragments, displayed by the precursor and neutral loss lines, enabling accurate identification with ultra-high resolution. Combining complementary fragmentation methods with 1D- and 2D FT-ICR MS provided an invaluable platform for the comprehensive characterisation of each lipid percussor ions.

P8

From grapevine to the dinning table: unprecedented approach using FT-ICR-MS

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Grapevine, *Vitis vinifera* L., has a long history of domestication and human manipulation for wine production and, is one of the most prominent fruit crops in the world. The wine industry plays a significant economic, social, and cultural role in several countries in Europe and South America. Wine is one of the most appreciated drinks over time and has a high degree of complexity, which results from several biochemical processes that ranges from the choice of grape varieties and yeasts suitable. The fermentation process is under the influence of environmental factors, such as temperature and humidity, as well as the choice of *terroir* and its origin and, in the final stage, in wooden barrels.

Tracing the chemical profile is extremely important to understand the physical and organoleptic properties of wine as well as the association of compounds present in the grapevine with the identification of biomarkers of resistance to disease[1] and the identification of the origin of metabolites with the *terroir*. Despite the complexity of the samples, it was possible with the FT-ICR-MS to accurately identify a large number of compounds, to associate the compounds with the grape varieties used[2] and even to identify pesticides and toxic compounds in the wines[3].

Untargeted metabolomics allows the study of multiple samples as well as following the evolution of complex processes such as wine production. This study is the first spark to a new concept of analysis of the whole wine production from the vineyard to the wine glass.

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P9

Observation of sodium-cationized polyaromatic hydrocarbons in the analysis of heavy oil samples with positive-ion ESI FT-ICR MS

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Aromatic or polyaromatic hydrocarbons (PAHs) are less frequently observed in the electrospray ionization (ESI) mass spectra of fossil fuels due to their low proton affinities. Here, we report on the observation of abundant sodium-cationized PAHs in ESI FT-ICR mass spectra of heavy oil samples. Three different oils, light cycle oil (LCO) and scrap tire pyrolysis oil (STPO) as well as their hydrotreated product (HTO), were analyzed on a 12-T FT-ICR instrument (Bruker solariX XR), coupled with positive-ion ESI. Sodium cation binding to hydrocarbons was further studied computationally for selected aromatic and saturated bicyclic hydrocarbons by DFT calculations, using the hybrid density functional B3LYP method in combination with the def-TZVP basis set. Compositional analysis of LCO, STPO and HTO samples with (+)ESI FT-ICR MS confirmed that the oils were composed of a wide variety of heteroatomic (NOS) compounds as well as aromatic and polyaromatic hydrocarbons. To our surprise, most PAHs were present as sodium adducts. To further understand preferential sodium cationization of PAHs, we performed DFT calculations with some model compounds. The lowest energy structures for the studied $[PAH + Na]^+$ complexes were generated and the absolute Na^+ binding energies were calculated. In each case the sodium ion prefers to form strong 6-coordinate π-bonds to the aromatic rings with a distance of ca. 2.4 Å to the centre of the ring (binding energies from –105 to –110 kJ/mol). In the absence of aromatic rings, Na^+ adopts a two-coordinate σ-bond (around –60 kJ/mol) with bond distances of around 2.7 Å.

P10

Vacuum photoionization high-resolution orbitrap mass spectrometry: online characterization of primary ship engine emission

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Anthropogenic air pollution significantly alters atmospheric physicochemistry globally, causing a significant environmental impact, *i.e.*, climate change. Not only large-scale environmental aspects have to be considered, but also more local effects on human health causing cardiovascular diseases by oxidative stress and inflammations. Consequently, a deep molecular understanding of emission sources is needed to trace compounds accountable for these impacts. One substantial contributor to air pollution is maritime transport[1]. In particular, the exhaust gases of ship engines carry a high fraction of aromatic compounds, which are known for their carcinogenic health effects. In this study, we attempted to complement the chemical picture for ship emissions by deploying a recently modified Exactive™ Orbitrap mass spectrometer (PhotOrbi)[2] using resonance enhanced multi-photon ionization (REMPI)[3]. The primary exhaust gas emissions of a research engine feed with different ship fuels and operated at different loading conditions were directly investigated online. This molecular-level analysis features the high dynamic range, resolution, and mass accuracy of the Orbitrap platform as well as the soft and selective ionization properties of REMPI. In particular, utilizing a KrF Excimer Laser (248 nm) for ionization allows us to directly address polycyclic aromatic hydrocarbons (PAHs). This novel approach, compared to established online time-of-flight process platforms, allowed us to tackle the high molecular complexity of these gases and trace harmful species directly, even during dynamic processes, with sub-ppm accuracy in sum formula attribution.

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P11

$[KrSF_5]^+$ – A new Krypton complex in the gas phase

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The gas-phase reactions of noble gas (Ng) cations, namely Kr^+ and Xe^+, with SF_6 were investigated experimentally by Fourier transform ion cyclotron resonance mass spectrometry (FTICR/MS) and computationally using MP2 and BCCD(T) methods [1]. The study revealed an unprecedented interaction between Kr^+ and neutral SF_6 that gave rise to a new cationic, weakly bound complex of Kr, $[KrSF_5]^+$, although the major reaction channel was dissociative electron transfer to yield SF_5^+ and {Kr,F} (Fig.1, center).

Experimental studies examined formation and stability of the new species, while computational studies addressed the energetics of the reaction and indicated that $[KrSF_5]^+$ is stable by ca. 1 kcal/mol (Fig.1, right-green). The same computational approach was used to examine the reaction of Xe^+ with SF_6 and showed it to be thermodynamically unfavourable by ca. 35 kcal/mol (Fig.1, right-red), confirming the non-observation of reaction in MS experiments. Analysis of the bonding in $[KrSF_5]^+$ clearly showed that it is a non-covalently bound species, while in its presumed precursor $[KrSF_6]^+$ a partially covalent Kr-F bond could be present.

By the combination of a low-pressure mass spectrometry technique, FTICR/MS, and *ab initio* computations, we were able to observe and characterise a new Kr species, specifically the $[KrSF_5]^+$ complex, contributing to the enrichment of noble gas chemistry since reports of new Kr species have been rare [2].

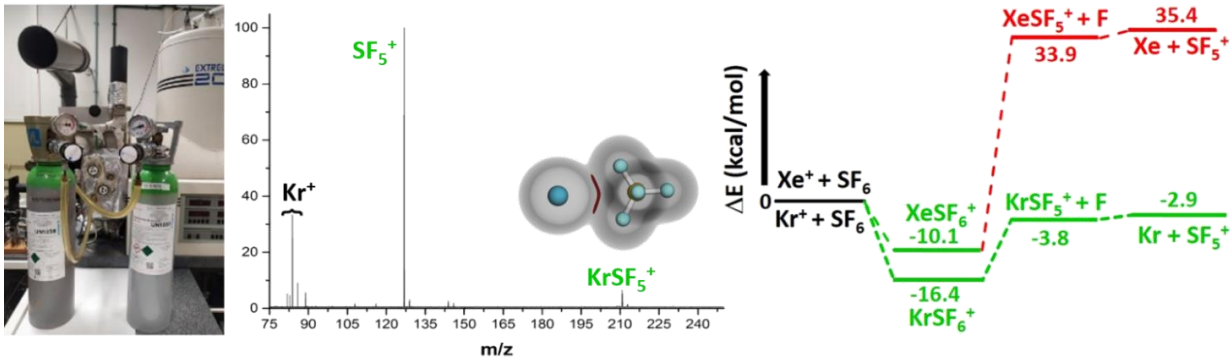

Fig. 1. Mass spectrum of a 0.5 s reaction of Kr^+ with SF_6 at a pressure of ca. 5.0 x10^{-7} Torr (center). Graphical display of the energetics (ΔE_0) of $Ng^+ + SF_6$ reactions (Ng = Kr, Xe), obtained computationally at the BCCD(T) level (right).

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P12

Two dimensional mass spectrometry as a tool for biotheraputics analysis using ultraviolet photodissociation and electron-based dissociation techniques

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Bioactive proteins in venom from scorpions has been widely used as alternative medicines for the treatment of a great array of conditions, such as cancers and blood coagulations. However, only partial genome sequences are available for these venom protein; which is challenging to determine biomarkers from the crude venom.

To fully cover the sequence of each protein in the venom, herein, we applied a 2DMS technique coupled with ultraviolet photodissociation (UVPD), electron-based dissociation (ExD), infrared multiple photon dissociation (IRMPD) fragmentation methods. 2DMS allows for an unbaised separation and detection of proteins which facilities the detection of proteins with different physical properties; while the combination of different fragmentation methods resulted in high protein coverages, hence a more confident determination of the biomarker sequence.

2DMS is a data independent analysis technique which has been shown to be applicable in the characterisation of complex mixtures without the need for prior separation. It allows for the correlation of all fragments to its respective precursors, without requiring quadrupole isolation or chromatographic separations. Based on our results, several unique proteins were solely observed with 2DMS compared to nano-LC tandem MS experiment due to the unbaised protein separation and identification in 2DMS, indicating 2DMS is a complementary experiment to nano-LC experiment which results in the ability to assign proteins of various properties. In addition, vertical precursor lines from a 2DMS plot allows the identification of the precursors that are producing common fragments which allows a fast screening and grouping of proteins with same backbone but various PTMs.

P13

In-hardware absorption mode capability in FTMS

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Ion signals contained in a time-domain transient in FTMS are characterized by: (i) amplitude, (ii) frequency, and (iii) initial phase. The initial phase is perhaps the ion signals' least known (or described) property. However, accurate information on the initial phases is critical for modern FTMS that needs the best possible performance to tackle the growing challenges in diverse applications. Most importantly, this information opens up a possibility to represent FT mass spectra in the absorption mode (aFT), well known for its benefits over the magnitude mode FT.

Nowadays, several software tools exist that may help to convert time-domain transients into the aFT mass spectra as the post-acquisition data processing. However, the in-hardware aFT capability is not readily available in commercial Orbitrap and ICR FTMS instruments for various reasons. To overcome this limitation, previously, we developed a specialized high-performance data acquisition system, which allows acquiring time-domain transients with in-phase ion signals, thus enabling the direct generation of aFT mass spectra, Figure [1, 2].

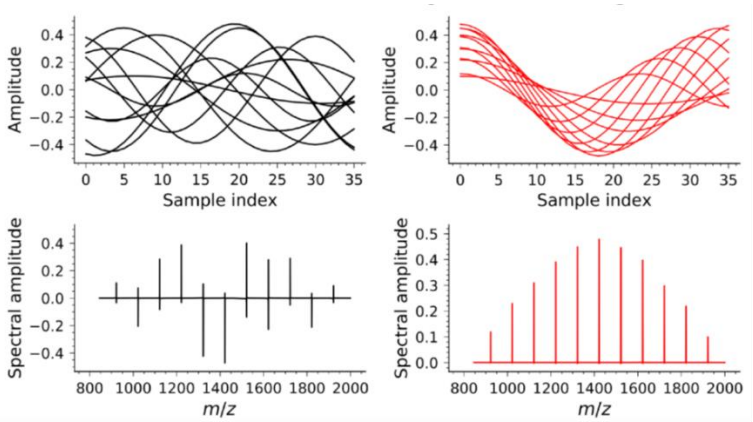

Figure: FT of a usual time-domain transient whose components have diverse phases (left, top) yields a mixed-mode mass spectrum (left, bottom) that requires post-processing for it to be represented in absorption mode. Right panels: FT of time-domain transients with in-phase ion signals (right, top) generates mass spectra directly in absorption mode (right, bottom).

Here, we report on this technology's analytical and applied advantages following its expansion to diverse FTMS platforms and application fields, including intact-mass and top-down proteomics, MALDI imaging, LC-MS applications, and isotopic ratio analysis.

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P14

Open modification of dynamically harmonized FT ICR cell for the new generation of FT ICR mass spectrometers with ultra-high magnetic field

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The dynamically harmonized FT-ICR cell (DHC) [1] provides the best mass-accuracy and resolving power of any other FT-ICR cell. This is due to the hyperbolic shape of the trapping potential averaged over the cyclotron motion. However, in DHC there is a problem with the maximum achievable quality of vacuum due to the closed shape of the cell: vacuum is not high enough to make mean free path greater than the length of trajectory ions coming during detection time, which linearly increases with magnetic fields. We present an open modification of the DHC [2], where the quality of the vacuum can be significantly improved. We analyzed the potential inside the new modification and proved that it can be as close to the ideally hyperbolic potential as needed. The new cell can be embedded into a vacuum tube, which has additional advantages. Firstly, the residual gas is reduced even more by reducing the area exposed by cell electrodes and connecting wires. Secondly, the preamplifier can be placed directly on the trap electrodes without using long wires from the trap to the feedthroughs. This causes the input capacitance of the measurement circuit to decrease from 75 [3] to ~4pF (limited manly by the input capacitor of InterFET 2N4416 transistor). Reducing the capacitance of the input circuit of the preamplifier allows to increase the S/N of output signal.

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P15

Revealing the Chemical World of Fingermarks through FT-ICR-MS

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Fingermarks are unique to each individual and commonly correspond to a complex pattern of ridges and valleys that are crucial in Forensic Sciences for human identification [1]. Besides the physical pattern, fingermarks contain a wide range of chemical constituents. Untargeted metabolomics based on extreme resolution, Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), enables the detailed description of the chemical complexity of dermopapillary residues [2,3], leading to the identification of hundreds of chemical species and metabolites. Exogenous substances such as pharmaceuticals, drugs, personal care products, and food additives are of great forensic importance. However, the development of latent fingermarks generally requires the use of different reagents [4], making their characterization by mass spectrometry an extremely complex affair in a realistic scenario. The purpose of this study was to obtain relevant chemical information from fingermarks in the presence of common fingermarks developers like Instant White, Dragon's Blood, and Magnetic Latent Print Powder. The extracted metabolites were analyzed by FT-ICR-MS in a 7 Tesla Solarix XR from Bruker, equipped with the ParaCell. All samples were analyzed by direct infusion in positive electrospray ionization mode and processed in absorption mode. Metabolite identification was performed using the Human Metabolome Database (HMDB) – pharmaco and sweat metabolites. The results obtained allowed to observe minimal differences in the identified chemical compounds between latent and developed fingermarks.

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P16

Identifying cathinone regioisomers by FT-ICR MS^2, using Monte Carlo simulation of ion abundance ratios

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According to the latest report of the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA, 2022), synthetic cathinones are the second most apprehended group on the drug market, with 162 being monitored by the Early Warning System [**1**]. The growing diversity of synthetic cathinones' structures presents a challenge for identifying these compounds using conventional mass spectrometry methods. In this work, we aimed to distinguish chloro-cathinone regioisomers in a test group of 20 synthetic ones.

All synthetized cathinones were analysed by Fourier-transform ion cyclotron resonance (FT-ICR) with electrospray ionization (ESI) and tandem mass spectrometry (MS/MS) with collision induced dissociation (CID). Identification was based on acceptance intervals of relative abundances (RA) of characteristic fragments on the MS^2 spectra simulated by the Monte Carlo method [**2**], considering the estimated dispersion and correlation of ion abundances.

Through the RA of the observed product ions, it was possible to distinguish both chlorine regioisomers and structural isomers, having at least one identifying fragment with a different RA for each regioisomer. Furthermore, a MS^2 pattern of the synthetic cathinones was established. The defined identification criteria were successfully applied to the identification of a specific regioisomer in seized samples.

This work showed, for the first time, that FT-ICR allows the identification of synthetic chloro-cathinones through MS^2 spectra and to discriminate between chlorine regioisomers. This method can be applied to the identifications of others similar compounds by FT-ICR-MS/MS.

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Fast photochemical oxidation of protein/nucleic acid complex coupled to high-resolution MS

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The methods of structural proteomics had undergone a remarkable growth in recent years, which had huge impact in the field of structural and molecular biology. These methods are able to address the questions related to structure and dynamics of protein complexes. Beyond well-established MS-based methods, radical covalent labelling has showed to be an effective analytical tool for characterization of biomolecular assemblies. In this study, we adopted the Fast Photochemical Oxidation of Proteins (FPOP) approach to study the dynamics of transcription factor/DNA response element complexes. Protein in the absence or presence of ds-DNA was irradiated by excimer laser (KrF, 248 nm) in the presence of hydrogen peroxide. Protein was digested using LysC and Trypsin. LC-MS/MS and LC-MS analyses were performed to identify and quantify the ratios between apo and holo form, respectively. To map the damage of DNA initiated by the reactive hydroxyl radicals, DNA alone and in the complex with protein undergone FPOP oxidation. Protein was digested using proteinase-K and DNA fragments were separated by methanol gradient in LC system that was directly coupled to a high-resolution FT-ICR-MS in negative ion mode. Analysis of oxidized peptides enabled the localisation and thus quantification of residues directly involved in protein-DNA interaction. Analysis of separated DNA fragments revealed that hydroxyl radical cut the DNA unspecifically creating 5'OH-[mer]-3'OH or 5'OH-[mer]-3'P terminal fragment ions, which were in the presence of the protein significantly less abundant. Obtaining the information about the solvent accessibility of both DNA and protein enabled *ab initio* design of protein/DNA structural model.

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From shale oil to pharmaceuticals – investigation of shale oil distillates and their sulfonated products by high-resolution mass spectrometry

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Bituminous shales are rocks that contain organic matter (kerogen). By dry distillation of these schists, the kerogen can be accessed due to pyrolytic decomposition, resulting in shale oil distillates. For pharmaceutical purposes, these precursor distillates are treated with sulfuric acid before being neutralized with bases like sodium hydroxide or ammonia. These bituminosulfonate salts and its precursor distillates are known to be of ultrahigh isobaric and isomeric complexity. Additionally, various pharmacological effects have been distinguished. Among other, anti-inflammatory and antibacterial properties are reported. Unfortunately, the literature regarding the in-depth chemical characterization of bituminosulfonates is limited. Nevertheless, to understand the production workflow and the biological working mechanism in detail, complete characterization of the bituminosulfonates and its precursors is a prerequisite.

Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) was applied to investigate the isobaric complexity of these matrices. Here, electrospray ionization was used to analyze the polar compounds such as sulfonates, while atmospheric pressure photoionization was applied to address aromatic and sulfur containing compounds. For the isomeric complexity, comprehensive two-dimensional gas chromatography (GCxGC) coupled to electron ionization high-resolution time of flight mass spectrometry (EI-HRToF-MS) was utilized as complementary method, resulting in a wide range of sensitive molecular-level speciation.

With this work, we present the characterization of pharmacologically active bituminosulfonates and its distillate precursors. Moreover, besides fingerprinting we were able to perform first data integration of FT-ICR-MS with GCxGC-HRToF data, which to our knowledge is presented for the first time. Therefore, these techniques allowed first assumptions on the complex reaction pathways of the sulfonation.

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Using Graph Properties of Mass-Difference Networks for Profiling and Discrimination in Untargeted Metabolomics

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Untargeted metabolomics experiments yield numerical matrices with thousands of signal intensities of detected metabolites, which can be highly variable. To minimize variability, data pre-treatments are applied to extract biological information for characterization and interpretation. In mass spectrometry data, Mass-Difference Networks are derived by transforming the less variable and seldom used spectral feature occurrence to nodes, linking them based on mass differences representing chemical transformations. This is usually used to assist formula annotation or analyze chemical transformation importance.

Here, we demonstrate that using graph properties of the Mass-Difference Networks of each sample (sMDiNs) as signatures for metabolic profiling and class discrimination is a viable alternative to using intensity-based matrices, while extracting new relevant information. As proof of concept, on diverse benchmark datasets, several statistical methods' performances in discriminating samples by class based on several graph properties (analogous to pre-treatments) computed from sMDiNs were compared to the corresponding pre-treated intensity-based matrix.

The Degree profile graph property led consistently to the best performance of clustering and classification methods, being competitive with the pre-treated intensity data matrices. Furthermore, using both the Degree profile and prevalence of chemical transformations between samples allowed us to rank the latter by importance for class discrimination, showing the potential of the methodology. Thus, sMDiNs are a viable alternative to the common intensity-based workflow, allowing the highlighting of usually overshadowed data, as illustrated by the ability to rank chemical transformations and more not yet explored, providing complementary information to give a fuller picture of the biological system in question.

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Accessing the molecular composition of polar species in carbonaceous aerosols by thermal analysis high-resolution mass spectrometry

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Anthropogenic air pollution exposes the environment and humanity to immense amounts of organic contaminants and inhalable aerosol particles. Biomass burning is considered a significant source of primary combustion aerosol, with a semivolatile organic fraction that, through reactions with atmospheric oxidants, plays a part in the formation of secondary organic aerosol (SOA). These biomass related emissions appear to be structurally more diverse with distinct optical and physicochemical properties, exhibiting harmful consequences on human health and climate. Therefore, detailed knowledge on the composition is essential. Due to the limited volatility and stability of the biomass combustion constitutes, a novel methodology to address those labile and more polar species is desirable. Evolved gas analysis techniques coupled with high-resolution mass spectrometry could be identified as a powerful analytical approach addressing the vast complexity of carbonaceous aerosols. Nonetheless, particularly labile carbonylic functionalities commonly found in SOA will undergo complex thermal fragmentation pathways. Derivatisation strategies, usually applied for gas chromatographic attempts, combined with direct inlet probe (DIP) make it possible to access an enlarged chemical space compared to classical DIP techniques with keeping minimum sample pre-treatment. Using in-situ derivatization inside the DIP capillary makes it feasible to induce a specific selectivity towards more polar and stable species, working as a complementary approach to the limitations of chromatographic techniques. Hence, along with DIP and atmospheric pressure sources, it is possible to combat the chemical heterogeneity and complexity gaining a deeper understanding of the impact of anthropogenic aerosols.

Orbitrap-based metabolomics benefits from a full window apodization absorption mode Fourier transform data processing

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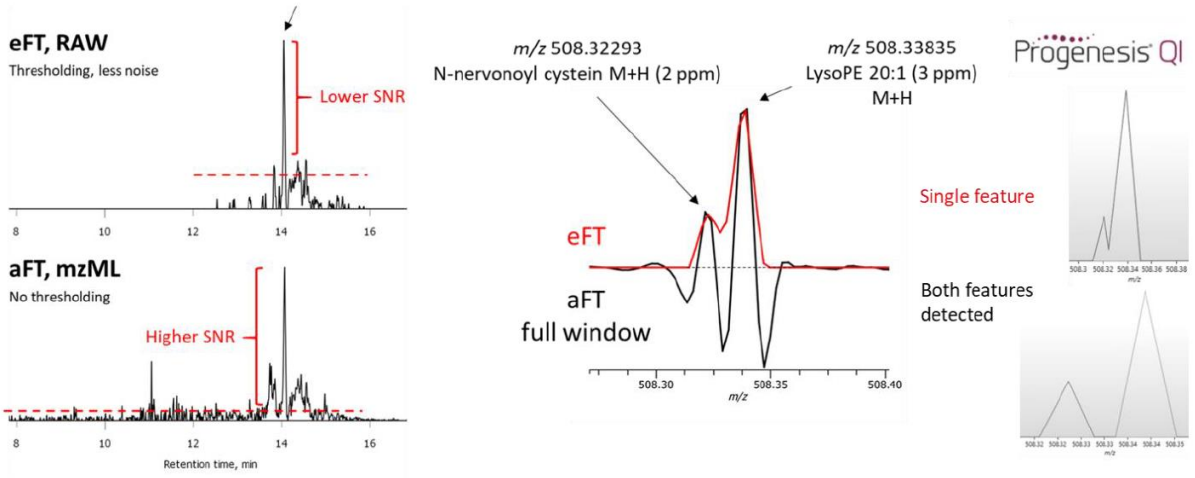
The majority of metabolomics workflows require compatibility with UHPLC time scales (e.g. 2-4 Hz scanning). Orbitraps provide these speeds at resolution (R) settings of 70-120k. Higher R requires slower scanning and potentially leads to a loss of quantitative information. On the other side, R>100k resolves almost all isobaric overlaps hence reducing the number of ambiguous features annotations and/or identifications. Generally, a half-window apodization function is applied to the recorded time-domain transients prior to Fourier transformation to obtain Orbitrap mass spectra. A full window apodization is typically applied to represent mass spectra in the magnitude mode FT or to increase effective R and signal-to-noise ratios in the ICR FTMS for absorption mode (aFT) mass spectra [1]. This work represents an attempt to use aFT with a full window apodization to increase effective resolution without affecting acquisition speed on a Q Exactive Focus Orbitrap (maximum R = 70k) for metabolomics. Mass spectra generated from the externally acquired transients were comparable to the original reduced profile Orbitrap data in terms of mass accuracy and ion abundances. Effective resolution was increased 1.2-1.8-fold depending on peak intensity, see Figure. Improvements in the dynamic range were observed due to the absence of noise thresholding. The eFT and aFT data were subjected to comparative analysis in multiplatform software package *ProgenesisQI* as vendor's Compound Discoverer software accepted only RAW format. Processing of a reference metabolomics eFT and aFT datasets required no modifications to standard workflows and revealed more confident annotations and roughly 1.5x more spectral features in the aFT data.



The aFT mode increases dynamic range (left) and effective R for feature detection (right) in Orbitrap data

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