39th INFORMAL MEETING ON MASS SPECTROMETRY

Credit P. Kitmacher @Sorbonne Université

Campus des Cordeliers

Sorbonne Université, Paris, France
15th – 17th May 2023

IMMS 23 PARIS

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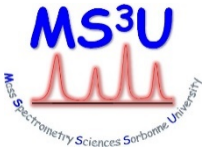
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Quantification

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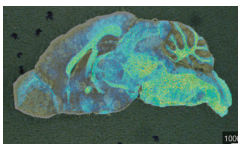
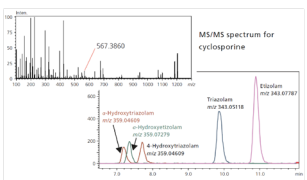


Superposition d'images au microscope optique avec des images MS

Les utilisateurs peuvent facilement basculer entre l'analyse d'imagerie et l'analyse LC-MS

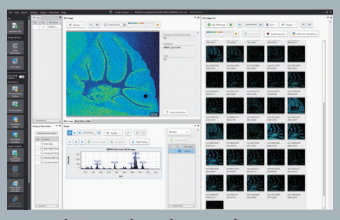
Le spectromètre de masse est équipé d'un microcope optique donc l'analyse de données peut faire correspondre les images du miscroscope optique aux images MS

LCMS Q-TOF LCMS-9030



Chromatogramme de masse de l'étizolam, du triazolam et de leurs métabolites, ajouté au sang total à une concentration de 10 ng/ml

Analyses quantitatives



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O01
Top-down and bottom-up two-dimensional mass spectrometry for protein structural characterisation

M. Polák[1),2)], M. Palasser[3)], A. Kádek[1),2)], D. Kavan[1),2)], M.-A. Delsuc[4),5)], K. Breuker[3)], P. Novák[1),2)], M. A. van Agthoven[1)]

1) Institute of Microbiology of the Czech Academy of Sciences, Průmyslová 595, 252 50 Vestec, Czech Republic
2) Faculty of Science, Charles University, Albertov 2038, 128 00 Nové Město, Prague, 12843, Czech Republic
3) Center for Chemistry and Biomedicine, University of Innsbruck, Innrain 80/82, 6020 Innsbruck, Austria
4) Institut de Génétique et de Biologie Moléculaire et Cellulaire, INSERM, U596, CNRS, UMR7104, Université de Strasbourg, 1 rue Laurent Fries, 67404, Illkirch-Graffenstaden, France
5) CASC4DE, Le Lodge 20 av. du Neuhof, 67100, Strasbourg, France

Two-dimensional mass spectrometry (2DMS) is a method for tandem mass spectrometry that relies on ion radius modulation instead of ion isolation to correlate between precursor and fragment ion peaks. 2D mass spectra show all the fragmentation patterns of the analytes in a sample. Signal multiplexing yields high signal-to-noise ratios and therefore complete sequence coverage (e.g., for biomolecules) [1]. Modifications can easily be assigned and located visually with precursor ion scans and dissociation lines. Previous studies have established the potential of 2D MS with electron capture dissociation (ECD) for bottom-up proteomics and for the label-free relative quantification for the top-down analysis of biomolecules [2,3]. Covalent labelling methods such as acetylation and oxidative foot-printing can be used to probe the three-dimensional structures of biomolecules, e.g., to study protein-ligand interactions [4,5]. In this study, we combine 2D MS and covalent labelling of proteins for both bottom-up and top-down analysis. We compare the performance of 2D MS vs. LC-MS/MS for the identification, location, and quantification for protein structural characterisation.

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O02
Elastomer identification by Pyrolysis GC-MS in museum collections

C. Bouvier[1)], E. Pellizzi[1)], F. Bauchau[2)], N. Bouillon[2)], L. Antonelli[2)], L. Royan[3)], N. Balcar[4)]

1) Laboratoire scientifique de la Bibliothèque nationale de France (BnF), 77600 Bussy-Saint-Georges, France;
2) Centre Interdisciplinaire de Conservation et Restauration du Patrimoine (CICRP), 13003, Marseille, France;
3) Musée National d'Art Moderne - Centre Georges Pompidou (MNAM-CGP), 75004, Paris, France;
4) Centre de Recherche et de Restauration des Musées de France (C2RMF), 75001, Paris, France

Elastomers cover a variety of formulations. Commonly referred to as *rubbers*, recognizing them still can be challenging, and their names in heritage collections can be imprecisely inventoried. Also, data about their behavior over time is limited, even if some pieces already require conservation-restoration treatments.

This requires both precise identification and knowledge of the chemical structure about rubber in collections. To address this while meeting the need to develop relevant approaches for their conservation, a joint project [1] involves the scientific laboratories of the Bibliothèque nationale de France (BnF), Centre de Recherche et de Restauration des Musées de France (C2RMF), Centre Interdisciplinaire de Conservation et Restauration du Patrimoine (CICRP), and conservators from the Musée National d'Art Moderne - Centre Georges Pompidou (MNAM-CGP). By correlating analytical characterization results with observations obtained from collections surveys and the expertise of each partner, a recognition protocol for elastomers was developed.

Using pyrolysis - gas chromatography - mass spectrometry (Py-GC/MS) on elastomers provides their molecular identification. A database was created by identifying and compiling marker molecules of 80 elastomers of known composition. A tool relying on this database was developed to process raw Py-GC/MS data automatically and to yield a direct identification of the elastomer. The tool will be openly shared to anyone interested in identifying elastomers.

The tool has been so far successfully used on 148 samples from the MNAM-CGP collections, and the identifications are being compared with the collection survey data, generating useful conclusions on artworks difficult to preserve.

References

1. ElaStomer identification by Pyrolysis GC-MS in museum cOllectioNs (ESPyON) a research project funded by the Fondation des Sciences du Patrimoine (FSP)

O03
Mass spectrometric analysis of intact proteins: the dark side of deconvolution

Á. Gömöry, K. Vékey, L. Drahos

Research Centre for Natural Sciences, Magyar Tudósok krt 2, H-1117 Budapest, Hungary

One of the most commonly used techniques for studying intact proteins, including monoclonal antibodies (MABs), is HPLC-MS. While studying pure, large amounts of protein is relatively easy, analyzing mixtures can be more complicated. The real challenge arises when small amounts of protein mixtures with large molecular masses are studied. In such cases, automatic evaluations (e.g. deconvolution of mass spectra) can yield incorrect results, making HPLC separation of the mixture increasingly important.

In this presentation, I will illustrate the process, problems, and difficulties of evaluating the mass spectra of proteins from small to complex antibody-drug conjugates. In the latter case, when the mixture is too complex, a reliable result can only be obtained using a detailed manual evaluation of the ion chromatograms and mass spectra.

O04
Mass Spectrometry Study of Host-Guest Complexes between Angle-Strained Alkyne-Containing Cycloparaphenylenes and Fullerenes

L. Ye[1)], M. Freiberger[1)], T. A. Schaub[2)], R. Jasti[2)], T. Drewello[1)]

1) Department of Chemistry and Pharmacy, Friedrich-Alexander Universität Erlangen-Nürnberg, 91058 Erlangen, Germany
2) Department of Chemistry & Biochemistry and Material Science Institute, University of Oregon, Eugene, OR, 97403, USA

Cycloparaphenylenes (CPPs) are strained ring molecules, comprising only sp2-hybridized carbon atoms. As a result of their both concave and convex extended π arrays, CPPs have been widely employed as ideal supramolecular hosts for fullerenes [1] and CPPs [2].

In this study, a variety of functionalized triazole-containing CPPs with an elliptic lasso-like shape are exploited as hosts for C60 and C70. Host-guest complexes of these Lasso-CPPs and closely related [12]CPP with C60/C70 are investigated by electrospray ionization mass spectrometry. The mass spectra show that [1:1] complexes of Lasso-CPPs with C60/C70 are formed as radical cations and protonated species, while [2:1] complexes mainly exist as protonated molecules. Energy-resolved collision (MS2) experiments reveal that Lasso-CPP⊃fullerene [1:1] complexes are more stable as radical cations than as protonated species. This is due to the fact that in the radical cation, the positive charge on Lasso-CPPs can be delocalized, thus enhancing the complex stability. Changes in the electron donating/-accepting nature of peripheral substituents on Lasso-CPPs, on the other hand, have little influence on the complex stability. Additionally, MS2 experiments indicate that [2:1] and [1:1] complexes of Lasso-CPPs with C70 are more stable than the corresponding C60 analogue, as reported for CPP-based complexes [2]. However, complexes of Lasso-CPPs with C60/C70 are found to be more stable than [12]CPP complexes.

Our results suggest that strain-promoted Lasso-CPPs with a series of unique properties are desirable host molecules for fullerenes. Mass spectrometry is a powerful tool for the study of these non-covalent host-guest complexes.

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2. M. B. Minameyer, Y. Xu, S. Frühwald, A. Görling, M. von Delius, T. Drewello, Chem. Eur. J., **26**, 8729 (2020)

O05
Monitoring of conformational changes in transmembrane proteins using HDX and FFAP radical labeling

L. Fojtík[1),2], J. Portašiková[1),2], Z. Kukačka[2)], P. Novák[1)], P. Man[1),2)]

1) Faculty of Science, Charles University, Prague, Czech Republic
2) Institute of Microbiology of the CAS, Prague, Czech Republic

The chloride channel family is divided into two groups: channels and antiporters. Channels are known for their large conformational changes of subunits during ion transport. In contrast, antiporters have only tiny movements near the transport pathway. Transporters are involved in many cellular processes, and their mutation can cause serious diseases [1]. The CLC-ec1 is a Cl-/H+ antiporter of a single proton for two chloride ions from Escherichia coli. Ion transport is supposed to be accompanied by a structural change between inward and outward-facing conformation, but this transition has not been captured so far. During the transport cycle, the protonation of key Glu residues should induce an outward-facing state. This was mimicked through a mutation of three Glu residues for Gln[3]. We used this mutation to reveal the role of protonation in the ion transport mechanism of CLC-ec1. CLC-ec1 and CLC-QQQ mutant were expressed in the E. coli system and purified by IMAC chromatography in η-decyl maltoside as a solubilization reagent[1]. Solubilized protein was transferred to the saposin nanodisc by size exclusion chromatography. We then used hydrogen/deuterium exchange and radical labeling with Togni reagent (FFAP)[4] coupled to high-resolution mass spectrometry to capture the structural changes alongside the backbone as well as on the side chains of aromatic residues The optimization of the digest workflow for both methods provided full sequence coverage with reasonable lengths of the peptides. As the best conditions for the HDX we determined online digest on the column of co-immobilized pepsin-nepentesin 2 whereas for FFAP we got better sequence coverage by offline digest via cyanogen bromide and trypsin. HDX and FFAP were subsequently used for the study of the structural differences between CLC-ec1 and the CLC-QQQ mutant at pH 7.4. To prove the role of protonation, a comparison experiment at four different pH levels, from 4.4 to pH 7.4, was performed for both proteins. Results showed a critical connection between protonation and structural changes in the ion transport path and conformational change in the transmembrane helixes.

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O06
Structural characterization of phospholipids by radical-driven fragmentations

S. Poyer[1)], J.-Y. Salpin[2)], D.Touboul[1),3)]

1) Université Paris-Saclay, CNRS, ICSN, UPR 2301, 91198, Gif-sur-Yvette, France;
2) Université Paris-Saclay, Univ Evry, CNRS, LAMBE, UMR 8587, 91025 Evry, France;
3) LCM, CNRS, Ecole polytechnique, Institut Polytechnique de Paris, Route de Saclay, 91120 Palaiseau, France

Structural characterization of glycerophospholipids (GPLs) at the fatty acid level presents an opportunity to improve the understanding of the complex relationship between lipid metabolism and diseases. The fine structural characterization of GPL ions generated by atmospheric pressure ionization sources is still not accessible without instrumental modification or derivatization [1]. Indeed, the fine description of structures remains challenging since the most popular tandem mass spectrometry (MS/MS) method is built on collision-induced dissociation (CID) of the gas-phase ion. With CID conditions, the weakest chemical bonds will be cleaved, leading to incomplete fragmentation and lack of structural information, especially in locating double bonds (DBs) on the aliphatic chains.

Since radical release can be obtained from copper reduction by CID in the gas phase and yield diagnostic fragment ions to locate DBs on fatty acids, we hypothesize that this alternative could be of interest for a more complete structural characterization of GPLs [2]. Unfortunately, the fragmentation of copper-adducted GPLs produces only even-electron fragments. As a result, no structural information for the DB has been obtained. To allow copper to chelate with DBs and thus generate a DB-reactive radical, we investigated the gas-phase formation of ternary complexes, which would attenuate the attraction of copper to the polar head of these lipids. The ternary complexes formed with an atomic or a molecular anion provided information on the location of the DB but were weakly observed due to their low stability. Adding a stabilizer ligand such as bipyridine to this complex significantly improved the formation of a reactive copper adduct. CID dissociation of these complexes for different classes of GPLs yields diagnostic fragment ions on both sides of the DB, allowing unambiguous location even for highly unsaturated analogs. This methodology was applied to complex GPL samples using LC-MS/MS analysis with a post-column introduction of CuCl2 and bipyridine.

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O07

An orphan cytochrome P450 from *M. tuberculosis* as a potential drug target for the metabolization of the antituberculous drug SQ109: identification and characterization of the resulting metabolite

E. Sadowski[1,2], N. Pietrancosta[1,3], J.-L. Boucher[4], A. Aubry[2,5], E. Sachon[1,6]

1) Sorbonne Université, École normale supérieure, PSL Université, CNRS, Laboratoire des Biomolécules, LBM, 4 place Jussieu, 75252 Cedex 05 Paris, France.
2) Sorbonne Université, INSERM U1135, Centre d'Immunologie et des Maladies Infectieuses, Cimi-Paris, 91 boulevard de l'hôpital, 75013 Paris, France.
3) Sorbonne Université, INSERM, CNRS, Neurosciences Paris Seine - Institut de Biologie Paris Seine (NPS - IBPS), 75005 Paris, France.
4) Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques, CNRS UMR 8601, Université Paris Cité, 45 rue des Saints-Pères, 75006 Paris, France
5) AP-HP, Centre National de Référence des Mycobactéries et de la Résistance des Mycobactéries aux Antituberculeux, Laboratoire de Bactériologie- Hygiène, Groupe Hospitalier Pitié-Salpêtrière, 47-83 Boulevard de l'Hôpital, 75013 Paris, France.
6) Sorbonne Université, MS3U Platform, Mass Spectrometry Sciences Sorbonne Université, 4 place Jussieu, 75252 Cedex 05 Paris, France.

The emergence of the multidrug-resistant tuberculosis (MDR-TB) strains generates the need for efficient drugs. Among the new potential anti-TB, SQ109 showed activity against resistant *Mycobacterium tuberculosis* (*Mtb*) and already advanced to Phase II clinical trials. SQ109 is an N-geranyl-N'-(2-adamantyl)ethane-1,2-diamine, degraded by human liver cytochrome P450 (P450) and by recombinant *Mtb* CYP124A1. In our aim to identify potential substrates to the orphan *Mtb* P450, CYPX, we found that it could bind and metabolize SQ109.

The haemoprotein CYPX displays typical UV-visible spectroscopic features which allow measuring binding constants for potential substrates. SQ109 strongly binds (Kd = 2.08 +/- 0.4 µM) to CYPX. *In vitro* enzymatic assays were carried out by reconstitution of the catalytic cycle of CYPX to test if SQ109 was a substrate. The SQ109 unique produced metabolite was analysed by ESI-FT-ICR-MS. The ion signal obtained at m/z 347.30586 ($C_{22}H_{39}N_2O^+$) allowed us to identify an additional oxygen atom in the SQ109 structure. To characterize the oxidation site and its nature, collision-induced dissociation (CID) experiments were performed on non-metabolised and oxidised SQ109 precursor ions. Based on the observed fragment ions, including neutral losses of water molecules, structures were proposed with a hydroxyl group on carbon 4 or 5 on the geranyl moiety. The localization of the hydroxyl is in good agreement with the position of SQ109 relative to the catalytic haeme group, that was obtained by docking of the SQ109 in a 3D homology model of CYPX. Overall, our findings identified *Mtb* CYPX as a new potential drug target which could contribute to the metabolization of the highly potent anti-TB SQ109.

O08
Structural Elucidation of Archeal Diether Phospholipids by High-Energy CID/tandem TOF-MS

E. Pittenauer and M. Marchetti-Deschmann

Institute of Chemical Technologies and Analytics, Vienna University of Technology, Vienna, Austria

In contrast to most other living organisms (animals, plants, bacteria etc.) archeae are representing nowadays an own kingdom. Even their cell wall lipids differ markedly as they possess typically ether bonds with only minor structural variations in their alkyl chains constituting regular methyl-branched C20-building blocks called phytanol instead of ester bonds. The identity of the polar head group ranges from PI, PC and PE to saccharide-moieties. Additionally, also C-C-linked tetraether lipids are formed representing very big carbocyclic compounds. Structural elucidation of such unusual phospholipids was previously typically performed with ESI low-energy CID MS/MS or MALDI-TOFMS with post source decay (PSD) yielding spectra of poor quality. Here, we present for the first time MALDI high-energy CID-data on selected diether phospholipids including two synthetic ether derivatives selecting all types of precursor ions formed ($[M-H]^-$, $[M+H]^+$, $[M+Na]^+$ and $[M+2Na-H]^+$). All diether derivatives forming $[M-H]^-$-precursor ions (PI, PE) show abundant charge-remote fragmentation of the alkyl chains with 14 Da-spacing except for the methyl-branchings spaced by 28 Da. Low mass ions typically identify the polar head group of the lipid species. Protonated precursor ions (PC, PE) yield strongly differing spectra as the PE derivative shows an unexpected loss of H3PO4 from the precursor ion and a McLafferty rearrangement. No diagnostic low-mass head group product ions could be detected. Among PC-derivatives, an unexpected rearrangement involving the transfer of one alkoxy group to the phosphate-moiety was detected - besides high-mass charge remote fragmentation - yielding most likely an O-alkyl-phosphocholine product ion. For sodiated and disodiated species only the latter ones seem to be of interest for high-energy CID-experiments as only these precursor ions yield abundant charge-remote fragmentation – comparable to high-energy CID-spectra of [M-H]--precursor ions. The low-mass region identifies the type of polar head group. In conclusion, several unique and new, previously undescribed fragmentations/ rearrangements are shown and discussed in detail.

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O09
Development of automated MS/MS methods on an Orbitrap Fusion™ and a spectral database for in-depth lipidomic analysis of human plasma

V. Marie, B. Colsch, F. Fenaille

Université Paris-Saclay, CEA, INRAE, Département Médicaments et Technologies pour la Santé (DMTS), MetaboHUB, F-91191 Gif sur Yvette, France

Liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) is the most widely used analytical method for untargeted lipidomic analysis of human biofluids. However, several issues still remain for the comprehensive structural characterization of lipids due to their naturally occurring huge structural diversity with difficult to resolve co-eluting isobaric and isomeric species. In this study, we have evaluated and optimized different new acquisition methods from targeted LC-HRMS/MS (tMS2) with inclusion lists to optimized data dependent (DDA) Top-N and data independent acquisition (DIA) SWATH (Sequential Window Acquisition of all THeoretical fragment ion spectra) workflows using an Orbitrap Fusion™ mass spectrometer (ThermoFisher Scientific) for cataloguing the human lipidome at high confidence.

Several parameters were optimized such as the Orbitrap mass resolution for the collection of MS and MS/MS spectra, the collision energy applied, and the number of windows for the SWATH method. The quality of the resulting spectra were first evaluated by matching to the *in silico* database LipidBlast using the software MS DIAL.

The tMS2 method uses inclusion lists to fragment the most relevant lipid precursor ions and allows the manual annotation of up to 406 unique lipid species in human plasma. The optimized DDA Top 10 method used a resolution of 120k for the MS and 30k for the MS^2 scans, using stepped collision energy conditions to generate meaningful MS/MS spectra for the different lipid classes. Under these conditions up to 355 unique lipid species were confidently identified after matching with the LipidBlast database and manual verification.

We also devised a DIA SWATH method with variable overlapping 15 windows to partition the whole set of precursor ions almost equally across the ~350-1000 m/z range, thus yielding 302 unique annotated lipid species following MS-DIAL deconvolution and LipidBlast search.

All the high-quality and manually curated MS/MS spectra of endogenous lipids were then compiled within a single database file for ~450 distinct lipid species. The efficiency of the MS DIAL software was also evaluated for the automatic identification of lipids by matching to both the LipidBlast database and the manually curated database. An in-depth study of the scoring system allowed us to define threshold values for dot and reverse dot product scores, above which MS/MS spectra can be considered as confidently matched. Overall, the generated data and knowledge can be highly useful for the lipidomics community.

O10
Mass spectrometry-based characterization of proteins exposed to inflammatory oxidants

P. Hägglund

Department of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark

Proteins are major targets of oxidants produced by activated immune cells, such as peroxynitrous acid (ONOOH) and hypochlorous acid (HOCl). These oxidants kill bacteria and other invading pathogens, but can also cause collateral damage to host cells and tissue, and contribute to the development of numerous inflammatory diseases. In this talk, I will outline the mass spectrometry-based workflows we are applying in our lab to study how oxidants influence protein structures and present some recent data where we have mapped modifications in oxidized proteins derived from extracellular traps.

O11
From grapevine to the glass: a metabolomics tale

M. Sousa Silva, M. Soeiro, M. Louro, J. Luz, F. Traquete, A.P. Marques, A. Ferreira, C. Cordeiro

Laboratório de FT-ICR e Espectrometria de Massa Estrutural, Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal

"*The whole universe is in a glass of wine*" [1]. This universe is the result of different biochemical processes that involve several players: the carefully selected grape varieties, the bacteria and yeasts present during fermentation, and the effects of terroir and geographical origin. The characterization of the chemical fingerprint of wine remains one the greatest challenges of analytical chemistry, defying all conventional approaches. Offering the highest resolution and mass accuracy possible and allowing the detection and identification of thousands of compounds in a single analysis, Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) is ideal for metabolomics studies of complex samples such as wine.

We have been using FT-ICR-MS to unfold the metabolomics wine tale, from the metabolic composition of grapevines [2,3], to the metabolic contribution from yeasts (both conventional and non-conventional), that goes beyond the fermentation process itself, and finally to the unique chemical signature of wine. This whole set of small molecules that contribute to the wine's physical, aromatic and organoleptic properties, as a signature of its origin and authenticity, can be unraveled by FT-ICR-MS, thus telling the wine tale, from the grapevine to the glass.

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O12
Measurement of the binding energy of the heme ligand in a cooled ion trap.

M. Aarabi[1)], S. Soorkia[2)], G.Grégoire[2)], M. Broquie[2)], A. de la Lande[3)], R. Omidyan[1)], B. Soep[2)], N. Shafizadeh[2)]

1) Department of Chemistry, University of Isfahan, 81746-73441 Isfahan, Iran
2) Institut des Sciences Moléculaires, CNRS UMR 8214, bat 520, Université Paris Saclay 91405, Orsay, France
3) Institut de Chimie-Physique, Université Paris Saclay CNRS, UMR 8000. 15, rue Jean Perrin, 91405 Orsay, France.

We have used a home-made experimental setup which couples an electrospray, with a cooled Paul ion trap and a time-of-flight detection. The temperature variation allows to access the thermodynamic properties of the complex and in particular the binding energy between a ligand and an organometallic compound. In my presentation I will show our results on the binding energy of iron protoporphyrin with different ligands such as O_2 , CO, H_2O,…. Indeed this system is a model for the reaction of oxygen fixation or release on a hemoprotein. The mechanism of this process has not been yet elucidated precisely. The active site of hemoglobin Heme is an Iron atom in the oxidation degree II ligated to Protoporphyrin. The nature of the iron-oxygen bond in the heme is a subject of debate since Pauling (1936) and only recently, quantum chemistry was been able to describe it. Thus, the Fe II - O2 binding has its origin in a transfer of charge between the iron atom and oxygen. The determination of Heme-O_2 binding energies in absence of other interactions is a way to validate this hypothesis by comparison with calculations. This energy can be measured simply in the gas phase. In this presentation, I will focus on the methods developed to measure the binding energy of metal-ligand on different model system in the gas phase.The bond formation enthalpies with [O_2, CO, H_2O,…] have been derived from Van't Hoff plots of experimentally determined equilibrium constants for ligand binding reactions in the gas phase. It is the first direct determination of the binding energy iron-ligand. I will discuss the different factors and present an overview of gas phase investigation of heme-ligand binding via the Vant'Hoff equation and high level calculations. This confrontation of the experimental and theoretical results on the binding energies allows a particular insight on the Heme-Ligand properties.

As in nature, the degree of oxidation of iron plays an important role in the binding of small molecules to hemoproteins; we measured the binding energies of ferrous and feric heme with different ligand. We have also characterized the critical influence of the water molecule on the reaction of ligadation of heme.

O13

Multiplex analysis by mass spectrometry: a strong and alternative tool for the detection of staphylococcal enterotoxins involved in food poisoning outbreaks

A. Hueber[1)]; J-A. Hennekine[1)]; Y. Nia[1)]

Laboratory for Food Safety, ANSES, Maisons-Alfort, France

Staphylococcal food poisoning outbreaks (SFPO) are caused by the ingestion of food contaminated with staphylococcal enterotoxins (SEs) produced by strains of coagulase positive Staphylococci, and their notification is mandatory. The suspected food is sampled and submitted to several type of analysis allowing the enumeration of the bacteria, detection of their enterotoxins genes and the detection of the enterotoxin produced in the food as the ingestion of is last one is responsible of the disease.

To date, 33 SEs are described in the literature but only 5 SEs (SEA to SEE) can be routinely detectable *via* commercially available immunoassays (e. g. EN ISO 19020). Even ELISA methods are the most commonly used and considered as the most sensitive, false positive results can be obtained in complex matrices as reported in the EN ISO 19020 method. Thus, the competent authority recommends the use of a confirmatory analysis based on a different principle.

In this work, we present an LC-MS method developed under our European Union Reference Laboratory mandate for detection of 8 SEs in food. SEs are extracted from food matrices by immuno-capture (IC), and then submitted to trypsin digestion before analysis by LC-HRMS/MS. For each type of SEs, between 3 and 8 proteotypic peptides were optimized using sequences (including sequence variants) obtained from more than 500 strains from different SFPO occurred in Europe. [1]

This LC-HRMS/MS method was implemented during the investigation of several SFPO occurred in France and Europe (Italy) between 2016 and 2022. The data obtained by mass spectrometry were compared to those from EILISA, PCR and genomic analyses. A correlation was observed between LC-MS, ELISA and molecular typing methods. The high specificity and multiplex analysis of mass spectrometry are the main advantage over commonly used ELISA methods.

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O14
Impact of ion mobility separation on optimal collision energies from a bottom-up proteomics point of view

K. Nagy[1,2]; G. Gellén[3]; D. Papp[2,3]; G. Schlosser[3], Á. Révész[1]

1) MS Proteomics Research Group, Research Centre for Natural Sciences, Magyar Tudósok körútja 2., H-1117, Budapest, Hungary
2) Hevesy György PhD School of Chemistry, ELTE Eötvös Loránd University, Faculty of Science, Institute of Chemistry, Pázmány Péter sétány 1/A, H-1117, Budapest, Hungary
3) MTA-ELTE Lendület Ion Mobility Mass Spectrometry Research Group, Eötvös Loránd University, Faculty of Science, Institute of Chemistry, Pázmány Péter sétány 1/A, H-1117, Budapest, Hungary

Ion mobility spectrometry (IMS) is a widely used separation technique that can enhance the performance of liquid chromatography – mass spectrometry (LC-MS/MS) methods by providing an additional dimension of separation.

In bottom-up proteomics, the collision energy (CE) applied greatly influences the information content of the obtained MS/MS spectra from peptides. The highest identification scores and the greatest number of successful identifications can be achieved with an instrument-specific optimal setting. When IMS is applied, significant ion heating can occur in the IMS cell as a result of collisions with the buffer gas, which can affect the optimal CE for MS/MS experiments.

The objective of this study was to examine the impact of ion mobility separation on the energetics of peptide ions through the investigation of the optimal CE choice for MS/MS experiments. LC-MS/MS measurements were performed using varied CE settings both with and without IMS on a Waters Select Series Cyclic IMS mass spectrometer, which has two consecutive collision induced dissociation (CID) cells; one before (trap) and one after (transfer) the ion mobility cell. Over a thousand tryptic peptides from HeLa tryptic digest standard were analyzed in terms of CE dependence of identification score using Byonic search engine. The trap cell and transfer cell were also compared as pre and post ion mobility fragmentation modes.

Results indicate that IMS significantly energizes peptides, and the use of lower CE is recommended when IMS is applied. On average, the optimal CE is 6.3 V lower when IMS is applied. This difference also manifests in the optimum CE value versus m/z trends. It was also determined by comparing the pre and post ion mobility fragmentation modes, that it is necessary to adjust the CE when the trap cell is used for activation instead of the transfer cell.

O15
Very low pressure CID experiments

K. Vékey, Á. Gömöry, L. Drahos

Research Centre for Natural Sciences, Magyar Tudósok krt 2, H-1117 Budapest, Hungary

CID is a very common technique, typically used under 'standard' conditions. Sometimes the collision energy is varied or optimized, but other parameters are rarely modified. We have decreased the collision pressure in a triple quadrupole type instrument, resulting in less collisions, which should have a similar effect than decreasing the collision energy. However, low pressure spectra were dissimilar to low energy spectra, and we have studied this effect in detail.

We have found, that decreasing the collision gas pressure to a ca. 50 times lower value than commonly used, we got into a range, where predominantly single collisions occurred. This allowed us to study ion fragmentation and energetics in detail. We have also got information on the efficiency of kinetic to internal energy conversion. Both simple calculations and detailed modeling indicates, that close to 100% of the center of mass collision energy can be converted into internal energy in a single collision.

O16
COMPetitive PAiring StatisticS (COMPASS) for Protein Structural Analysis: Insights Into α-Synuclein Conformational Changes Upon Liquid-Liquid Phase Separation

C. Iacobucci[1),2),3)], *D. Ubbiali*[2),3)], *A. Sinz*[2),3)]

1) Department of Physical and Chemical Sciences, University of L'Aquila, Via Vetoio, Coppito, 67100 L'Aquila (Italy)
2) Department of Pharmaceutical Chemistry and Bioanalytics, Institute of Pharmacy, Martin Luther University Halle-Wittenberg Kurt-Mothes-Str. 3, 06120 Halle/Saale (Germany)
3) Center for Structural Mass Spectrometry, Martin Luther University Halle-Wittenberg, Kurt-Mothes-Str. 3, 06120 Halle/Saale (Germany)

α-Synuclein (α-syn) is an intrinsically disordered protein (IDP) that undergoes liquid-liquid phase separation (LLPS), fibrillation, and forms insoluble intracellular Lewy bodies in neurons, which are the hallmark of Parkinson's Disease (PD). Neurotoxicity precedes the formation of aggregates and might be related to α-syn LLPS. The molecular mechanisms underlying the early stages of LLPS are still elusive.

To obtain structural insights into α-syn upon LLPS, we take advantage of cross-linking/mass spectrometry (XL–MS) and introduce an innovative approach, termed COMPASS (COMPetitive PAiring StatisticS). In this presentation, COMPASS method will be introduced and its application on α-syn described. In fact, COMPASS revealed that the conformational ensemble of α-syn shifts from a "hairpin-like" structure towards more "elongated" conformational states upon LLPS.[1]

We obtain insights into the critical initial stages of LLPS and establish a novel mass spectrometry-based approach that will aid to solve open questions in LLPS structural biology.

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O17
Estimation of the thermodynamic and physicochemical properties of the alkali astatides: how weak are the bonds in molecular astatine (At2) and tennessine (Ts2)?

P. C. Burgers[1)], *J. L. Holmes*[2)]

1) Department of Neurology, Laboratory of Neuro-Oncology, Erasmus Medical Center, Rotterdam, 3015 CN, The Netherlands
2) Department of Chemistry and Biological Sciences, University of Ottawa, 10 Marie Curie, Ottawa, Ontario, K1N 6N5, Canada

Astatine, element 85, occurs on earth with an estimated total abundance of less than 1 g [1]. One of its longest-lived isotopes, 211At, with a half-life of 7.2 h, is of considerable interest as an α particle emitting radionuclide for targeted alpha therapy (TAT), but only nanogram quantities are available through synthetic methods. Recently, sodium astatide, NaAt, was produced and successfully used as a therapeutic drug, leading to marked tumor regression effects in mice that had received grafts of thyroid cancer cells [2]. Thus, there is renewed interest in the properties of astatine and astatine containing compounds.The recent [3] accurate and precise determination of the electron affinity (*EA*) of the astatine atom At0 warrants a re-investigation of the estimated thermodynamic properties of At0 and astatine containing molecules as this *EA* was found to be much lower (by 0.4 eV) than previous estimated values. In this contribution we estimate, from available data sources, the following thermodynamic and physicochemical properties of the alkali astatides (MAt, M = Li, Na, K, Rb, Cs): their solid and gaseous heats of formation, lattice and gas-phase binding enthalpies, sublimation energies and melting temperatures. Use of Born-Haber cycles together with the new *EA*(At0) value allows the evaluation of ΔHf(At0)g from which it is concluded that At_2 (and Ts_2) are weakly bonded species (De < 50 kJ/mol), in agreement with the finding from theory that spin-orbit coupling considerably reduces these bond strengths [4]. Bond order assessments of the dihalogens X_2 (X = F, Cl, Br, I, At, Ts) with and without spin-orbit coupling confirm these findings. Like F2, At2 belongs to the charge-shift bonding systems [5], but for a different reason. A comparison of the potential energy curves for the X_2 diatomics will be presented.

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O18
Analysis of cholesterol in the tissue sections by MALDI MSI approach

A. Bodzon-Kulakowska, P. Mielczarek, M. Harężlak, A. Samek, P. Suder

Department of Analytical Chemistry and Biochemistry, Faculty of Materials Science and Ceramics, AGH University of Science and Technology, A. Mickiewicza 30 Ave., 30-059 Krakow, Poland

Cholesterol is a very crucial molecule from a biochemical point of view. For example, about 25% of the whole-body cholesterol is present in our brain, where it is synthesized *de novo.* Therefore, it means that the changes in the level of this molecule may be responsible for different pathological conditions such as Alzheimer's disease, Niemann-Pick disease, or Rett syndrome [1].

The brain is a very complex organ with a lot of different structures. It means that the possibility of retaining spatial information during the analysis is exceptionally important in the case of this organ. MALDI mass spectrometry imaging offers such an opportunity. Still, in the case of cholesterol, there could be a problem with such analysis, mainly because cholesterol is characterized by low proton affinity and low acidity.

Cholesterol may be derivatized with the aid of betaine aldehyde [2]. This compound reacts selectively with the hydroxyl group of alcohols. In this reaction, hemiacetal salt is formed, and the obtained product is labeled with charge. In our work, we have optimized the way of cholesterol derivatization by betaine aldehyde with the use of the SunCollect® system, which is recognized as the wet-interface technique. Two parameters that are crucial for this system – the sputtering nozzle position over the sample and the number of betaine aldehyde solution layers were optimized. Moreover, in our study, we were able to perform quantitative cholesterol analysis in the rat brain cerebellum based on the calibration curve obtained with the aid of rat brain homogenate. We hope that our study will be helpful for those interested in cholesterol analysis.

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O19
Application of mass spectrometry to study electronic ligand effects on gold organometallic complexes

L. Bourehil[1),2)], C. Soep[1)], S. Dutrannoy[1)], S. Seng[1)], D. Lesage[1)], B. Bertrand[1)], H. Dossmann[1)]

1) Sorbonne Université, CNRS, Institut Parisien de Chimie Moléculaire, IPCM, F-75005 Paris, France
2) Synchrotron SOLEIL, L'Orme des Merisiers, St Aubin, BP 48, F-91192 Gif-sur-Yvette, France

Ligand dissociation is a key process in homogenous catalysis and gives access to a wide range of products and reactions. Through their steric and electronic effects, ligands are able to orientate the reactivity of the complexes and can thus influence the selectivity in the chemical transformations. Usually, the metal-ligand bond is depicted by the Dewar-Chatt-Duncanson model [1,2] involving two opposite interactions, a σ-donor and a π-acceptor effect. Over the years, many experimental methods have been developed to measure these electronic effects, the most common being that of Tolman by Tolman electronic parameter (TEP), which is limited to organometallic complexes possessing probe ligands such as CO and based on the A1-symmetrical CO-stretching frequency shift [3]. We have recently developed the use of new experimental gas-phase approaches to measure these effects, one based on mass spectrometry using activation by higher energy collision dissociation (HCD). Under this method, the satisfying results obtained on model systems [4] have encouraged us to apply this later on gold (III) complexes of the type $[(C\hat{}C)Au(NHC/PPh_3)L]^+$ and $[(C\hat{}N\hat{}C)AuL]^+$ with L a differently substituted pyridine ligand and where a direct measurement of the ligand effect is possible. The effect of pyridine substitution on the enrichment of the metal center was evaluated by determining the dissociation energy (BDE), M-L+ -> M+ + L. Rationalization of the experimental results is made using kinetic modelling, density functional theory and bond description methods to get information on the electronic structure of the complexes and thus on the metal-ligand interaction.

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O20
Amyloidosis: how proteomics can make a difference in clinical practice

J. Courraud[1,2], P.E. Nikolaou[3], D. Canetti[4], G. Médard[2], J. Gilbertson[4], A. Wechalekar[4], I. Andreadou[3], N. Thomaidis[2], E. Kastritis[1]

1) Dep. of Clinical Therapeutics, School of Medicine, National and Kapodistrian University of Athens, Alexandra Hospital, Leof. Vasilissis Sofias 80, Athens 11528, Greece
2) Laboratory of Analytical Chemistry, Dep. of Chemistry, National and Kapodistrian University of Athens, Panepistimiopolis Zografou, 15771, Athens, Greece
(3) Laboratory of Pharmacology, Faculty of Pharmacy, National and Kapodistrian University of Athens, Panepistimioupolis, Zografou, 15771, Athens, Greece
(4) Centre for Amyloidosis University College London, and Royal Free London NHS Foundation Trust Rowland Hill Street, London NW3 2PF, UK

Amyloidoses are a group of diseases caused by accumulation, in various tissues, of amyloid fibrils derived from different misfolded proteins. Because several organs are often affected, symptoms are non-specific, delaying accurate diagnosis and treatment. Correct typing of amyloid deposits is imperative to distinguish the various types of amyloidosis, for which specific therapies are available. Alongside immuno-histochemistry, mass spectrometry (MS)-based methods are considered the gold standard, but they are only routinely used in a few places in the world.

Here we present how we are implementing a proteomic analysis[1] of laser-microdissected amyloid depots via LC-MS/MS using a nano LC coupled with a timsTOF flex instrument. While learning from the experience of other centers, we are testing several alternatives and ideas emerging from other approaches to proteomics to improve sensitivity and decrease analysis time. Exploiting the capacities of the Parallel Accumulation–Serial Fragmentation (PASEF) technology, we generate high-quality data, which are then processed using MaxQuant (Swiss-Prot database for identification) and R for inspecting proteins relevant to the diagnosis. As a preliminary step to validate our method, we have analyzed 16 fat tissue samples of patients with various immunoglobulin light chain (kappa or lambda) amyloidoses and compared our results with the findings of the UK National Amyloidosis Centre in London. We are also testing our method on kidney biopsies. Although proteomics is not (yet) used widely in clinical practice, here is an example where it makes a difference for the patients. Local – and therefore timely and 'cheap' – processing of samples can have a real impact on treatment course, and ultimately on prognosis.

Reference

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O21

Simultaneous absolute quantification and structural characterization of therapeutic monoclonal antibodies after administration to patients using capillary electrophoresis-tandem mass spectrometry

T. Reinert[1),2)], P. Houzé[1)], N. Mignet[1)], A. Kulus[2)], M. Allez[3)], R. Gahoual[1)], Y-N François[2)]

1) Unité de Technologies Chimiques et Biologiques pour la Santé, Faculté de Pharmacie, Université Paris Cité, CNRS Inserm Paris (France)
2) Laboratoire de spectrométrie de masse des interactions et des systèmes (LSMIS), Université de Strasbourg, CNRS, Strasbourg (France)
3) Saint-Louis Hospital, Assistance Publique – Hôpitaux de Paris (AP-HP), Paris (France)

Infliximab (IFX) is a chimeric monoclonal antibody (mAbs) approved mainly for the treatment of Crohn's disease. Because IFX is administered over long-term periods for the treatment of a chronic pathology, it is important to monitor its concentration in patient serums in order to adjust the treatment if necessary. Also, the clinical practice recently pointed that some patients may show unexpected response without providing any tangible interpretation. Patients follow-up is currently performed only through quantification using ELISA immunoassay, however in some cases specificity may be problematic due to matrix effects.

We developed a novel analytical strategy based on capillary electrophoresis hyphenated to tandem mass spectrometry (CE-MS/MS) for the absolute quantification and concomitant structural characterization of IFX in human serum. A dedicated serum purification process was designed to provide optimal sensitivity and compatibility with the CE-MS/MS analysis. Purified IFX peptides obtained from tryptic digestion were separated and characterized by CE-MS/MS. CE-MS/MS method demonstrated the successful quantification of IFX in spiked serum for concentration ranging from 0.4 to 25 µg/mL. Structural characterization of IFX was performed simultaneously using the same dataset. CE-MS/MS data allowed to successfully characterize the structures of six major N-glycosylation and establish a detailed glycoprofiling of IFX in serum samples. Also, six PTMs of interest, including asparagine deamidation and aspartic acid isomerization, were precisely characterize regarding localization and modification levels. CE-MS/MS analytical strategy was applied to serum samples originating from 24 patients treated for Crohn's disease using IFX. Results exhibited an important disparity regarding the evolution of IFX concentration after administration between the different patients. Also, CE-MS/MS data demonstrated a gradual Asp57 deamidation during IFX residence time in the patient's system that was not described in the literature. This residue is located in the region of IFX directly interacting with TNF-α, and therefore the modification could alter the activity of IFX. As a consequence, the study emphasized the possibility provided by CE-MS/MS to achieve an additional dimension of characterization regarding the outcome of mAbs after administration.

O22
Chemical ionization in a compact FT-ICR mass spectrometer for real-time analysis of pathological biomarkers in sweat

T. Abar, H. Mestdagh, J. Lemaire, M. Heninger

Institut de Chimie Physique UMR8000 - Université Paris-Saclay, 15, avenue Jean Perrin, 91405; Orsay, France

Health issues are a significant problem for our societies. Early diagnosis can significantly contribute to a decrease in mortality caused by diseases. In this respect, a non-invasive and rapid medical analysis would be a promising alternative to the usual clinical diagnostic methods requiring invasive sampling and/or extended biomedical analysis. It would provide access to disease-specific biomarkers with minimal inconveniences. It has been known for a long time that some diseases can change the body odors by emitting various substances, classified as VOCs (Volatile Organic Compounds), in the different biological fluids (urine, sweat, blood, exhaled breath….). Identifying some of these VOCs in biological matrices is an exciting project that presents new challenges. The main objective of my thesis is to develop an analytical device based on mass spectrometry to analyze sweat samples and identify volatile biomarkers characteristic of diseases.

In the present work, we used an FT-ICR mass spectrometer with a permanent magnet coupled to chemical ionization and a tubular furnace for real-time analysis of isovaleric, lactic, pyruvic acids, and urea. The presence of those molecules at high concentrations (mM) in sweat indicates a health problem related to isovaleric acidemia [1], pressure ischemia [2], and/or kidney failure [3].
Test analyses are performed as follows: sample solutions were soaked on a sterile gauze, vaporized by heating in the furnace, and introduced in the mass spectrometer to be analyzed under controlled conditions.

For this purpose, several parameters were optimized, such as i) the heating program of the furnace, ii) the flow of the carrier gas iii) the device allowing sample introduction in the mass spectrometer: three-way or two-way valve. Those optimizations were performed with 100 ppm in mass toluene aqueous solution.

Results will be shown on analysis of aqueous solutions of the four biomarkers, first individually, then as a mixture, at different concentrations.

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O23

In situ Chemical Diversity Characterization of *Sextonia rubra* fruits by MALDI-CID-FT-ICR Imaging and Molecular Networks

M. Levasseur[1)], E. Nicol[2)], Y.Estevez[3)], N. Amusant[3)], V. Eparvier[1)], E. Houël[4)], D. Touboul[1,2)]

1) Université Paris-Saclay, CNRS, Institut de Chimie des Substances Naturelles, UPR 2301, 91198, Gif-sur-Yvette, France
2) LCM, CNRS, Ecole polytechnique, Institut Polytechnique de Paris, Route de Saclay, 91120 Palaiseau, France
3) CNRS & Cirad, UMR EcoFoG, AgroParisTech, INRAE, Université des Antilles, Université de Guyane, 97300 Cayenne, France
4) Sorbonne Université, CNRS, Laboratoire de Biodiversité et Biotechnologies Microbiennes, LBBM, Observatoire Océanologique, 66650 Banyuls-sur-Mer, France

Sextonia rubra is a tropical tree endemic to the Guiana Shield and the Brazilian Amazon. It is known for its heartwood natural durability that comprises numerous lactone derivatives [1]. However its fruits have not been studied chemically. Here we propose analytical methods to explore the chemical diversity of these fruits using imaging MS and chemoinformatics analysis.

We analyzed fruit sections by MALDI-CID-FT-ICR. All MS^2 spectra were aggregated and analyzed using the MetGem software [2]. This metabolomic tool allows the annotation of the detected ions by querying fragmentation spectra databases.

Thus, lactone derivatives were detected in all tissues, flavonoids in internal tissues and alkaloids in external tissues. These results suggest a distribution of specialized metabolites according to their ecological role. Our methodology proves to be relevant for annotating the structures of the molecules while preserving the information of their spatial distribution.

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O24
Improved Quantitative Approach for Monitorization of Gangliosides Structural Diversity in Fungal Cell Factories by LC-MS/MS

J.-F. Montero-Bullón, J. Martín-González, G. Muñoz-Fernández, A. Jiménez, J. L. Revuelta

University of Salamanca, Department of microbiology and genetics.

Current advancements in MS metabolomics gravitates towards enhanced high-throughput analysis with unequivocal identification of hundreds of biomolecules in a run. These new methods aim at monitoring - in a quantitative manner, all species concerning a biological problem. Thereby, for discrimination of a set of structure-related and/or building blocks-sharing metabolites, fine chemical elucidation is essential. Such is the case of this work, dealing with a wide plethora of glycolipids – recombinant gangliosides, that comprises chimeric structures arising from metabolic engineering.

Gangliosides naturally occur in higher animals with important (patho)-physiological properties and are composed of a sphingoid core bound to a glycan moiety including several units of sialic acid. The combinatorial diversity grows exponentially in synthetic biology approaches, e.g., use of microbial cell factories, with an expanded building blocks assortment. A dedicated platform accounting for this complexity, meeting the high-throughput and unequivocal identification requirements, is herein presented. The targeted LC-MS/MS methodology developed includes internal standard analogues-based absolute quantification, adapted to gangliosides bioavailability in fungal – recombinant - organisms and includes hitherto uncharacterized structures, with unusual sphingoid bases and both simple and hydroxylated fatty acids. The addition of glycans to the polar head was also successfully monitored for up to 4 monomers including hexoses and acidic residues. Specific endeavour was required to avoid interferences between more than 100 species, often isobaric species, by separation in the chromatographic stage and discrimination via MS and MSn *info* in a high-end device (Vanquish/Q-Exactive, Thermo™). This platform represents an improved methodology to study the biochemical diversity associated to gangliosides for natural and metabolically engineered biosynthetic pathways.

O25
Green waste valorization: Microwave-assisted modifications of the hemolytic and antifungal saponins contained in *Aesculus hippocastanum* seed

P. Savarino[1),2)], N. Desoignies[2)], P. Gerbaux[1)]

1) Organic Synthesis and Mass Spectrometry laboratory (S²MOs) – Université de Mons, 19 Avenue Maistriau, B-7000 Mons, Belgium;
2) Phytopathology, Microbial and Molecular Farming laboratory (PMMF) – Haute Ecole de la Province de Hainaut, 11 Rue Paul Pastur, B-7800 Ath, Belgium

Saponins are natural amphiphilic molecules that are found in some marine animals, e.g., sea cucumbers or sea stars, but abundantly in the plant kingdom. These molecules are specialized metabolites and are reputed to be essential for the relationships between plants/animals and their ecosystem since they intervene in the communication and defense processes. For decades, their surfactant properties have been valorized as soaps and detergents and their cytotoxicity presents great avenues in cosmetic, pharmaceutical, and medical research [1]. Part of our recent research focuses on the valorization of biomasses with a special interest in the development of biointrants in the context of biological agriculture. In the present study on horse chestnut saponins, we report on the mass spectrometry identification and the targeted chemical modifications of these specialized metabolites. We further evaluated the biological activities, i.e., cytotoxicity and antifungal properties, of the (modified) saponins in the context of defining the structure/activity relationship and proposing high-added value applications of this green waste. The horse chestnut is one of the most abundant ornamental trees in Europe. Chestnut seeds are nevertheless discarded due to the presence of toxic compounds, such as esculin and saponins. These saponins, the so-called Escins, are monodesmosidic saponins based on a(n) sapogenin/aglycone (apolar part) substituted in different positions by different acyl chains. The glycan (polar part) is always a branched trisaccharide. The first step of the project consisted of the structural characterization of the seed-extracted saponins by mass spectrometry techniques. The identification and quantification of saponins were carried out by MALDI-ToF and LC-MSMS experiments. Nine compositions have been identified, for a total of twenty-four isomers. Flash chromatography was further used to generate an Escins I, II and III enriched-extract. Secondly, since the isolated Escins present two ester groups on their aglycones (tigloyl/angloyl group on C21, and acetyl group on C22 or C28), we selectively hydrolyzed these ester groups upon microwave activation. During our optimization, we detected new saponins in which the acetyl group is displaced from C22 to C16. Finally, we evaluated the impact of the chemical modifications on the cytotoxicity of the saponins based on a hemolytic activity assay and the antifungal activities of the (modified) saponins against *Alternaria solani*, a fungal pathogen of tomato and potato crops, have also been shown to be tuned by the chemical modifications.

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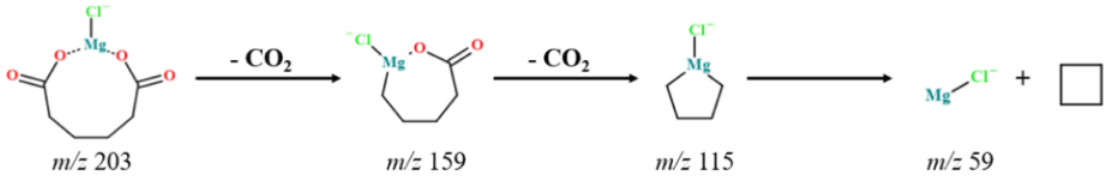
O26

Reaction model for the formation of the new C-C bond by magnesium promoted decarboxylation of the Adipic Acid – a mass spectrometry study.

K. Podgórniak[1)], A. Błaziak[2)], K. Błaziak[3)]

1) Faculty of Chemistry, University of Warsaw, ul. Pasteura 1, 01-224 Warsaw, Poland
2) Institute of Physical Chemistry, Polish Academy of Sciences, Warsaw 01-224, Poland
3) Biological and Chemical Research Center, University of Warsaw, ul. Żwirki i Wigury 101, 01-224 Warsaw, Poland



The mixture of adipic acid and magnesium chloride was used to study the gas-phase reactivity of negatively charged bicarboxylate-magnesium chloride complex ions in the mass spectrometer. The kinetic properties and detailed reaction mechanism was investigated by both: the pressure controlled ER-CID experimental technique and DFT methods. It has been shown, that bicarboxylate-metal complexes can undergo under kinetic control the sequential decarboxylation leading to the formation of the new C-C bond within the cyblobutane. A very first step of the reaction, the loss of the neutral CO2 leads to the formation of an intermediate structure in which the carbon-magnesium bond is essential in organometallic cyclic geometry form. The second decarboxylation leads to next intermediate in which the -C-Mg(Cl)-C- binding motif play a key role, from which at the very end of the mechanism the loss of MgCl promotes the new carbon-carbon bond stabilization within the cyclobutane structure. In this project, the modified mass spectrometry instrumentation with upgraded gas inlet system was used to measure the activation barriers of each reaction step. The differences in adipic acid decarboxylation abilities with and without a second, catalytic body was also analyzed. Ultimately, the comprehensive reaction mechanism was proposed by utilization of the DFT-derived reaction model and experimental data.

O27

Identification of insoluble oil paint film structure after innovative soft chemical depolymerization and high resolution MALDI FTICR MS analysis. Application to Cultural Heritage and environment samples

B. Almasri[1,2]), A. Mazzah[1)], Y. Bakkour[2)], C. Rolando[1)]

1) University of Lille, Faculty of Sciences & Technologies, Villeneuve d'Ascq, France;
2) Lebanese University, Faculty of Sciences, Tripoli, Lebanon

Oil paint binders are made from triacylglycerol of polyunsaturated fatty acids which crosslink by exposition to light and atmosphere by a mechanism named siccativation. Despite their wide use as binders in artworks for centuries; little is known about the structure of the 3D-crosslinked polymeric film of oil paints, and the mechanism of their siccativation is still under debate. Three oils were used: walnut, linseed and poppy oil which differ only by the number of unsaturations of their fatty acids. Until now there is no robust method to identify which oil was used from a painting sample, which is an important information for historians of arts and restorers.

To solve this problem, we developed a new methodology for the analysis of insoluble paint films starting by an innovative soft chemical depolymerization protocol which is a transamidation reaction using dimethylaminopropylamine (DMAPA) which breaks the ester bonds. This catalyst was selected also to improve the mass spectrometry ionization and to enhance the cleavage by participation. The resulting depolymerized paint films were then analyzed using MALDI ionization on an ultra-high resolution Fourier transform ion cyclotron resonance mass spectrometry (FTICR MS) 9.4 Tesla. In a first step, we made mock-up samples using different oils, pigments, driers and ageing conditions as temperature or UV exposure. We identified successfully the remaining traces polyunsaturated fatty acids of the original paint, their modifications induced by the siccativation process and the crosslinking dimers and oligomers. The products obtained were shown to be very dependent and characteristic of the initial oil. In a second step, this sensitive method was applied to museum size samples on painting from the XIXth and XXth centuries to identify the oil used by the artist according to the period but also to the pigments which bring the desired colour.

We will show that this methodology works for other polyester polymers such as alkyd paints which are formed of phthalic or isophthalic part, polyols and unsaturated fatty acids and polyethylene terephthalate after natural or accelerated ageing. For identifying the diols or polyols which are water soluble, we modified slightly the above protocol starting by a water-free transesterification with methanol and derivatization of the alcohols by benzoylation with dimethylaminobenzoyl chloride.

O28
Alkyne hydroarylation catalyzed by (P,C)-cyclometalated Au(III) complexes: energetic aspects

M. Régnacq[1)], K. Miqueu[2)], D. Bourissou[3)], D. Lesage[1)], Y. Gimbert[4)]

1) IPCM UMR 8232, CNRS/SU, 4 Place Jussieu, 75252 Paris Cedex 05, France
2) IPREM UMR 5254, 64053 Pau Cedex 09, France
3) LHFA UMR 5069, CNRS/Université de Toulouse, UPS, 31062 Toulouse Cedex 09, France
4) DCM UMR 5250, CNRS/UGA, 38000 Grenoble, France

Over the last decade, gold(III) catalysis has grown intensively. It offers now many innovative synthetic routes. For most of them, mechanistic insights are yet to be investigated. We particularly focused on the intermolecular hydroarylation of alkynes catalyzed by (P,C)-cyclometalated Au(III) complexes. Experimental studies, supported by DFT, showed that a Wheland intermediate can be easily obtained (resting state). In solution, different aryle/alkyne combinations have been tested, and their relative anti-addition product have been obtained, indicating an outer-sphere mechanism[1]. So, the endothermic exchange between the electron-rich aryle and the incoming alkyne takes place, before the outer-sphere attack of the aryle. This study therefore concerns both the determination of dissociation energies between the Au(III) complexes and the different substrates, and the comparison of electronic properties of the ligand/substrate.

Complexes have been generated in the gas phase in a triple quadrupole (Quattro II, Waters) by ion-molecule reaction, and then fragmented by CID. We have chosen to work under high-pressure conditions with argon as collision gas, since the formed complexes are very fragile and T-CID conditions have not been met. Kinetic modeling of the survival yield curves using MassKinetics software has been applied to collect critical energies of dissociation[2,3]. A good agreement was found between these experimental critical energies and bond dissociation energies obtained by molecular modeling. That consolidates both the models used and the mechanistic insights.

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O29
Quantification of cholesterol metabolites in the brain by on-tissue derivatization mass spectrometry imaging in a mouse model of Huntington's disease

A. Passoni, A. M. Siciliano, M. Favagrossa, A. Lanno, L. Colombo, M. Salmona, R. Bagnati, E. Davoli

Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milan (Italy)

Huntington's disease (HD) is an autosomal neurodegenerative disorder caused by a mutation of the IT15 gene on chromosome 4 [1], translating for the mutant huntingtin protein (mHTT). mHTT reduces the sterol-regulatory element-binding protein (SREBP) transcription factor translocation, essential for cholesterol (Chol) synthesis, leading to a significant reduction of Chol metabolism in the brain. Notably, different Chol metabolites have been shown to be down-regulated in the brain of R6/2 mice [2].

Here, we proposed a high-resolution imaging-mass spectrometry (IMS) approach, for the study of the spatial distribution of Chol brain metabolites and their simultaneous quantitation using in-house developed software.

In the present study, the whole brain of both WT and R6/2 mice at 12 weeks was employed and three to five sagittal sections were cut and mounted on the target at–20 °C as replicates.

The IMS method was developed on an AP-MALDI source (Mass-Tech), installed on an LTQ Orbitrap XLmass spectrometer (Thermo Scientific), and allowed the simultaneous quantitation and spatial distribution study of the free form of 24OHC, Chol, desmosterol, and 7-dehydrocholesterol in the brain.

We set up the IMS method to evaluate the distribution of the selected Chol metabolites in the brain from R6/2 mice, focusing on the striatum, the most affected area by neurodegeneration in HD. Our IMS approach included two-derivatization steps directly on tissue slices that use Chol oxidase and Girard's T reagent and MS/MS experiments. The evidences confirmed the data previously obtained with LC-MS analysis *(unpublished data),* highlighting a significant reduction of desmosterol, 24OHC and chol levels. As desmosterol is a Chol metabolite representative of the Bloch pathway, our results suggested that this is the most affected pathway by HD.

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O30
Experimental and theoretical determination of the collision cross sections of phosphoric acid clusters: anions compared to cations

H. Lavanant[1)], V. Calabrese[2)], F. Rosu[3)], V. Gabelica[4)], C. Afonso[1)]

1) Normandie Univ, UNIROUEN, INSA Rouen, CNRS, COBRA, 76000 Rouen, France
2) University of Lyon, University Claude Bernard 1 of Lyon, Institute of Analytical Sciences, CNRS, UMR 5280 5 rue de la Douan, 69100 Villeurbanne, France
3) CNRS, UAR 3033, Institut Européen de Chimie et Biologie (IECB), Pessac, France
4) University of Bordeaux, INSERM and CNRS, ARNA Laboratory, IECB site, 2 rue Robert Escarpit, 33600 Pessac, France

Electrospray ionization of phosphoric acid solutions readily produces hydrogen bonded phosphoric acid cluster anions and cations, in the negative and positive ion mode, respectively. Using drift tube ion mobility experiments, we determined and compared series of collision cross sections (DTCCS) in He and N_2 for phosphoric acid cluster anions and cations with different aggregation number n (4 to 90) and different charge states (1 to 4). We obtained different growth rate and CCS properties as a function of the aggregation number (*n*) for positively and negatively charged cluster ions. Then, we set up coarse-grain models using cluster of spheres and theoretical CCS calculations with either the projection approximation or the trajectory methods.

The DTCCS values were always found larger for a given aggregation number *n* for positive cluster ions than for negative cluster ions, both in He and in N2 drift gas. The aggregation number *n* for which charge states increased was lower for negative cluster ions than for positive cluster ions, expectedly for cluster of acid molecules.

Using the projection approximation method for CCS calculation in helium, we found that, consistently with the observed trend, the coarse-grain model that fitted negative cluster ions had lower sphere radii than the positive cluster ions. From this simple aggregation of sphere model, we calculated a charge density for both negative and positive cluster ions and compared it to the Rayleigh limit of the circumscribed sphere.

O31
Real-Time Analysis of Anionic Polymerizations by Electrospray-Ionization Mass Spectrometry

K. Koszinowski, N. F. Eisele, F. Kreyenschmidt

Universität Göttingen, Institut für Organische und Biomolekulare Chemie, Tammannstr. 2, 37077 Göttingen, Germany

Anionic polymerizations are of enormous practical importance. Due to the high reactivity of the growing anionic polymer chains, in-situ measurements have been notoriously difficult. Here we show that electrospray-ionization mass spectrometry holds great promise for the real-time analysis of such reactions. Under carefully optimized conditions, the active anionic intermediates of the ring-opening polymerization of an acceptor-substituted cyclopropane as well as the organocobaltate anions formed in the coordinative anionic polymerization of isoprene can be detected [1,2]. The molar-mass distributions derived from the ESI mass spectra agree well with those of the isolated polymers determined independently by well-established methods, thus giving confidence in the quantitative accuracy of the ESI-mass spectrometric results. Besides simple mass distributions, the new approach affords individual rate constants and succeeds in the observation of side products. This wealth of mechanistic information improves our understanding of anionic polymerizations and should be instrumental for optimizing practical applications.

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O32
Negative Ion Mode Proteomics: An MS/MS-Free Approach for Increased Proteome Coverage

P. A. Penanes[1)], V. Gorshkov[1)], M. V. Ivanov[2)], M. V. Gorshkov[2)], F. Kjeldsen[1)]

1) Department of Biochemistry and Molecular Biology, University of Southern Denmark, DK 5230, Odense M, Denmark;
2) V. L. Talrose Institute for Energy Problems of Chemical Physics, N. N. Semenov Federal Research Center for Chemical Physics, Russian Academy of Sciences, Moscow 119334, Russia.

Negative ion mode proteomics has the potential to increase both sequence coverage and depth of proteome analysis. However, challenges such as corona discharge, spray stability and inefficient ionization need to be addressed by optimizing both chromatographic and mass spectrometric conditions. Moreover, despite demonstration of some MS/MS techniques for negatively charged peptides, their implementation in routine proteomics experiments is not yet standard. To address this, we have explored an alternative approach using MS1-only data in conjunction with predicted peptide chromatographic retention times.

Negative mode analyses were performed using an Orbitrap Eclipse mass spectrometer with alkaline mobile phases, and short 5-15 min. chromatographic gradients. For protein identification, Biosaur feature detection and ms1searchpy search engine based on accurate mass and retention time were applied.

The best results in terms of spray stability and signal intensity were achieved using mobile buffers at 2.5 mM imidazole and 3% isopropanol. The method was validated on a *HeLa* standard, showing comparable performance to the positive mode, with over 1,000 proteins identified in a single-shot 10-minute gradient. Results highlighted the complementarity of data for protein identification and sequence coverage, with 15% of proteins and 40% of peptide features detected only in the negative mode. Evaluating the proteins as a function of pI showed a preference for the acidic part of the proteome for negative mode data. When all replicates for the 10-minute gradients in both polarities were combined, it allowed to increase the number of identified proteins to more than 1,773. We also looked at how using four different proteases (LysC, GluC, AspN, and trypsin) affected the performance of our method, and found that trypsin and LysC performed best in terms of protein identification, indicating that the same lysis and digestion procedures as for positive mode proteomics can also be utilized for analysis in the negative ion mode.

O33
Pseudo-MRM and the Survival Yield Technique for the accurate quantification of a tryptic peptide despite isobaric co-elution

A. Maroto[1)], D. Jeanne Dit Fouque[1)], R. Lartia[2)], A. Memboeuf[1)]

1) UMR CNRS 6521, CEMCA, Université de Bretagne-Occidentale, Brest, France
2) UMR CNRS 5250, ICMG FR-2607, Université Joseph-Fourier, Grenoble, France

Multiple Reaction Monitoring (MRM) is a quantification technique, usually performed in triple-quadrupole instruments (QqQ), consisting in the monitoring of intensities of at least two diagnostic fragment ions (transitions) in CID MS/MS experiments. In the context of iso-baric/meric interferences, pseudo-MRM is an alternative mode, applied for the analysis of difficult-to-fragment compounds, which relies on the monitoring, at high collision energy, of the precursor ions peak only [1]. Pseudo-MRM has shown good performances for the analysis of complex samples, due to the reduction, inside the collision cell, of isobaric co-elution. However, there is not yet a mean for determining optimal conditions for it. We propose to assess optimal conditions by using Gas Phase Collisional Purification (GPCP) [2] that relies on Energy Resolved Tandem Mass Spectrometry (ER-MS) and the Survival Yield technique. This way, optimal excitation voltage can be selected to fully fragment the iso-baric/meric interference, while keeping the analyte of interest.

Going a step further by using an internal standard (IS), the monitoring of analyte/IS ratio at several collision voltages is shown to clearly indicate the complete fragmentation of the interference with the appearance of a plateau. This is a clear and very robust indication of the total purification of the analyte. The concentration of the analyte after GPCP can then be calculated with an IS calibration curve.[3] We have applied the above-mentioned technique to quantify a tryptic peptide, at m/z 780.402, in the presence of a co-eluted isobaric interference with m/z 780.370. A triply deuterated analogue of the tryptic peptide was used as IS. This approach was applied in two cases: 1) ion trap with an unconventionally 8 m/z wide isolation window (which can be seen as a modified parallel pseudo-MRM [4]); and 2) single quadrupolar LC-MS with in-source fragmentation. In both cases, reference samples intentionally contaminated were correctly quantified despite the isobaric interference with: ~1% deviation, a linear dynamic range up to 25 µM, detection limit about 0.1 µM and a quantification limit of 0.25 µM.

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O34

Application of LEDA Algorithm for the characterization of Isomers in Simultaneous Degradation Study in Human Plasma by HPLC-MS/MS

M. Pallecchi[1)], L. Braconi[1)], M. Menicatti[1)], S. Giachetti[2)], S. Dei[1)], E. Teodori[1)], G. Bartolucci[1)]

1) NEUROFARBA Department, Section of Pharmaceutical and Nutraceutical Sciences, University of Florence, Via U. Schiff 6, Sesto Fiorentino, 50019 (FI), Italy;
2) Chemistry Department DICUS 'Ugo Schiff', University of Florence, Polo Scientifico, Via della Lastruccia 3, Sesto Fiorentino, 50019 (FI), Italy

Design and synthesis of new candidate drugs produces a large number of compounds that must be qualified and tested to evaluate their characteristics and potential applications. Therefore, many studies will be scheduled and, consequently, it will be necessary to arrange specific, reliable, fast and relatively cheap analytic methods to support this research.

For these reasons, a tandem mass spectrometry (MS/MS) approach in isomer recognition by playing with the "energetic dimension" of the experiment was proposed[1]. The chromatographic set up (HPLC) was tuned to minimize the run time, without requiring high efficiency or resolution between the isomers. Then, the MS/MS properties to solve the signal assignment were explored by performing a series of energy resolved experiments in order to optimize the parameters, and by applying an interesting post-processing data elaboration tool (LEDA)[2-4]. The reliability of this approach was evaluated, determining the accuracy and precision of the quantitative results through analysis of the isomer mixture solutions. Next, the proposed method was applied in a chemical stability study of human plasma samples through the simultaneous addition of each pair of isomers. In the studied case, only one of the isomers suffered of enzymatic hydrolysis; therefore, the influence of the stable isomer on the degradation rate of the other was verified. In order to monitor this process correctly, it must be possible to distinguish each isomer present in the sample, quantify it, and plot its degradation profile. The reported results demonstrated the effectiveness of the LEDA algorithm in separating the isomers, without chromatographic resolution, and monitoring their behavior in human plasma samples.

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O35
Chiral mass spectrometry analysis for metabolomics

C. Cao[1)], J;-C. Tabet[1),2)], S. Alves[2)], C. Chalet[2)], E. Dariy[3)], E. Rathahao-Paris[2)], D. Lesage[2)], Y. Gimbert[2)], A. Perret[3)], F. Fenaille[1)], C. Junot[1)], A. Damont[1)]

1) Département Médicaments et Technologies pour la Santé (DMTS), MetaboHUB, Université Paris-Saclay, CEA, INRAE, Gif-sur-Yvette, France;
2) Faculté des Sciences et de l'Ingénierie,Institut Parisien de Chimie Moléculaire (IPCM), Sorbonne Université, Paris, France;
3) Génomique Métabolique, Genoscope, Institut François Jacob, CEA CNRS, Univ Evry, Université Paris-Saclay, 91057 Evry, France.

Chirality presents a unique challenge in life science domain since individual enantiomer may exhibit different biological activities and be involved in different biological pathways. In the field of metabolomics, chiral recognition may be a key to highlight specific disease biomarkers. Today, absolute stereochemical determination remains largely unexplored and extremely challenging in large scale metabolomics studies due to the complexity of biological samples and the structural chemical variety of molecules constituting the metabolome. Therefore, there is still a need to explore and develop efficient chiral analytical methods applicable to the untargeted profiling of biological samples. As high-resolution mass spectrometry (HRMS) is the most frequently used analytical platform to explore the metabolome, the development of gas-phase chiral recognition methods are of particular interest.

In this context, we aimed to develop an innovative method for fast and sensitive chiral analysis based on mass spectrometry and its application in metabolomics. The key issue is based on chiral recognition through the formation of non covalent diastereoisomeric multimer ions displaying specific gas phase behaviors. For this, a chiral reference (R) displaying specific gas phase behavior towards an enantiomer (E) of interest has to be employed in combination with a metal (M) ion (e.g., Cu^{2+}, Fe^{2+} and others).

The approach for the search of R on the one hand and promising M on the other hand to the development of an enantioselective method will be presented. The optimal conditions for the implementation of a sufficiently sensitive chiral analysis on an Orbitrap instrument (Fusion, Thermo Scientific) will also be discussed. Finally, the first results of chiral analysis through the study of the fragmentation of non-covalent diastereomeric ions formed to differentiate L- and D-glutamine, as well as cis-hydroxy-L- and D-proline, will be introduced. In particular, the implementation of calibration ranges for determining enantiomeric excess (ee) in the buffer with ions of the type $[E+R-H+M^{2+}]^{+}$ will be demonstrated. The approach that will be detailed here is currently under development for the analysis of enantiomeric mixtures in the biological matrix.

O36
Dynamics of ion-molecule reactions for astrochemistry; study of isomerism and internal or collision energy

R. Thissen[1),2)], N. Solem[1)], C. Romanzin[1),2)], C. Alacaraz[1),2)]

1) Institut de Chimie-Physique, Université Paris-Saclay, CNRS, UMR8000, 91405 Orsay, France
2) Synchrotron SOLEIL, L'Orme des Merisiers, F-92292 Saint-Aubin, France

HCO+ and HOC+ are two isomeric cations detected in Photon Dominated Regions [1], diffuse clouds [2], and Mars atmosphere [3,4]. They correspond to the protonation of carbon monoxide on either the carbon or oxygen atom and are important intermediates in the formation of complex molecules as HCO+ is the most abundant ion in molecular clouds. There is a difference in reactivity between the two isomers and isomerization is possible when energy is provided or through collision. The isomers have a difference of energy of 1.7 eV (HCO+ being the more stable) and are separated by an isomerization barrier of 3.2 eV [5]. Previous studies of their reactivity have mostly been obtained by electron ionization [6,7] which does not allow the formation of pure HOC+ nor precise internal energy determination.

The CERISES [8] instrument, connected on the DESIRS beamline at SOLEIL Synchrotron, was used to produce (i) HCO+ from formaldehyde (H2CO) and (ii) HOC+ from deuterated methanol (CD3OH). We have studied the reactivity of the two isomers with a series of neutral targets to (i) characterize the ionic purity and internal energy content of the two isomers and (ii) study complex reactivity (with a potential increase in chemical complexity). From this extensive data set, we have determined the isomeric purity, the evolution of internal energy as a function of photon energy, and that the reactivity of both isomers with methanol is dominated by Proton Transfer (PT) and dissociative PT. Attempts to evaluate the collision-mediated isomerization are also being performed through an innovative method that aims to combine time-of-flight spectrometry with SIMION simulation.

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O37
Mass spectrometric investigation of isobaric peptides of biological interest: ESI-MS versus ToF-SIMS

A. Distefano[1)], N. Tuccitto[1)], A. Auditore[1)], A. Licciardello[1)], V. Cunsolo[1)], A. Di Francesco[1)], O. Monasson[2),3),4)], E. Peroni[2),3),4)], G. Grasso[1)]

1) Chemical Sciences Department, University of Catania, Viale Andrea Doria 6, 95125, Catania, Italy
2) 2 CY Cergy Paris Université, CNRS, BioCIS, 95000, Cergy Pontoise, France
3) Université Paris-Saclay, CNRS, BioCIS, 92290, Châtenay-Malabry, France
4) CY Cergy Paris Université, CY PeptLab, 95000, Cergy Pontoise, France

A small peptide (TAT1; sequence: GRKKRRQRRRPS) derived from the transcriptional activator TAR (TAT) protein has recently drawn some attention due to its capability to stimulate proteasome activity [1]. ESI MS/MS experiments revealed a great stability of this peptide against fragmentation, mainly related to the high content of arginine residues. Consequently, peptide fragmentation leads to a predominant loss of neutral molecules from the side-chain of arginine rather than the cleavage of peptide bonds. So we have chosen the TAT1 amino-acidic sequence as a base to construct three model isobaric peptides TAT1-Car, Car-TAT1 and T-Car-T, simply adding carnosine (Car) to the C- and N-terminus as well as in the middle of the peptide sequence, respectively. The addition of the Car moiety to the TAT-1 sequence was dictated by the interest that our group has towards Car [2,3]. Due to the peptides high stability, differentiation of the investigated isobaric peptides, in particular Car-TAT1 and T-Car-T, by MS/MS experiments is not easy. On the contrary, ToF-SIMS analysis, combined with multivariate data treatment of spectra, can discriminate among these oligopeptides. Results are discussed and a mechanism of fragmentation is proposed.

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O38
Cold-Spray Ionization mass spectrometry: evidence of cooling effect in term of ion internal energy and application in deep eutectic solvent analysis

É. Bertrand, D. Rondeau, T. Delhaye, X. Castel, M. Himdi

Univ Rennes, CNRS, IETR – UMR 6164, F-35000 Rennes, France

The *Cold-Spray Ionization* (CSI) source is a variant of the *Electrospray Ionization* (ESI) source where the nebulizing gas is cooled by liquid nitrogen at a temperature of -40°C [1]. Thereby, the liquid emerging from the capillary is considerably cooled but the temperature of gaseous ions produce is not known. For the presented studies, CSI was used to characterize Deep Eutectic Solvents (DES) a new class of green solvents [2]. In this context, the evaluation of internal energy distribution of so-called “thermometer ions” has been first performed in CSI and compared to the distribution observed in ESI. The "Vibrational Temperatures" calculated from the mean internal energies of the dissociating benzhylpyridinium cations have been compared to the “Characteristic Temperatures” (*T*char) referenced to be the temperature required for an ion population in thermal equilibrium leads to the same ion Survival Yields (*SY*) as experimentally observed with the same mass spectrometer (JEOL AccuTOF) [3,4]. The modelling of ion’s behavior in mass spectrometer, the calculation of P(Eint) and then Tchar have been performed thanks to the software “Mass Kinetics” [5]. Results shows that the temperature of ions generated by CSI is approximately 100K cooler in comparison by the ones formed by ESI. This temperature difference should explain why the CSI mode allows the characterization of labile non-covalent complexes unobserved by ESI such as the Deep Eutectic Solvent (DES) [6]. Indeed, Deep Eutectic Solvent are a new generation of green solvent able to form non-covalent molecular networks by the combination of hydrogen bond acceptor (HBA) and donor (HBD) and electrostatic interaction. A previous study shows that it is possible to characterize the structural affinities of constituent molecules of DES by mass spectrometry and especially with the CSI method. The chosen DES is the Reline, a mix of choline chloride salt and two urea molecules. To complete this work, other DES having choline chloride salt as hydrogen bond acceptor were also analysed by CSI-MS, namely the assembly of choline chloride/thiourea (1:1) and choline chloride/glycerol (1:1). This work allowed to testify the ability of the CSI method to characterise DES in gaseous phase.

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O39
Use of Advanced Mass Spectrometry Techniques to Help in the Chemical Storage of Solar Energy

B. Tassignon[1),2)], G. Henrard[1),2)], J. De Winter[1)], J. Cornil[2)], P. Gerbaux[1)]

1) Organic Synthesis and Mass Spectrometry Laboratory, Interdisciplinary Center for Mass Spectrometry, University of Mons – UMONS, 23 Place du Parc, B-7000 Mons, Belgium;
2) Laboratory for Chemistry of Novels Materials, Center of Innovation and Research in Materials and Polymers, University of Mons – UMONS, 23 Place du Parc, B-7000 Mons, Belgium

Storing solar energy represents a major challenge in modern science. Chemical storage with **MO**lecular **S**olar **T**hermal systems (**MOST**) appears promising though challenging. The working principle of those systems is based on iterative closed cycles of photoisomerization and back-isomerization between a parent compound and its metastable isomer. Energy is stored within the metastable isomer which possesses a certain half-life time and thermal energy is released during the thermal back-isomerization process. Among the MOST systems, azobenzene with its E->Z photoisomerization has been studied but characteristics such as storage enthalpy and half-life time must be improved. To do so, anchoring chromophores on a macromolecular backbone appears to be an elegant strategy since cooperative effects between chromophores may help enhancing these properties [1]. Our work consists of producing new MOST systems based on a peptoid-type backbone supporting azobenzene chromophores at key positions. This backbone could allow cooperative effects since peptoids belong to foldamers family and can thus adopt specific secondary structures in solution. We synthesized different peptoids containing azobenzene(s) at key positions with solid support protocol. Primary structures of the molecules were confirmed with MS and MS/MS analyses based on B/Y and A/Y fragmentation patterns. With UV irradiation, different isomers are produced and their relative proportions against irradiation time are monitored using LC-MS analyses. LC-MS also allows the determination of the crucial photoisomers half-live times ($t_{1/2}$) by performing continuous analyses while the solutions are kept in the dark at controlled temperatures. Determination of $t_{1/2}$ showed that azobenzene behaves differently depending on its position on the backbone, showing interesting influence from our peptoid support. We will show that repeating $t_{1/2}$ measurement at different temperatures allows to have insights on the activation barrier in solution, which is another crucial MOST parameter. Finally, thermal back-isomerization behaviour of each photoisomer will be further discussed since LC-MS allows their separation and independent analyses.

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O40
Metformin reacts directly with glucose following the Maillard reaction pathway

M. Pallecchi[1)], G. Bartolucci[1)], L. Braconi[1)], S. Dei[1)], E. Teodori[1)], A. Lapolla[2)], G. Sartore[2)], P. Traldi[3)]

1) NEUROFARBA Department, Section of Pharmaceutical and Nutraceutical Sciences, University of Florence, Via U. Schiff 6, Sesto Fiorentino, 50019 Firenze, Italy;
2) Department of Medicine, University of Padova, 35128 Padova, Italy
3) Istituto di Ricerca Pediatrica Città della Speranza, Corso Stati Uniti 4, Padova, Italy

Metformin (dimethylbiguanide) is the preferred first-line oral blood glucose-lowering agent to manage type 2 diabetes. Looking to its activity, Sterne in 1963 suggested for this compound the name 'glucophage' (meaning glucose eater).[1] The generally accepted mechanism of metformin effect is based on the stimulation of adenosine monophosphate (AMP)-activated protein kinase (AMPK). In its turn AMPK is directly activated by an increase in AMP:ATP ratio in metabolic stress conditions, including hypoxia and glucose deprivation. Recently, many novel pathways, besides AMPK induction, have been revealed, which can explain some of other metformin's beneficial effects (reduction in cardiovascular disease and mortality compared with non-intensive treatment and a possible reduction in cancer incidence). The molecular details of metformin mechanism of action continue to be an area of vigorous research. In the review of Pearson et al [2] the known and unknown aspects about the molecular action of metformin have been widely discussed.

In the present study we investigate on an alternative pathway, i.e. on the possible reaction between metformin and circulating glucose, which is present in the range 140-200 mg/dL in diabetic patients. It must be considered that oral doses (from 500 to1000 mg) of metformin are rapidly absorbed in the small intestine, typically giving a peak plasma concentration (Cmax) of about 2 μg/mL (rarely >4 μg/mL), with a steady-state concentration range of 0.3–1.5 μg/mL. Its distribution is extensive (usual volume of distribution [Vd], 100–300 L). Metformin has an elimination half-life (T1/2) of ∼6–7 h. [3]

These values suggest that the reaction of metformin (MET) with glucose (GLU) can in principle occur, following the Maillard reaction pathway. This aspect has been studied and the obtained results are reported and discussed in the present investigation. Glucose and metformin reacts with the formation of [GLU+MET – H2O] (***a***) i.e. the final product of the Maillard reaction. [GLU+MET – 2H2O] (***b***) species are also observed in higher abundance. Both ***a*** and ***b*** are detected by HPLC/ESI mass spectrometry operating in high resolution conditions, showing that for both accurate mass measurements are in agreement with the proposed compositions. ***b*** species reasonably originate by the further reactivity of ***a***, due to the presence of other amino groups. Their possible structures have been investigated by MS/MS experiments performed on their protonated species. Interestingly they have been detected in urine samples of subjects under metformin therapy. Consequently not only the action of the unreacted metformin can be considered, but also the products originated by reaction with glucose must be taken in account for their possible activity at systemic level.

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P01
Study of phosphate-based electrospray ionization enhancement

A. Boulghobra, M. Bonose

Université Paris-Saclay, CNRS, Institut de Chimie Physique, UMR 8000; 91400 Orsay, France

Electrospray ionization is an ionization technique particularly inclined to alkali metal ion adduct formation in positive mode. These adducts may reduce the ionization efficiency of the analyzed molecules and, thus, the sensitivity. Therefore, eliminating sodium and potassium adducts allows to improve the sensitivity and the precision of electrospray ionization signal, especially in biological fluids. In the present work, we propose the use of phosphate as additive in the mobile phase to reduce, nay to eliminate, alkali metal ion adducts. Phosphate was used at concentrations ranging between 5 µM and 20 µM and allowed to enhance electrospray ionization signal for 3-ortho-methyl-DOPA, 5-hydroxytryptophan, 3-metoxy-4-hydroxy-phenylglycol, homovanillic acid and 5- hydroxyindole acetic acid. Phosphate-based positive electrospray ionization enhancement was significantly affected by the mobile phase buffer nature and pH, and by the analyte. Nevertheless, in negative mode, the opposite effect was observed with a signal decrease with phosphate addition. To determine the optimal conditions for each analyte, a full-factorial design of experiment (3 buffers x 5 pHs x 4 phosphate concentrations) was set up. The enhancement was confirmed in positive mode for five other compounds, namely, tetrahydrobiopterin, dihydrobiopterin, biopterin, dihydroneopterin and neopterin. Mass spectrometry spectra exhibited a decrease of alkali metal ion adducts, when increasing phosphate concentration in the mobile phase. Phosphate addition allowed to improve the signal to noise ratios with gain factors between 1.4 and 103.4 depending on the analyte and the mobile phase. Therefore, the proposed desalting method is easy-to-handle and highly effective.

P02
Optimisation of resolution and sensitivity in absorptive FTICR-MS spectra

M. van Agthoven[1)], A. Kadek[1)], P. Novak[1)], M.-A. Delsuc[2)]

1) Institute of Microbiology of the Czech Academy of Sciences, Prague, 14220, Czech Republic; Faculty of Science, Charles University, Prague, 12843, Czech Republic;
2) IGBMC, INSERM, U596, CNRS,UMR7104, Université de Strasbourg, France.

FT-ICR-MS uses a superconducting magnet to trap ions and measure their cyclotron frequency, which is inversely proportional to their *m/z* ratio. After excitation, electrodes record the trajectories of the ions, and a Fourier transform (FT) of the recorded dataset generates the frequency spectrum. A simple axis inversion produces the mass spectrum.

Current FT-ICR-MS spectrometers achieve extreme resolutions. However, the phase of the ions (related to the initial time of their trajectory) is usually unknown, and a *magnitude* Fourier spectrum has to be computed, nearly halving the resolution compared to an *absorption* spectrum computed in the same conditions.

Kilgour et al[1] have proposed a simple procedure to estimate the phase of a FTICR-MS spectrum, thus providing access to the full resolution. This procedure was recently extended to tandem 2DMS measurements[2]. However, the strong correction that must be performed produces baseline artefacts, distorting the spectra and hampers the detection of small peaks. A pre-processing apodisation approach was proposed[3], which mitigates this difficulty without completely removing it.

Here we show that the baseline artefacts originate from cross-talk with the dispersion tails of nearby peaks, thus degrading the beginning of the transient. We propose a post-processing reconstruction of the erroneous points, which restores an artefact-free spectrum, allowing an improved sensitivity to small peaks and an ultimate resolution, and provide guidelines to produce the best possible *absorptive* FTICR spectra. We present this approach on a mixture of peptides fragmented by UVPD

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P03
Charge transfer dissociation (CTD): a new activation method in Evry for probing the structure of natural or synthetic compounds

J.-Y. Salpin[1)], S. Poyer[2)], W. Buchmann[1)], R. Spezia[3)], R. Daniel[1)], G. P. Jackson[4)]

1) Université Paris-Saclay, Univ Evry, CNRS, LAMBE, UMR 8587, 91025 Evry, France.
1) Université Paris-Saclay, CNRS, ICSN, UPR 2301, 91198 Gif-sur-Yvette, France
3) Sorbonne Université, CNRS, LCT, UMR 7616, 75052, Paris, France
4) West Virginia University, Morgantown, United States

The combination of soft ionization methods with tandem mass spectrometry (MS/MS or MSn) has become an essential tool for the fine structural analysis of biomolecules. The most popular MS/MS method is based on collision-induced dissociation (CID) of the gas-phase analyte. However, this method suffers several limitations, such as incomplete fragmentation or the loss of labile chemical functional groups, leading to a loss of structural information.

To reach a more complete characterization, especially for biomolecules with high isomerism, new activation methods have been developed for several years, including Charge Transfer Dissociation (CTD). We have implemented the CTD fragmentation method in 2022 on an ion trap of the LAMBE mass spectrometry platform.

Charge Transfer Dissociation has been recently introduced. [1] CTD is based on the collision between strongly accelerated (keV) He^+ ions and a precursor ion. This method allows a charge exchange process, leading to the formation of radical ions, whose fragmentation appear complementary to CID processes. Several examples of application will be presented.

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P04
Collisional cooling in CID tandem MS ion trap experiments.

A. Memboeuf[1], D. Jeanne Dit Fouque[1], M. Pallecchi[2], A.Maroto[1] and G.Bartolucci[2]

1) Univ Brest, CNRS, UMR 6521 CEMCA, F-29200 Brest, France;
2) NEUROFARBA Department, Section of Pharmaceutical and Nutraceutical Sciences, University of Florence, Via U. Schiff 6, Sesto Fiorentino, 50019 (FI), Italy

Injection of a gas inside a quadrupole ion trap mass spectrometer at a sufficiently high pressure (10-5-10-3 mbar) increases the efficiency in trapping the ions to be mass-analyzed. This higher trapping efficiency is achieved through the collapsing of the ions orbits to the ion trap centre by collisional damping of the ionic motion[1]. The storage of ions over long period of time (tens to hundreds of milliseconds) together with the high trapping gas pressure typically used, eventually leads to a very large collision number (up to 106)[2] that are extensively used to generate tandem mass spectra.

Several years ago, Ichou & al.[3] reported Energy-Resolved tandem MS analysis on protonated leucine enkephalin peptide using a triple quadrupole instrument. They compared the Survival Yields (portion of the precursor ions surviving the CID process at constant experimental conditions) obtained at different acceleration voltages by drawing SY curves at different Ar collision gas pressures. When the Ar pressure inside the collision cell was increased, they observed a shift of the SY curve to lower acceleration voltages. This shift was attributed to an increase in the number of collisions experienced by the precursor ions and in their residence time in their journey to the detector.

We conducted similar ER-MS experiments to draw SY curves at different He pressures however on two different ion trap instruments. We observed a completely opposite behavior with SY curves shifted to larger excitation voltages when collision gas pressure is increased. This observation was confirmed on a large m/z range with: different types of compounds (synthetic and biologic polymers) and, different adductions (protonated, sodiated and lithiated). These experimental results are discussed in light of collisional cooling, which may predominate inside the ion trap on contrary to CID MS/MS experiments in triple quadrupole instruments.

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P05

Mass spectrometry and photochemical study of photoisomerization and thermal back-isomerization of heteroaryl azobenzene anchored on peptoids for the chemical storage of solar energy

G. Henrard[1),2)], T. Robert[1)], B. Tassignon[1),2)], J. De Winter[1)], G Corni[2)], P. Gerbaux[1)]

1) Organic Synthesis and Mass Spectrometry Laboratory, University of Mons – UMONS, 23 Place du Parc, B-7000 Mons, Belgium
2) Laboratory for Chemistry of Novels Materials, University of Mons – UMONS, 23 Place du Parc, B-7000 Mons, Belgium

Molecules that undergo light-induced isomerization to a metastable isomer can be used to store solar energy. Such systems are known as MOlecular Solar Thermal systems. Exposing compound to sunlight generates a high energy photoisomer whose lifetime is considered as a key-criteria for storage purpose. When energy is needed, the photoisomer is converted back to the stable compound, releasing the excess energy in the form of heat. Azobenzenes (ABs) with their E → Z N=N photoisomerization are among the most widely studied molecular photoswitches, but their properties such as the storage enthalpy, the half-life time of retro isomerization and the photochemical properties need to be improved [1]. At UMONS, two strategies are considered to optimize azobenzene-based chromophores for MOST applications; (i) the replacement of one phenyl group by a thiazolyl moiety is envisaged to red shift the absorption of the chromophores in the visible region, and (ii) the grafting of several azobenzene residues on peptoids appears as an elegant strategy to enhance the storage enthalpy and the metastable isomer half-life time upon cooperating effects. Anchoring AB photoswitches on a peptoid chain is performed using an on-resin step-by-step synthetic procedure allowing to incorporate different side chains at selected positions. Three different residues are incorporated in our peptoids; i.e. methylamine (N*me*), (S)-phenylethylamine (N*spe*) and (E)-4-(thiazol-2-yldiazenyl) aniline (N*azo*). Two peptoids have been successfully synthesized, namely N*spe*N*azo*N*spe* and N*me*N*azo*N*me*, and their sequences are confirmed based on MSMS analysis. LC-MS experiments are carried out before and after irradiation to separate/identify/quantify the stereoisomers. Before irradiation, only the stable E isomers are detected for both structures. After UV-vis irradiation, only the Z-isomers of the N*spe* containing peptoid are detected, pointing to a fast retro isomerization of the Z-isomers of the methyl-containing peptoid. The stabilizing effect of the phenyl ring of the N*spe* residue within the Z-isomers is currently tested by preparing sequence-defined peptoids incorporating the N*azo*, the N*spe* and the N*me* residues at key positions.

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P06
Noncovalent Binding Between Fullerenes and Protonated Porphyrin-tweezer in the Gas-phase Studied by ESI-MS

M. Kinzelmann[1)], B. Scholz[2)], P. Gazetas[2)], M. Ruppel[2)], N. Jux[2)], M. E. Pérez-Ojeda[2)], A. Hirsch[2)], T. Drewello[1)]

1) Physical Chemistry I, Friedrich-Alexander-Universität Erlangen-Nürnberg, Egerlandstr. 3, 91058 Erlangen, Germany
2) Organic Chemistry II, Friedrich-Alexander-Universität Erlangen-Nürnberg, Nikolaus-Fiebiger-Straße 10, 91058 Erlangen, Germany

The complex formation and stability of one flexible and two rigidly linked tetrabenzoporphyrin-tweezers (TBP) with up to four fullerene guest molecules was investigated by electrospray-ionization mass spectrometry (ESI-MS). The doubly protonated TBP-tweezers readily form complexes with C_{60} and C_{70}. Energy-dependent collision-induced dissociation experiments show a successive loss of the neutral fullerenes, indicating noncovalent binding between host and guest molecules.

There are two likely complex geometries: A “tweezer-like” complex were one fullerene is enclosed from both TBP moieties while the other fullerenes bind to the outside of the TBP-tweezer, or an “open” geometry in which the fullerenes bind above and below the TBP units. Experimental results indicate that all [1:1] complexes adopt the “tweezer-like” geometry, while the structure cannot be determined experimentally for the [1:2] and [1:3] complexes. However, for the [1:4] complex, the structure opens up to provide a fourth porphyrin binding site for the fullerenes. In general, the [1:1] complexes are more stable than the larger complexes, the stability decreases with increasing number of fullerenes. The C_{70} complexes are more stable than the corresponding complexes with C_{60} due to the larger interaction area. This study shows that ESI-MS is a powerful tool for the study of noncovalently bound supramolecular complexes.

P07
Gas phase structures of alkali-cationized cereulide responsible for regioselective dissociation by collisional activation, effect of the missed ions

S. Liuu[1)], K. Trinh[1)], E. Darii[2)], C. Cao[3)], A. Damont[3)], J.-C. Tabet[3),4)], Y. Gimbert[4),5)], François Fenaille[3)], Y. Makni[1)], I. Chanthadary[1)], G. Lavison–Bompard[1)], J.-A. Hennekinne[1)], O. Firmesse[1)]

1) Food Safety Laboratory, ANSES, Maisons-Alfort, France
2) Génomique métabolique, CEA Genoscope, Evry, France.
3) LI-MS, CEA Saclay -Gif-sur-Yvette, France
4) IPCM - Sorbonne Université, Paris, France
5) UMR CNRS 5250, Grenoble, France

Emetic toxin is increasingly reported worldwide for its involvement in *Bacillus cereus* food poisoning outbreaks. The preformed toxin in food called cereulide is a heat-stable cyclodepsipeptide, $[^{(D)}$Ala-O$^{(L)}$Val-$^{(L)}$Val-O$^{(D)}$Leu$]_3$ and an ionophore selective to K^+ in solution. In electrospray (ESI), the K^+ selectivity is reduced since the Na^+, $NH4^+$ adduct ions are also detected in competition without adding salt to introduced sample solutions.

Here, by infusing synthetic cereulide into ESI positive ion source, K^+/Na^+ cationized cereulide coexists in two forms: *charge-solvated* (CS) form and *protonated salt* (PS). Various high resolution tandem instruments based on resonant (LTQ/Orbitrap) and non-resonant (Qq/TOF, Qq/Orbitrap) excitations were used. Energy resolved mass spectrometry breakdowns (ERMS, 0 eV to 150 eV) were compared for different involved alkali cation ions to highlight coexisting forms.

The $[M+Na]^+$ and $[M+K]^+$ ions within PS form dissociate through covalent bond cleavages resulting in alkali retention in the **a**, **b** ion series accompanied by *exotic* product ions, detected with tandem based on TOF and Orbitrap analyzers. From $[M+K]^+$, a lot of product ions are lost from the latter analyzer compared to those detected using the former. Indeed, the lost ions are essentially K^+ (for Orbitrap, m/z scale is ≥ m/z 50). This behavior differs to that observed for $[M+Na]^+$, which does not display abundant Na+ using Qq/TOFMS. The direct K^+ loss occurs only from the cationized cereulide within CS form. This is confirmed by the sequential MS^3 experiments on the $[M+K]^+$ and $[M+Na]^+$ product ions that display exclusive 2nd generation product ions with K^+/Na^+ retention. This suggests that the CS form is favored for $[M+K]^+$ and conversely for $[M+Na]^+$. The existence of the missed alkali cation (*vide supra*) is highlighted by the loss of the total ionic current compa-red to that observed during the dissociation of $[M+NH4]^+$. Finally, consideration of the various currents (total ion, product ion, alkali cation) based on the absolute abundances [1] will be discussed as well as the different product ion series.

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P08
Stability and Charge Localization of [1:1] and [2:1] Complex Ions of [n]CPPs and (Li+@)C60/C70

M. Freiberger[1], I. Solymosi[2], S. Frühwald[3], M. E. Pérez-Ojeda[2], A. Hirsch[2], A. Görling[3], T. Drewello[1]

1) Physical Chemistry I, Friedrich-Alexander-Universität Erlangen-Nürnberg, Egerlandstraße 3, 91058 Erlangen, Germany.
2) Organic Chemistry II, Friedrich-Alexander-Universität Erlangen-Nürnberg, Nikolaus-Fiebiger-Straße 10, 91058 Erlangen, Germany.
3) Theoretical Chemistry, Friedrich-Alexander-Universität Erlangen-Nürnberg, Egerlandstraße 3, 91058 Erlangen, Germany.

[n]Cycloparaphenylenes ([n]CPPs) are strained macrocycles, comprising only sp2- hybridized carbon atoms. In recent years, [n]CPPs have become of great research interest in the field of supramolecular chemistry since their special structure enables the formation of novel host-guest complexes. In this work, we investigate the gas-phase chemistry of noncovalent complexes of [n]CPPs with C60, C70 and the endohedral Li+@C60. The [1:1] complexes, [10]CPP⊃C60 and [10]CPP⊃C70, are observed as radical cations and anions, while [10]CPP⊃Li+@C60 is only observed as cationic species. Their stability and charge distribution are studied using energy-resolved collision-induced dissociation (ER-CID).

Considering the complexes with C60 and C70, the ER-CID measurements reveal that complexes with the C70 core exhibit a greater stability and, on the other hand, that the radical cations are more stable than the respective radical anions. [2:1] complexes of the ([10]CPP2⊃C60/70)+•/−• type are observed for the first time as isolated solitary gas-phase species. Here, C60-based [2:1] complexes are less stable than the respective C70 analogues.

Regarding complexes with Li+@C60, our experiments reveal a significant increase in stability for complexes featuring [n]CPPs.

This study contributes to a better understanding of host-guest complexes between [n]CPPs and (endohedral metallo) fullerenes on a molecular level, which is the prerequisite for future applications.

P09
Chemical storage of solar energy: measurement of activation energies by ion mobility and mass spectrometry

T. Robert[1)], B. Tassignon[1,2)], G. Henrard[1,2)], J. De Winter[1)], F. Chirot[3)], P. Dugourd[3)] and P. Gerbaux[1)]

1) Organic Synthesis and Mass Spectrometry laboratory (S²Mos)
2) Laboratory for Chemicals of Novel Materials (CMN) University of Mons, 23 place du parc, B-7000 Mons Belgium
3) Univ Lyon, Université Claude Bernard Lyon 1, CNRS, Institut Lumière Matière, F- 69622, Lyon, France

To meet the challenge of the ever-increasing energy consumption producing greenhouse gases and other atmospheric pollutants, the development of solar energy solutions has been widely investigated over the past decades. Molecular Solar Thermal systems (MOST) are molecules that undergo light-induced isomerization into high-energy metastable isomers which can be used to store solar energy in closed and carbon neutral cycles [1]. Azobenzenes have emerged as promising MOST candidates, but key parameters need to be optimized through different strategies for their versatile synthesis. The half-life time of metastable isomers is directly related to the activation barrier of thermal back-isomerization [1]. The main objective of this work is to develop a method for the rapid determination of these kinetic parameters, and in this regard, mass spectrometry offers a considerable potential. On the one hand, liquid chromatography coupled to mass spectrometry allows to determine the kinetic parameters in solution by monitoring the evolution of the photoisomers distribution over time at different temperatures [2]. On the other hand, ion mobility spectrometry can be used to measure the distribution of photoisomers resulting from collisional activation induced gas-phase back-isomerization in our Synapt G2-Si. To determine the activation energies, we use a method recently proposed by Donor et al. to calibrate the Synapt G2-Si in internal energy [3] using reference values measured in the gas phase by thermal activation in an original tandem ion mobility device at ULyon [4].

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P10
« Phytocuisson » project: Determination of degradation and volatilization products from active substances during thermal processing

F. Dubocq[1),3)], P. L'Yvonnet[2)], G. Vial[2)], X. Sarda[2)], A. Duboisset[2)], S. Mahouche-Chergui[3)], B. Carbonnier[3)], J. Parinet[1)]

1) ANSES, Laboratory for Food Safety, 14 rue Pierre et Marie Curie, 94701 Maisons-Alfort, France
2) ANSES, Direction of Regulated Products Evaluation, 14 rue Pierre et Marie Curie, 94701 Maisons-Alfort, France
3) University Paris Est Creteil, CNRS, ICMPE, UMR 7182, 2 rue Henri Dunant, 94320 Thiais, France

Plant Protection Products (PPP) are used to protect crops from diseases and pests. In the EU regulatory framework of PPP approval, studies investigating the degradation pathway of active substances during process, called hydrolysis studies, are required between 90 °C and 120 °C. However, some other processes reach temperatures above 120 °C and may lead to the formation of new degradation products. "Phytocuisson" project aims at investigating degradation and volatilization products between 90 °C and 240 °C in order to detect some compounds that could be overlooked when processing up to 120 °C.

First, litterature review was conducted. However, because of the lack of information, clear conclusions cannot be drawn about the possible degradation products that are overlooked by the regulatory studies [1]. Then, ten molecules were selected from the list of commercialized substances in Europe according to an *ad-hoc* prioritization approach. Thermal degradation studies were conducted on pure substances between 90 and 240 °C to study thermal and time kinetics. Chemical analysis was performed using liquid hyphenated to high resolution mass spectrometer (LC-HRMS) and data processing was conducted using suspect screening and non-target analysis. Degradation and volatilization of the active substances were confirmed using thermogravimetric analysis as well as differential scanning calorimetry.

Degradation products were detected in seven of the ten molecules. Out of these seven molecules, two had some degradation products reported in their respective regulatory hydrolysis studies. Most of the other substances had their degradation products reported in regulatory reports but in other sections than hydrolysis studies. Moreover, various volatilization products were tentatively identified from studied active substances. Investigating degradation products above 120 °C enabled to tentatively identify compounds that were not reported in regulatory process studies. This demonstrated that conducting tests beyond 120 °C would better characterize the degradation and the volatilization of active substances and improve consumer risk assessment.

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P11

Mass Spectrometry in a step by step following of the CO_2 reduction

H. Elseedy[1)] , E. Louarn[1)], A. Aukauloo[2)], Z. Halime [2)]

1) Institut de Chimie Physique UMR 8000 CNRS, Université Paris Saclay,Bât 350, Centre Universitaire d'Orsay, 91405 Orsay CEDEX;
2) Institut de Chimie Moléculaire et des Matériaux d'Orsay, UMR 8182 CNRS, Université Paris Saclay, 17 rue des sciences, 91405 Orsay CEDEX

Artificial photosynthesis as inspired from nature has been a very hot topic and an urge to overcome fossil fuels over consumption. CO_2 reduction powered by photosensitizers and catalysts like iron porphyrins has been studied to mimic the naturally efficient (CODH) carbon monoxide dehydrogenase enzyme [1]. Due to the lack of the detailed mechanistic approach of binding and activating the CO_2 and the constraint structure of the active site, Scientists suffer from difficulty producing a more efficient catalysts with rewarding turn over frequencies (TOF)and turn over numbers (TON). New findings have shown not only the involvement of Fe^0 oxidation state but also Fe^1 in the activation of CO_2 [2]. We design a new approach using the MS-MS instrument and the high-resolution FT-ICR/MS to provide more insights about the mechanism of the CO_2 reduction process by studying and identifying more intermediates. An emerging funded project of our group that aims at developing a new interface for real-time mass spectrometry that if succeeded will lead us to an In-operando monitoring of the reaction in the liquid phase.

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P12
Structural analysis of cyclic bioactive peptides using *« tricky »* multistage CID experiments

L. Labous[1)], A. Maroto[1)], F. Arioz[1)], C. Enjalbal[2)], S. Dutertre[2)], A. Memboeuf[1)]

1) Univ Brest, CNRS, UMR 6521 CEMCA, F-29200 Brest, France;
2) Univ Montpellier, CNRS, ENSCM, IBMM, Montpellier, France

Bioactive peptides are catching great interests for the last few decades. They find several applications such as preservatives in food chemistry and also as therapeutics. There are actually more than 60 bioactive peptides on the pharmaceutical market and this figure shows a yearly increase. Among these peptides approximately 2/3 are composed of macrocylic structures. This shape indeed usually improves the bioactivity of the peptides, their stability and their pharmacokinetic properties.[1]

If some of those therapeutic peptides are cyclic analogues of linear compounds, some of them are natural cyclic peptides that can be found in various living organisms. In both cases, it is essential to develop robust, efficient and sensitive techniques to determine their structures and/or their quality after synthesis or purification as natural extracts. Depending on the origin of the compounds and, eventually on the synthetic procedure, peptides may have different forms that can lead to complications for the structural analysis.

We have performed multistage mass spectrometric experiments on a quadrupole ion trap to evaluate the potential of CID MS/MS and Energy-Resolved Mass Spectrometry (ER-MS) for extensive structural information. Experiments were conducted on purely synthetic model peptides consisting in either simple macrocycles obtained by intramolecular end-to-end cyclization using click-chemistry [2] or with synthetic analogues of natural venom peptides from cone snails.[3]

We have determined that an increase in the amount of structural information can be obtained: 1) by careful and appropriate tuning of the mass spectrometer CID conditions[4,5], and 2) by using specific methodologies such as the «*Gas-Phase Collisional Purification*»[6-8]. Here we show some preliminary results towards this direction.

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P13
Application of mass spectrometry methods to determine the structure of by-products in peptide synthesis

A. Misicka-Kęsik[1),2)], B. Wilenska[1),2)], J. Dyniewicz[1)], K. Błaziak[1),2)], M. Popławska[1)], K. Witoszka[1)], E. Witkowska[1)]

1) Neuro-Opiomel Ltd, 22 Krasińskiego St., 05-820 Piastów, Poland;
2) Faculty of Chemistry, University of Warsaw, 1 Pasteur St., 02-093 Warsaw, Poland

During peptide synthesis carried out on a polymer support, various by-products are often formed, and most of them are successfully eliminated from the final product, usually by various type of chromatography (flash, HPLC) purification methods. But in some cases, the structure of the by-product is very similar to the main compound, which makes the separation of such two (or more) compounds very troublesome and sometimes impossible. This can be a serious problem in the development process of potential drugs because the regulations of medical agencies (FDA and EMA) on pharmaceutical substances recommend that all impurities present in Active Pharmaceutical Ingredient (API) above 0.1% should be characterized, especially those above 1%.

In the case of peptides, mass spectrometry methods are the best option for characterizing by-product structures, because other methods, such as Edman degradation, are poorly applicable for peptides containing Trp, Asp, His or Met amino acids in the sequence. Tracing the fragmentation pathway by mass spectrometry allows us to confirm or propose the structure of byproducts (impurities).

We will present the results of our studies to determine the structure of a difficult to separate by-product formed during the synthesis of a cyclic peptidomimetic, a potential drug for the treatment of neuropathic pain.

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P14

Characterization of auto-inducing molecules from bacterial *quorum sensing* using Paternò-Büchi reaction and tandem mass spectrometry

C. Gosset-Erard[1)], B. Nay[2)], V. Eparvier[1)], D. Touboul[1),3)]

1) Université Paris-Saclay, CNRS, Institut de Chimie des Substances Naturelles, UPR 2301, 91198 Gif-sur-Yvette, France
2) LSO, ENSTA, CNRS, Ecole Polytechnique, Institut Polytechnique de Paris, Route de Saclay, 91120 Palaiseau, France
3) LCM, CNRS, Ecole Polytechnique, Institut Polytechnique de Paris, Route de Saclay, 91120 Palaiseau, France

The quorum sensing is a chemical language allowing bacteria to interact [1], through the excretion of a set of molecules called auto-inducers. From a threshold population density, and thus a threshold concentration of auto-inducing molecules, their detection by an intracellular receptor regulates bacterial gene expression, including resistance or virulence genes.

Our study focuses on the auto-inducing *N*-acyl-homoserine lactones (AHL) [2] of Gram-negative bacteria from the *Burkholderia* and *Paraburkholderia* genus as some of these bacteria species are described as opportunistic pathogens for humans [2]. The AHL differ in their acyl-chain length (generally from C4 to C18) and may be modified by a 3-oxo or 3-hydroxy substituent, a terminal methyl branch, or various degrees of unsaturations [3]. As the bacterial signal specificity depends on all of those, including the instauration's position on the acyl chain, it is essential to obtain a complete structural charaterization of AHL to better understand the population regulation and virulence phenomenon.

Although several mass spectrometry (MS) methods have been developed to study AHL [4], only NMR analyses requiring the purification of a large amount of product enable the localization of the double bonds on the acyl chain. Thus, this study aims to develop a new method that will significantly reduce the amount of biological material needed. This method is based on LC-MS/MS or/and SFC-MS/MS analyses of extracts derived by a Paternò-Büchi reaction in solution or post-column [5]. In parallel, *in situ* characterization will be performed by the deposition of a photoreactive matrix on the bacterial cultures followed by imaging MS analyses.

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P15
Edition of heparan sulfate by endosulfatases monitored by HILIC chromatography coupled to mass spectrometry

N. El Omrani[1)] and S. Poyer[2)], Y. Takeda-Uchimura[3)], K. Uchimura[3)], R. Vivès[4)], F. Gonnet[1)], R. Daniel[1)]

1) Université Paris-Saclay, CNRS, Univ Evry, LAMBE, UMR 8587, Evry-Courcouronnes, 91025, France.
2) Université Paris-Saclay ,ICSN, CNRS
3) Univ. Lille, CNRS, UMR 8576 - UGSF - Unité de Glycobiologie Structurale et Fonctionnelle, Lille, France.
4) Univ. Grenoble Alpes, CNRS, CEA, IBS, Grenoble, France.

Glycosaminoglycans (GAGs) are anionic polysaccharides of remarkable molecular complexity involved in various biological and physio-pathological processes. The determination of structure-function relationships among these molecules is of great interest; however, the complex structure of GAGs, of which heparan sulfate (HS) is the most challenging representative, and the lack of tools for deciphering complex GAG sequences has restricted advances in the GAGs field. In fact, at the molecular level, HS constitutive disaccharide units can be modified by acetylation, epimerization, and sulfation at multiple positions by highly regulated biosynthetic machinery. These modifications are completed by a post-synthetic editing process involving endosulfatases that finely tune the sulfate code along the HS chain. In humans, HSulf-1 and HSulf-2 are extracellular sulfatases that regioselectively remove the 6-O-sulfate groups from HS. HSulfs action alters HS ligand binding properties and modulates multiple signaling pathways. To gain new insights into the functional properties of HSulf enzymes, we set up a robust and resolving analytical method based on hydrophilic interaction liquid chromatography (HILIC) coupled with mass spectrometry (MS). This method allowed the structural determination of the enzyme products from various sulfated oligosaccharide substrates and the monitoring of the 6-O-sulfate hydrolysis of natural sulfated substrates by HSulf enzymes. HILIC-MS methods are developed in our laboratory to allow the separation of GAG-sulfated oligosaccharides by size and sulfate pattern. A specific methodology was developed to monitor the progress of the enzyme reaction catalyzed by the endosulfatase HSulfs on various heparin (Hp)-derived oligosaccharides and characterize both the structure and the kinetics of the formation of the enzyme products. We followed the desulfation reaction on various heparin-oligosaccharide substrates over time. The reaction conditions of the heparin oligosaccharide substrates with the enzymes were optimized by adjusting buffer parameters to allow monitoring of the reaction by HILIC-MS. We observed the sequential hydrolysis of 6-O-sulfate groups within the sulfated oligosaccharides, confirming that HSulfs act processively while desulfating their substrates. In addition, we compared a variety of HP oligosaccharides to determine the influence of the sulfated motifs on the HSulf enzyme activity, taking us a step further in deciphering the catalytic mechanism of these specific editing enzymes.

P16
Characterization of chemical modifications of RNAs combining MS(/MS) approaches

V. Guérineau[1)], L. Tay[2)], C. Wang[3)], N. Ulryck[3)], M. Graille[3)], D. Touboul[1),2)]

1) Université Paris-Saclay, CNRS, Institut de Chimie des Substances Naturelles, UPR 2301, 91198, Gif-sur-Yvette, France
2) LCM, CNRS, Ecole polytechnique, Institut Polytechnique de Paris, Route de Saclay, 91120 Palaiseau, France
3) CNRS; Laboratoire de Biologie Structurale de la Cellule (BIOC), UMR 7654, Ecole polytechnique, Institut Polytechnique de Paris, Route de Saclay, 91128 Palaiseau, France

RNA modifications were previously considered as molecular decorations only ornamenting abundant and non-coding RNAs and offering an amazing variety of chemical structures (more than 150 different modified nucleosides). They have now jumped under the spotlights of both academic and applied research with the recent development and constant improvements of highly sensitive methods (next-generation sequencing or NGS, mass spectrometry or MS, etc.) as well as genome-wide association studies on patients that have identified mutations in RNA modification enzymes (writers). These led to the emergence of a new "-omics" area, called epitranscriptomics, which aims at decoding the fundamental principles controlling the RNA modification landscapes beyond the combination of four canonical nucleotides. In order to identify, quantify and position modifications on RNA, two methods have been implemented in the MS group at ICSN based on MALDI-MS/MS and LC-HRMS. Their complementarity will be exemplified with a study on tRNA modification enzyme Trm11 either from human or from the A. fulgidus archaeon.

After enzymatic digestion, small tRNA fragments were analyzed by MALDI-MS/MS. This method allows the sequencing of the small tRNA and thus the identification and location of the modification on the known sequence. LC-ESI-HRMS approach allows the quantification of guanosine (G), N2-methylguanosine (m^2G) and N2,N2-dimethylguanosine ($m^{2,2}G$) with limits of detection at 50 fmol injected. This led to the determination of the rate of methylation of the tRNA.

The implemented techniques showed a large complimentarity to identify, quantify and map modifications on RNA thus expanded knowledge in epitranscriptomics. Decreasing sensitivity and improving the number of chemical modifications remains a challenge for analytical chemistry.

P17
Effects of protein glycosylation on the stability of different β-galactosidase enzymes under heat and pH stress

M. Király[1)], B. Dalmadi Kiss[1)], L. Drahos[2)], I. Antal[1)], Á. Kiss[1)], Krisztina Ludányi[1)]

1) Department of Pharmaceutics, Faculty of Pharmacy, Semmelweis University, Hőgyes Endre u. 7., 1092, Budapest, Hungary
2) MS Proteomics Research Group, Research Centre for Natural Sciences, Magyar Tudósok körútja 2., 1117, Budapest, Hungary

Biological pharmaceuticals have been offering a novel trend in the pharmaceutical industry regarding the research of protein based biological medicines since the past decade. Biologically active proteins and biopharmaceuticals carry a variety of modifications, some of which, including glycosylation, are essential to their function and specificity.

Lactase (β-galactosidase) is an enzyme responsible for breaking down lactose, a disaccharide of milk. The lack or shortage of this digestive enzyme in the human small intestine results in the inability of the human organism to assimilate milk. The prevalence of the lactose intolerance is more than 60% amongst the human population.

The resistance of lactase enzymes extracted from different organisms can vary in a wide range. The most common sources used by the the food and pharmaceutical industry are filamentous fungus (A. oryzae) and yeast (K. lactis). The enzyme extracted from the fungus shows higher activity in acidic medium, and its thermal stability is better. On the contrary, the other enzyme can catalyze properly only at neutral pH and lower temperatures. The structural differences behind this phenomenon have not been explored yet, but it is very likely that the glycan structures play an important role in the stabilization and effectivity of the protein.

Our previous investigations [1] suggested reversible and irreversible changes as well, due to submitting the solid enzyme to heat stress testing. These changes were related to the alterations in the glycan structure of the protein. Deriving from this, the glycosylation of enzymes from different biological sources can affect their resistance to external conditions.

The goal of the research project is to reveal stability indicative changes in the glycan structure of different lactase enzymes under heat and pH stressed conditions.

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P18
Screening of Proanthocyanidins in commercial enological tannins by HPLC-HRMS

F. Martinelli, T. Nardin, R. Larcher

Fondazione Edmund Mach, Via Edmund Mach 1, 38098 San Michele all'Adige (TN), Italy

Proanthocyanidins (PA) are the second most abundant natural phenolics after lignin, and occur ubiquitously in plants. The name proanthocyanidins derives from the fact that when undergoing an oxidative depolymerization reaction in acidic medium this compounds result in colored anthocyanidins. [1]. Chemically PA are defined as oligomers and polymers whose building blocks are flavan-3-ols, such as catechin and epicatechin [2].

PA are better known as condensed tannins, in contraposition to hydrolyzable tannins which are polymers of gallic acid or ellagic acid.

PA can be naturally found in red wines, while hydrolyzable tannins can also be present in wines, mostly originating from the oak barrels used as containers during the ageing process. However enological tannins (ET) can also be directly added to the wines (both red and whites) to take advantage of their properties: to ensure palate balance and complexity, to stabilize the color in red wines, to precipitate the proteins in white wines, to protect from the oxidation process. Enological tannins are usually natural substances extracted from plants of different species [3].

The content of different PA classes in enological tannins and their degree of polymerization (number of monomers) have, to our knowledge, never been investigated.

In this work we carried on the characterization of PA profile of 50 commercial enological tannins of different origins (grape, grape seeds, grape skin, common oak, sessile oak, quebracho, chestnut, gall nuts) using UHPLC coupled with HRMS.

Chromatographic separation was achieved with a Thermo Scientific Ultimate 3000 UHPLC system equipped with Thermo Scientific ACCLAIM vanquish PA 2 column and a gradient of formic acid 100 mM and 20 mM ammonium formiate, acetonitrile and water. All the chromatograms were recorded in profile mode through a full MS-data dependent MS/MS experiment employing a Q-Exactive™ hybrid quadrupole-orbitrap mass spectrometer (HQOMS) equipped with heated electrospray ionization (HESI-II) interface. Targeted and untargeted studies were performed using 6 analytical standards and a homemade database.

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P19
Observation and characterization of Chlorophylls and Pheophytins anions and cations by mass spectrometry

A. Muhieddine[1)], M. Diop[1)], J. Attard[2)], M. el-Hayek[2)], S. Soorkia[1)], P. Carbonnière[3)], A. de la Lande[2)], B. Soep[1)], N. Shafizadeh[1)]

1) Institut des Sciences Moléculaires d'Orsay (ISMO) France
2) Institut de Chimie-Physique d' Orsay (ICP) France
3) Institut des sciences analytiques et de physico-chimie pour l'environnement et les materiaux (IPREM) Pau-France

Chlorophylls and Pheophytins are two essential pigments for the process of photosynthesis. It is well known that the Chlorophyll is a very efficient antenna for absorbing sunlight. The absorbed energy is then transferred to a set of four Chlorophylls and two Pheophytins within the reaction system 2 called PSII. PSII is the site of a charge separation and ultimately leads to the dissociation of water.

To understand the electron donor and acceptor properties of these compounds, we studied Chlorophyll and Pheophytin in positive and negative modes by mass spectrometry. In this poster we will discuss the stability of the different charge sites in these compounds that can accept or donate an electron, by comparing the calculated structures with experimental results.

In a second part, of this poster, we will present our recent results on the observation of the photoelectron spectrum of the Pheophytin anion which provides information on the vibrational structure of neutral Pheophytin.

P20
Determination of α-pinene oxidation products by liquid chromatography – mass spectrometry study

A. Błaziak, R.W. Szmigielski

Institute of Physical Chemistry, Polish Academy of Sciences

The atmospheric aqueous phase has been recognized in recent years as an important reaction media for the processing of water-soluble organic compounds. A large amount of water dispersed in the atmosphere in various forms, including fog, rain, clouds, and wet aerosol, makes it an important medium in which the volatile precursors are degraded and consequently – form aqueous secondary organic aerosols (aqSOA). The currently poorly characterized monoterpenes aqueous-phase processes are becoming an emerging topic of interest in the field of atmospheric chemistry.[1] The aqueous phase oxidation by different oxidizing species (like OH radical, NO_3 radical, ozone) can be an important removal mechanism of water-soluble α-pinene oxidation products. Important components of secondary organic aerosols are α-pinene oxidation products, which are formed through the different oxidizing reactions of α-pinene. These are a number of compounds having in the structure a functional group as carboxylic acid, carbonyl, aldehyde etc..[2]

Considering that the atmospheric aqueous phase plays an important role in our atmosphere. There is a need to develop a simple and efficient method for quantifying components of the atmospheric aqueous phase. The one challenge is a low concentration of analytes, therefore mass spectrometry seems to be the best technique for such challenging analysis. Therefore, in this study, we optimized and validated the method to quantify a number of α-pinene oxidation products. Our method revealed the high sensitivity and low values of LOQ, which is crucial in the analysis of samples of atmospheric origin. The optimized LC-MS method was used to analyze a number of filters and rain samples collected during the summer campaign in Diabla Góra (Poland).

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P21
Automation of resolution-enhanced Kendrick mass defect plots to characterize phospholipids by CID MS/MS

N. Elie[1)], D. Touboul[1),2)], S. Poyer[1)]

1) Université Paris-Saclay, CNRS, Institut de Chimie des Substances Naturelles, UPR 2301, 91198, Gif-sur-Yvette, France
2) LCM, CNRS, Ecole polytechnique, Institut Polytechnique de Paris, Route de Saclay, 91120 Palaiseau, France

Glycerophospholipids (GPLs) are among the most studied lipids since they composed at 80% the lipidic membrane of mammalian cells. Nonetheless their fine structural characterization is still not completely achieved. Indeed, while fragmentations obtained by collision-induced dissociation (CID) lead to the characterization of polar headgroup from protonated PC-species and aliphatic chain length from deprotonated PC-species for instance, a lack of structural information is still observed to locate double bonds (DBs) positions. We showed that DB location of GPLs such as phosphatidylcholines (PC) or phosphatidylethanolamines (PE) could be obtained by CID MS/MS from copper quaternary complexes, using bipyridine (BPY) and an anion. The formed quaternary complexes yield diagnostic fragment ions providing unambiguous location of the DB but at low intensity.

In order to highlight these DB diagnostic fragment ions, we used resolution-enhanced-KMD (RE-KMD) plots [1,2]. In fact, the fragment ion series of interest are separated by CH_2 units or C_2H_2 when an unsaturation is present. As a result, KMD plots will be specific and a divisor of 5 allowed to easily highlight C=C location from MS/MS spectra of copper-adducted PC. In this work, we also developed a program able to read GPL MS/MS spectra, in mzML format, and describe the nature of the GPL from diagnostic fragment ions, but also to locate and count its unsaturations, by following successive fragmentations along the aliphatic chains thanks to both the exact mass and the RE-KMD values. The program [3], written in Python, using freely available libraries such as pandas, numpy and pymzml, can identify, in a few seconds, the nature of the GPL, the length and DB locations of the aliphatic chains composing the GPL, leaving only the question of sn-1 and sn-2 positional isomers unanswered.

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P22
Towards an instrument-independent databank of product ion spectra recorded in non-resonant mode

A. Hueber[1),2)], A. Damont[3)], M. Green[4)], H. Kulyk[1)], E. Dariy[5)], E. Nicol[6)], S. Liuu[2)], D. Lesage[7)], Y. Gimbert[7),8)], G. Lavison–Bompard[2)], O. Firmesse[2)], Y. Nia[2)], J.-A. Hennekinne[2)], F. Fenaille[3)], C. Junot[3)], A. Perret[5)], J. Bertrand-Michel[1)], N. Cenac[8)], J.-C. Tabet[3),7)]

1) MetaToul (Toulouse Metabolomics & Fluxomics facilities) Toulouse, France
2) Food Safety Laboratory, ANSES, Maisons-Alfort, France
3) LI-MS, CEA Saclay -Gif-sur-Yvette, France
4) Waters Corporation, Wilmslow, United Kingdom
5) Génomique métabolique, CEA Genoscope, Evry, France
6) LCM, CNRS – IP Paris, Ecole polytechnique, Palaiseau, 91128, France
7) IPCM - Sorbonne Université, Paris, France
8) Departement de Chimie, UMR CNRS 5250, Grenoble, France
9) IRSD, Université de Toulouse, INSERM, INRA, INPENVT, Toulouse, France

For many years, various attempts have been made to generate reproducible product ion spectra under low energy collision conditions. In fact, in addition to the dependence on the dissociation process characteristics of ionized molecules (activation energy(ies), transition state properties), obtaining an interoperable spectral database can also be instrument-dependent. Therefore, it is required to harmonize the acquisition procedures with an inter-instrument calibration scale (i.e., choice of collision energy according to the instrument). These act on the dissociation rate constants (the internal energy E of the precursor ions after collision and the process kinetic shifts). Would solving such difficulties be a chimera?

Recently, a study on the deprotonated lipo-glutamate fragmentations showed, *via* an ion-dipole intermediate, the unexpected regeneration of the fatty acid carboxylate and its complementary ion. The product ion profiles constituting the ERMS of these deprotonated molecules showed reproducible “camel” and/or “dromedary” shapes [1]. These curious profiles have been shown reproducible independently on the used instruments as QTOFs from different companies and different tandems based on FT (orbitrap and ICR in different labs). Consequently, we have tried to find, through these profiles, a way to answer the question of reproducibility by removing instrumental effects. By comparing the product ion spectra under particular energies linked to "singular" point(s) of these profiles, it appears spectacularly a very strong analogy of the fingerprints characterizing these collision spectra recorded from about ten instruments. We will discuss these singular points which allow us to removing the instrumental effects following spectral recalibration (internal energy and kinetic displacement). A simulation using the MassKinetic program should confirm the relevance of our approach.

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P23
Statistical approach for HR-MS analysis of contaminants of emerging concern (CECs) in complex mixtures

E. Nicol[1)], Y. Xu[2)], S. Bourcier[1)], S. Bouchonnet[1)], and M. Lavielle[2)]

1) Laboratoire de Chimie Moléculaire, CNRS – IP Paris, Ecole polytechnique, Route de Saclay, Palaiseau, 91128, France;
2) Centre de Mathématiques Appliquées, CNRS – IP Paris, Ecole polytechnique, Route de Saclay, Palaiseau, 91128, France.

The impact on health and environment in terms of toxicity and ecotoxicity of contaminants of emerging concern (CECs) is one of the major issues of our time. Most of the degradation products of CECs remain unknown, studies about photodegradation of several CECs have already been carried out, often showing an increased toxicity of the irradiated samples. Photoreactions between the CEC and matrix have started to be considered and this work follows on this pathway.

This study focus on UV-visible irradiation of a neonicotinoid insecticide, acetamiprid, in commercial formulations (Polysect and Roseclear). A significant matrix effect was observed, highlighting the need to complement modelling studies with real-life studies. All these investigations were carried out with LC-HR-MS/MS, to obtain precise identification of the structures and to propose photochemical and fragmentation mechanisms. The results of in-silico and in-vitro toxicity studies showed that most of the photoproducts have a toxicity similar or superior to the parent compound.

However, it is not an option to systematically apply a model-based approach to the comprehensive study of each emerging pollutant of interest. A faster approach, such as screening, should be considered. UV-Photolysis of acetamiprid at 40 µg/l in water with 20 mg/l of fulvic acid to model environmental conditions was done. Direct infusion ESI-HRMS was performed without preconcentration. An in-house developed freeware (SPIX) was used to follow statistically relevant changes in complex mixtures and to model the kinetics of appearance of the transformation products.[1] SPIX software showed all the changes, photoproducts and parent compounds. This is a fast and reliable way to obtain the peaks which were relevant during the reaction.

These two approaches, laboratory modelling and statistical approach, are described and compared for the identification of CEC degradation products.

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P24
Influence of Search Engines on Results of Fast Photochemical Oxidation of Proteins Analysis

M. Zákopčaník[1),2)], D. S. Loginov[2)], P. Novak[1)]

1) Department of Biochemistry, Faculty of Science, Charles University, Hlavova 8, Prague, Czech Republic;
2) Institute of Microbiology of the CAS, Videnska 1083, Prague, Czech Republic

The study of protein folding and interactions are integral parts of the structural biology. One of the methods that proved capable of providing valuable data is fast photochemical oxidation of proteins (FPOP). This method of structural mass spectrometry uses covalent labeling of amino acid side chains by hydroxyl radicals. These radicals are usually generated directly in solution by KrF laser photolysis of H_2O_2, which allows to probe protein surface exposed to the solvent. The ability to detect modifications of up to 14 amino acid residues by employing bottom-up proteomics brings high spatial resolution and allows an investigation of protein structure and dynamics [1].

The achievement of these benefits is conditioned by a reliable identification of the protein modifications and, more importantly, their positions within the peptides. In this study we focused on describing and validating the modifications identified by MASCOT and PEAKS, which differ in their search algorithms. FPOP data analyses of hemoglobin-haptoglobin complex (HbHp) were downloaded from ProteomeXchange [2] (data set identifier PXD020509).

For each condition (Hb, Hp, HbHp), the modifications identified by MASCOT and PEAKS have been compared, with a focus on modifications identified by both search engines. Furthermore, using selected parameters (the search engines PSM score, position determination probability, and signal intensity), a principal component analysis (PCA) has been carried out. Empowering PCA to visualize and further evaluate the identifications has shown distribution of identified modifications into several clusters, which can be used to assess the reliability of the identifications.

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P25
Isotopic Ratio Mass Spectroscopy (EA-LC/IRMS) in conjunction with High Resolution Mass Spectroscopy (LC-HRMS) applied to honey authenticity discovering

G. Quaglia, A. Quaglia

Floramo Lifeanalyics Srl, Via Lime, 4 12047 Rocca de'Baldi (CN) Italy

Honey is high value healthy natural food globally consumed, due this high value is one of the most worldwide adulterated healthy natural food of animal origin.

Honey, due this high interest, is worldwide regulated: at international level by Codex Alimentarius since 1981 [1], in the European Union since 2001 by Council Directive 2001/110/EC of 20 December 2001 relating to honey.

Due this strictly regulation, no extraneous sugar or any other ingredient is admitted at any level of concentration, it is of utmost importance to develop sensitivity and specific, analytical methods to solve this big topic.

The hyphenated analytical techniques of Isotopic Ratio Mass Spectroscopy (IRMS) coupled with elemental analyzer [3] and liquid chromatography (EA/LC-IRMS) [4,5], in conjunction with High Resolution Mass Spectroscopy (LC-HRMS) play an important role to solve the honey purity.

The identification of adulteration by sugars addition can be based on studies the differences between 13C/12C stable carbon isotope ratios of honey protein and sugar composition by EA-LC/IRMS [4,5] evaluated with the identification of sugar syrup markers by LC-HRMS [6] like Oligosaccharides extraneous Dp 9-12, Difructose anhydride (DFA), 2-acetylfuran-3-glucopyranoside (AFGP), indicating the exogenous sugar syrup presence or contamination.

With these emerging powerful LC-HRMS and EA-LC/IRMS techniques applied on honey authenticity discovering, you will get reliable results with reasonable low LOD/LOQ to detect C3/C4 origin exogenous sugar syrup type, to solve honey authenticity definition.

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P26
Multimodal mass spectrometry analysis of organic compounds in old paintings

G. Rosé[1),2)], M. Sablier[2)], A. Brunelle[1)]

1) Sorbonne Université, CNRS, Laboratoire d'Archéologie Moléculaire et Structurale (LAMS UMR8220), Paris
2) Muséum National d'Histoire Naturelle, CNRS, Centre de Recherche sur la Conservation (CRC UAR3224), Paris

In cultural heritage, and particularly with old paintings, organic materials (binders) may have degraded and interacted with inorganic materials (pigments), thus requiring scientific analyses to understand these changes over centuries. Among them, varnishes, which are based on dissolved resins, are applied to the surface of the paintings in order to protect it and/or provide an artistic effect. These have been used in pictorial layers, in order to adjust the pigments' colour [1].

A chemical mapping at the surface of cross-sections of painting samples can be carried out with a Time-of-Flight Secondary Ion Mass Spectrometer (TOF-SIMS). The use of this instrument in heritage has already been demonstrated on multiple matrices, since it is the only one enabling molecular analyses of both the organic and inorganic contents at a submicrometric scale [2]. The secondary ions analysed are generated by the impact of keV-energy primary ions, which are known to generate multiple fragmentations, even when using cluster projectiles.

Pyrolysis comprehensive two-dimensional Gas Chromatography / Mass Spectrometry (Py-GC×GC/MS) is a separative method which offers a complete analysis of a sample, but without localization [3]. The two methods are thus used in parallel in a multimodal analysis, to provide a complete characterisation of the composition and together with the localisation of resins in pictorial works.

The most commonly used resins (colophony, copal, sandarac, turpentine, dammar and mastic…) were analysed with both methods, with several references from multiple geographical origins, in order to have a solid statistical basis. Py-GC×GC/MS helped find specific markers that can be used to enlighten the processing of the TOF-SIMS data. For example, GC technique allowed the identification specific fragments, allowing differentiation of turpentine and its distillation product, colophony. Finally, optimised reconstructed varnish recipes were analysed by TOF-SIMS, to justify the capacity of the new parallel workflow to discriminate different components in complex mixtures. The GC-MS data allowed the differentiation of complex mixtures analysed by TOF-SIMS, which was also validated by a principal component analysis.

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P27
Mass spectrometry for the characterization of environmental macroscopic fungi

J. Segret[1)], D. Touboul[1),2)], V. Eparvier[1)]

1) Université Paris-Saclay, CNRS, Institut de Chimie des Substances Naturelles, UPR 2301, 91198, Gif-sur-Yvette, France
2) LCM, CNRS, Ecole polytechnique, Institut Polytechnique de Paris, Route de Saclay, 91120 Palaiseau, France

Fungi belong to one of the most diverse kingdoms of life, yet it is estimated that hundreds of thousands of species, remain to be discovered and thus chemically studied [1]. More specifically, knowledge of their macroscopic forms is disparate and few studies have focused on their metabolome [2]. In particular, the metabolites produced by macromycetes from French Guiana, an area of high biodiversity, remains virtually unexplored.

To access this chemical diversity, we developed three mass spectrometry (MS) methods to acquire chemical profiles of volatile and non-volatile compounds of mushrooms collected in French Guiana. These profiles were then compared to determine if chemical diversity could be a reflection of phylogenetic classification.

The first approach is based on prior work developed at ICSN for microorganism biotyping by comparing lipid and protein fingerprints obtained by MALDI-ToF-MS [3] using MetGem [4]. While most samples showed no correlation in relation to their species, genera or orders, encouraging correlations were observed to the specie level in the Xylariale order.

The second approach relates to Volatile Organic Compounds (VOCs). A Headspace-Solid Phase Micro Extraction-GC-MS (HS-SPME-GC-MS) method was developed to generate VOC profiles on fresh mushrooms. Statistical analysis of the data on our limited set of 38 specimens showed species correlation but no systematic match for similar genera or order. Lastly, to give access to non-volatile compounds, ethyl acetate extracts were analyzed by RPLC-HRMS/MS. Three different ionization techniques were optimized in negative and positive modes. Both VOCs and non-VOCs data were then used to generate molecular networks to *in silico* annotate the metabolites produced by the fungi.

This work aims to propose a classification of fungi samples based on the chemical analysis of varied metabolites by MALDI-ToF-MS, HS-SPME-GC-MS or RPLC-HRMS/MS.

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P28

Direct introduction-ion mobility - mass spectrometry for fast isomer quantification: Application to the determination of 2'-fucosyllactose (2'FL) in human milk

S. Alves[1)], S. Abdoun[1),2)], K. Adel-Patient[2)], E. Rathahao-Paris[1),2)]

1) Sorbonne Université, Institut Parisien de Chimie Moléculaire, 75005 Paris, France;
2) Université Paris-Saclay, CEA, INRAE, Département Médicaments et Technologies pour la Santé, LIAA, 91191 Gif-sur-Yvette, France

The quantification of isomers in biological matrices represents a real analytical challenge. Despite enhanced mass resolving power able to resolve isobaric ions, high-resolution mass spectrometry (HRMS) cannot resolve structural isomers without a preliminary adequate separation step. Furthermore their accurate quantification in a biological matrix is also complicated by matrix or ion suppression effects, their low concentrations, and the lack of a true blank matrix to prepare calibration solutions. Then, mass spectrometry coupling with a separation technique such as liquid chromatography (LC) or gas chromatography (GC) is often used to separate and identify organic compounds.

Unlike conventional chromatographic technique, ion mobility (IM) separation is based on gas-phase ion mobility rather than polarity and is not limited by solvent or stationary-phase constraints. Hence, its coupling with mass spectrometry (IM–MS) offers an additional separation dimension without lengthening the MS acquisition time. In addition its potential in characterizing isomers that are not easily separated by LC or GC techniques has been demonstrated in various analysis fields such as pharmaceuticals, for lipids, and metabolites.

In this work, we demonstrated the possibility of quantifying isomeric compounds by direct introduction combined with IM-MS. Two quantitative approaches, standard addition method (using a matrix spiked with different concentrations of analyte) and external standard method were compared in the analysis of human milk. Despite the presence of various isomers of oligosaccharides in breast milk, the determination of 2'-fucosyllactose (2'FL) could be achieved thanks to the single ion mobility monitoring (SIM^2) mode. SIM^2 allowing to increase the IM resolving power, improving thus isomer separation and highly selective isomer detection.

P29

Rapid identification and quantification of xenobiotic exposure markers in an epidemiological study using direct introduction High-Resolution Mass Spectrometry

Estelle Rathahao-Paris[1,2)], Baninia Habchi[2)], Sylvie Streel[3)], Michèle Guillaume[3)], Anne-Françoise Donneau[3)], Brice Appenzeller[4)], Douglas N. Rutledge[5)], Alain Paris[5)], Sandra Alves[2)]

1) Université Paris-Saclay, CEA, INRAE, Département Médicaments et Technologies pour la Santé, 91191 Gif-sur-Yvette, France;
2) Sorbonne Université, Institut Parisien de Chimie Moléculaire, 75005 Paris, France;
3) Department of Public Health, University of Liège, 4000 Liège, Belgium;
4) Human Biomonitoring Research Unit, Luxembourg Institute of Health, 4354, Esch-sur- Alzette, Luxembourg;
5) Muséum national d'Histoire naturelle, MCAM, UMR7245 CNRS, 75005 Paris, France;

Cohort studies aim to establish a possible relationship between the occurrence of certain diseases and external risk factors such as exposure to chemicals. Analytical developments in metabolomics offer very attractive opportunities for large-scale phenotyping, in particular, for the detection of a large number of molecules present in biological matrices, including xenobiotics and their metabolites. Methods involving a chromatographic system (e.g., liquid or gas chromatography, LC or GC) hyphenated with mass spectrometry are commonly used. However, they are less suitable for performing large-scale analyses in epidemiological studies due to its throughput limited by analysis time. Direct Introduction coupled with Mass Spectrometry (DIMS) allows a striking reduction in analysis time and the use of High-Resolution Mass Spectrometry (HRMS) offers the possibility of discriminating isobaric compounds, thus increasing the number of detectable ions. In this study, a cohort phenotyping of more than 500 individuals was performed in less than 74 hours thanks to direct analysis using an FT-ICR instrument. Markers of xenobiotic exposure were detected using unsupervised Principal Component Analysis (PCA). The detection of their fine structures at high resolution (Rp > 900,000) supported the assignment of their identity. Their identification as dimethylbiguanide and sotalol was achieved at level 1 using commercially available standards and tandem mass spectrometry. These results were then reinforced by the cohort metadata providing information on the intake of these two drugs, dimethylbiguanide and sotalol, prescribed for type-II diabetes and cardiac arrhythmia, respectively. Regression analysis showed that their concentrations estimated from the calibration curve of standard solutions, in the discriminated subjects correlated well with the PCA score plots. The detection of these xenobiotic exposure markers proved the potential of our high-throughput approach without a priori as an emerging tool for rapid large- scale phenotyping in epidemiological studies.

P30
Rapid chiral characterization of amino acids using non-covalent Cu^{II} complexes and TIMS-ToF separation

C. Chalet[1)], E. Rathahao-Paris[1),2)], S. Alves[1)]

1) Institut Parisien de Chimie Moléculaire, Sorbonne Université, 75005 Paris, France
2) Université Paris-Saclay, CEA, INRAE, Département Médicaments et Technologies pour la Santé, LIAA, 91191 Gif-sur-Yvette, France

Chiral molecules constitute a major challenge in life sciences, since enantiomers may possess different biological activity and/or toxicity while having similar physical and chemical properties. Chiral analysis using mass spectrometry (MS) provides sensitive and specific detection and relies mostly on derivatization or formation of non-covalent complexes in the gas phase. For the latter, various metal or alkali cations and chiral selectors such as cyclodextrins or carbohydrates have been successfully used in many studies [1,2].

Recently, ion-mobility spectrometry (IMS) has emerged as a promising tool for the rapid analysis of isomeric compounds by being able to separate ions based on their *m/z* ratio and their collision cross section (CCS). In particular, a major advantage of the TIMS-ToF instrument used in this work is the ability to increase its resolving power and thus improve the ion-mobility separation [3].

Here, we developed a fast-screening method for 19 D- and L-amino acids (AAs) using non-covalent Cu^{II} complexes with phenylalanine (Phe) and proline (Pro) as chiral references. Chiral analyses were performed on the TIMS-ToF instrument using single ion mobility monitoring (SIM^2) mode providing high mobility resolving power. Each enantiomeric pair of AA could be discriminated with at least one combination of references and analyte or through self-association. This developed method will also be applied to chiral analysis in complex mixtures.

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P31
Does *in-vitro* fertilization change the lipid metabolism in early embryogenesis?

S. Gitta, É. Szabó, L. Márk

Department of Analytical Biochemistry, Institute of Biochemistry and Medical Chemistry, Medical School, University of Pécs, 7624-Pécs, Szigeti str. 12., Hungary

Although the assisted reproduction technologies (ART) are improving, the pregnancy per aspiration and live birth per aspiration is still low. Lipids have very diverse and important functions in cells, including lipid signaling. It is a well-known fact, that the lipid metabolism and signaling of early stage pregnancy are of a vital importance in successful embryogenesis. Our aim was to investigate the effects of IVF on the lipids of both maternal and embryonal tissues with mass spectrometry imaging during early pregnancy.

We investigated CD-1 mouse uteri after normal and *in-vitro* fertilization (IVF) at 6.5, 8.5 and 10.5 days of pregnancy. After collecting cryosections, histology (with hematoxylin-eosin) and matrix-assisted laser desorption ionization time-of-flight imaging mass spectrometry (MALDI TOF MSI) were used for detection of lipids. The MALDI measurements were performed in positive linear mode, so we mainly could find phosphatidylcholines. The lipids were accurately identified by liquid and gas chromatography coupled ultra-high-resolution tandem MS.

On day 6 of normal pregnancy we found an increase for phosphatidylcholine (PC) 34:0 (16:0/18:0) PC 34:1 (16:0/18:1) and PC 34:2(16:0/18:2) showed higher expressions in uterine stromal cells at implantation sites, while PC 36:1 (18:0/18:1) and PC 36:2 (18:0/18:2) in the PDZ. In contrast, in transferred animals the PCs showed an increase in glandular epithel at interimplantation sites. On day 8 of normal pregnancy PC 32:0 (16:0/16:0), PC 34:0 (16:0/18:0) and PC 34:1 (16:0/18:1) showed higher levels in the mesometrial pole (M-pole). In contrast, in transferred uterus samples PC 36:2 (18:0/18:2) and PC 40:6 (18:0/22:6) showed higher expression in both M-pole and AM-pole. On day 10 of normal pregnancy PC 32:0 (16:0/16:0) showed higher intensity in the placenta and PC 34:1 (16:0/18:1), PC 34:2 (16:0/18:2), PC 36:2 (18:0/18:2) in the mesometrial decidua. In transferred uterus samples higher expression of PC 34:0 (16:0/18:0), PC 34:1 (16:0/18:1), PC 34:2 (16:0/18:2), PC 36:1 (18:0/18:1) and PC 36:2 (18:0/18:2) in mesometrial decidua was seen, whereas PC 36:4 (16:0/20:4) and PC 38:4 (18:0/20:4) showed increased expression in the AM and lateral decidua.

P32
Single-cell lipidomics - mammalian oocytes analysis by MALDI mass spectrometry imaging

A. Bodzon-Kulakowska[1)], W. Młodawska[2)], P. Mielczarek[3)], D. Lachowicz[4)], P. Suder[1)], M. Smoluch[1)]

1) Department of Analytical Chemistry and Biochemistry, Faculty of Materials Science and Ceramics, AGH University of Science and Technology, A. Mickiewicza 30, 30-059 Krakow, Poland
2) Department of Animal Reproduction, Anatomy and Genomics, University of Agriculture in Krakow, Al. Mickiewicza 24/28, 30-059 Kraków, Poland
3) Laboratory of Proteomics and Mass Spectrometry, Maj Institute of Pharmacology, Polish Academy of Sciences, Smetna 12, 31-343 Krakow, Poland
4) Academic Centre for Materials and Nanotechnology, AGH University of Science and Technology, A. Mickiewicza 30, 30-059 Krakow, Poland

Oocytes are an exceptional kind of biological material. Here the individual variability of a single cell is significant. Even oocytes derived from the same ovary may differ in the amount of certain unsaturated and saturated fatty acids, which may influence/affect their quality and developmental competence in vitro. Therefore, the possibility of analyzing the lipid content from a single cell is crucial for elucidation of the biological process staying behind these reproductive cells' fate. Here, we present the method for lipid analysis for a single cell based on the MALDI-based mass spectrometry imaging (MSI) approach with wet interface matrix deposition. This technique allows for unusual insight into the cell membranes and intracytoplasmic molecular composition, allowing for the prediction of viability and efficiency of the fertilization process. Technical considerations of the analysis process, such as number of matrix layers and the position of the spraying nozzle during matrix deposition process, are presented in the article. Especially our findings considering the analysis of paraformaldehyde-fixed versus nonfixed cells seem to be interesting. We hope that our approach will be helpful for those working on lipid analyses in such extraordinary material as a single oocyte. Our study may also offer some clues for anybody interested in the single cell analysis with the aid of MALDI mass spectrometry imaging and wet-interface matrix deposition method.

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P33
Untargeted Lipidomics: Development of a SWATH Strategy Using Supercritical Fluid Chromatography Coupled to High Resolution Mass Spectrometry

M. Valmori[1),2)], *B. Colsch*[2)], *F. Fenaille*[2)], *D. Touboul*[1),3)]

1) Université Paris-Saclay, CNRS, Institut de Chimie Des Substances Naturelles, UPR 2301, 91198, Gif-Sur-Yvette, France;
2) Université Paris-Saclay, CEA, INRAE, Département Médicaments et Technologies pour La Santé (DMTS), MetaboHUB, F-91191, Gif-sur-Yvette, France;
3) Laboratoire de Chimie Moléculaire (LCM), CNRS UMR 9168, École Polytechnique, Institut Polytechnique de Paris, 91128, Palaiseau, France

Over the past two decades, lipidomics has seen significant advancements thanks to the development and application of separation techniques coupled to High Resolution Mass Spectrometry (HRMS). One particularly effective technique for lipidomic analysis is supercritical fluid chromatography (SFC), which is appreciated for its ability to separate the wide range of lipid classes and its short acquisition time [1].

Targeted and untargeted MS approaches for lipidomics by SFC-HRMS/MS have been previously described in the literature [2]. In order to improve the lipidome coverage within biological complex matrices and gain in structural information, implementation of Sequential Window Acquisition of All Theoretical mass spectra (SWATH), a method which originates from Data-Independent Acquisition [3], has been implemented on human plasma and vegetal extract. Untargeted DIA lipidomic analyses were thus conducted on SFC with Diethylamine column hyphenated to Q-TOF analyzer (6500 series, Agilent) in both positive and negative electrospray ionization modes.

The mass spectrometry analytical method was optimized by designing variable isolation windows that are specifically linked to the elution profiles of different lipid classes [4]. The deconvolution and identification of SFC-HRMS/MS data were achieved using the MS-DIAL software and an *in-silico* lipids library. Through the workflow, the structural elucidation of wide ranges of concentration of diverse lipids was achieved. This study presents the first application of SWATH acquisition on SFC-Q-TOF for untargeted lipidomics in human plasma and vegetal extract.

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P34
Identification of Biomarkers for Sulfur Mustard Exposure in Tears and Plasma of rabbits by Mass Spectrometry-based metabolomics

J. Bouhlel[1)], F. Caffin[2)], F. Gros-Désormeaux[2)], F. Castelli[1)], E. Chu-Van[1)], C. Piérard[2)], C. Junot[1)], F. Fenaille[1)]

1) Université Paris-Saclay, CEA, INRAE, Département Médicaments et Technologies pour la Santé (MTS), MetaboHUB, F-91191, Gif sur Yvette, France
2) Institut de Recherche Biomédicale des Armées (IRBA),91223 Brétigny-sur-Orge, France

Sulfur mustard (SM), or bis (2-chloroethyl) sulfide is a chemical warfare agent. SM victims mainly suffered from painful ocular, cutaneous and pulmonary lesions. Although research efforts have been expended to understand how SM exerts its toxic effects, its mechanism of action remains poorly understood [1]. In addition, there is no well-established prognostic SM biomarkers of ocular damages. In this way, the metabolomic approach in tears represents an innovative biomarker search strategy [2]. Animal experiments were established by exposing rabbits to SM vapors during 6 minutes, and their tear and plasma metabolomes were analyzed to highlight potential biomarker candidates of SM exposure. Metabolic profiles were studied up to 35 days following SM exposure by untargeted metabolomic analyses using Liquid Chromatography coupled to High Resolution Mass Spectrometry (LC-HRMS). Data processing was performed using the Workflow4Metabolomics platform. The annotation of up to 300 metabolites in tears and plasma samples and belonging to diverse chemical classes, was obtained using our in-house database by matching accurate masses, retention times, and MS/MS spectra to those of pure standards.

Comparison of plasma metabolic signatures in control and SM-exposed groups pointed out significant alterations of sulfur-containing amino acids, such as taurine, cysteine and cystathionine.

Metabolomic analyses of the corresponding tear samples were also performed in parallel to evaluate local effects of SM. At first, tears sampling and metabolite extraction procedures were optimized to obtain unbiased and reproducible snapshots of the tear metabolome. Results of tears metabolomics confirmed the significant alteration of taurine abundances. Interestingly, taurine has been reported as an antioxidant molecule whose abundance can be correlated to retinal diseases [3]. Altogether, tear and plasma metabolomes demonstrated good complementarity to get better knowledge of SM action mechanism and identifiy potential biomarkers of SM exposure.

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P35
Hidden in plain sight: Fingermark metabolomics revealed by MRMS

C. Cordeiro[1)], A. Madureira-Carvalho[2),3)], N.G.M. Gomes[2),3)], M. Pereira[1)], M. Cruz[1)], M. Witt[4)], M. Sousa Silva[1)]

1) Laboratório de FT-ICR e Espectrometria de Massa Estrutural, Faculdade de Ciências da Universidade de Lisboa, Lisboa, Portugal
2) TOXRUN – Toxicology Research Unit, University Institute of Health Sciences, CESPU, Gandra, Portugal
3) REQUIMTE/LAQV, Laboratory of Pharmacognosy, Department of Chemistry, Faculty of Pharmacy, University of Porto, Portugal
4) Bruker Daltonics, Bremen, Germany

The unique pattern of a fingermark is the staple of an individual identification through the collection of the image that is produced on objects and surfaces. The image is defined by a complex mixture of both endogenous and exogenous substances left behind by a subject that may reveal further information of forensic value. Pioneering work using low resolution mass spectrometry methods revealed the potential of fingermark analysis for sex identification [1], dating [2], drugs of abuse [3] and explosives [4]. Given the complexity and chemical diversity, only extreme resolution MS approaches can harness the potential of fingermark analysis in forensics and other applications. Magnetic Resonance Mass Spectrometry (MRMS) provides the highest figures of merit concerning mass accuracy (sub ppm) and resolution (106 at m/z 1000 or better if needed). Direct infusion by electrospray can be used, thus increasing throughput. MRMS analysis of fingermarks allows the simultaneous identification of hundreds of compounds, based on accurate mass and detailed isotopic pattern analysis as well as database annotation. Statistical methods enable the application of this methodology for reliable sex discrimination and age group classification and to gain insight on the metabolic basis for this discrimination, opening the way for other applications [5].

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P36
^{13}C-labeled Mouse Urine Metabolomics by LC-HRMS: effectively improving metabolite identification

A. Legrand, K. Rousseau, B. Fischmeister, S. Dechaumet, C. Junot, J.-J. Leguay, E. Ezan, F. Fenaille, A. Damont

Université Paris-Saclay, CEA, INRAE, Département Médicaments et Technologies pour la santé (DMTS), SPI, MetaboHUB-IDF, 91191 Gif-sur-Yvette, France

The ability to detect and confidently identify metabolites is a key factor to evaluate metabolic dysfunctions and to discriminate between control and diseased groups. Nowadays, the prevalent method for global metabolome profiling is liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS). However, despite the method performances to detect metabolites, more than 97% of the estimated human metabolome remains unknown [1]. In this context, in vivo stable isotope labeling enables to more accurately characterize and potentially identify new metabolites by LC-HRMS [2]. The murine urinary metabolome, close to the human metabolome, was successfully labeled by feeding three mice with a ^{13}C-labeled diet during six weeks. Increasingly ^{13}C-labeled mouse urines were then collected throughout this period and analyzed by LC-HRMS-based metabolomics using an Orbitrap FusionTM instrument (ThermoFisher Scientific). More than 130 metabolites from the internal chemical library were identified with high confidence (level 1, [3]) and 120 metabolites were annotated as acylcarnitines, acylglycines or acylglucuronides (levels 2 and 3, [3]). Results showed that the isotopic data provided improved metabolite identification: distinction of relevant signals, unambiguous assignment of chemical formula, better understanding of the fragmentation data, and greater confidence in the structural identification. Moreover, enrichment data were used as a new annotation tool as most of the metabolite showed an enrichment rate higher than 90% but an enrichment pattern characteristic of their chemical classes. Thus, an overall presentation will highlight the value of in vivo ^{13}C-labeling in terms of detection, annotation, identification, and metabolism investigations of mouse urinary metabolites. It will also be shown that it is a relevant tool to explore the “dark metabolome”.

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P37
Mass Spectrometry-Based Metabolomic Assessment of Environmental Platinoids-Induced Disruptions in two Species of Terrestrial Mosses

A. Paris[1)], B. Salam[1)], R. Puppo[1)], A. Marie[1)], C. Meyer[2)], S. Leblond[2)]

1) UMR 7245, MCAM, MNHN, CNRS, 63 Buffon Street, 75005 PARIS, FRANCE;
2) PatriNat, OFB, MNHN,12 Buffon Street, 75005 PARIS, FRANCE

The transport sector is one of the main contributors to atmospheric contamination. Since 1990s, many countries have made catalytic converters compulsory for petrol and diesel engines to limit emissions of three pollutants (CO, NOx and unburnt hydrocarbons) [1, 2]. However, this technology generates emergent pollution problems linked to platinum group elements (PGE). PGEs, depending on their nature of emission, are known to be allergenic, cytotoxic, mutagenic, and carcinogenic. In France, few data are available on PGE levels in the environment and very few studies focus on their ecological impacts. The objective of the present study is to get significant canonical links between metabolomic fingerprints obtained by FIA-ESI-MS or by FIA-ESI-MS/MS using bb-CID activation, in either positive or negative ionization modes from direct injection of extracts (2 µL methanol-60%) prepared from dry powder of terrestrial mosses (two species collected on 405 geographical sites and as well as PGEs / metals measured by ICP-MS on the same dry powder samples. Multidimensional analytical parameters were checked to repeatedly assess data quality thanks to "Blank" samples and "Quality Control" (QC) ones, repeatedly analyzed all along the analytical series. Two primary datasets were obtained from FIA-MS phenotyping which correspond to MS and MS-MS information. From PCAs, the whole analytical process was validated with Blanks and QC samples displaying a low variability. Few Principal Components (5 and 4 PCs in positive and negative modes, respectively) were sufficient to concentrate more than 90% of the total metabolomic information. In positive mode, a clear "Species" effect is detected from PC2 with a partial differentiation of *Pseudoscleropodium purum* (Pp) extracts from *Hypnum cupressiforme* (Hc) ones.

Different chemometric approaches are evaluated to get significant canonical links between some candidate biomarkers selected from MS or MS/MS data obtained in either positive or negative ionization modes and taking into account the complementary exposure metadata in metals and/or PGEs.

This work is a contribution of the BIPlatE project (Développement d'indicateurs de biosurveillance des teneurs en platinoïdes dans l'écosystème forestier). BIPlatE is supported by the Agence de l'Environnement et de la Maîtrise de l'Energie (PNR EST- ADEME project n° 2166D0009).

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P38
HPLC-PDA-ESI-HRMS-based profiling of plant secondary metabolites

B. Wilenska[1)], MR. Naliwajski[2)], K. Sykłowska-Baranek[3)]

1) Faculty of Chemistry, University of Warsaw, 1 Pasteura St., 02-093 Warsaw, Poland;
2) Department of Plant Physiology and Biochemistry, Faculty of Biology and Environmental Protection, University of Lodz, 12/16 Banacha St., 90-237 Lodz, Poland;
3) Department of Pharmaceutical Biology, Faculty of Pharmacy, Medical University of Warsaw, 1 Banacha St., 02-097 Warsaw, Poland

In vitro plant cell platforms are continuously explored for application in the biosynthesis of secondary metabolites that are active ingredients of medicines and cosmetics [1]. To cope with environmental harmful conditions, plant cells developed adaptive strategy that involves production of a wide variety of complex secondary metabolites. The spectrum and quantity of biosynthesized compounds in specific plant species is determined by its genotype, tissue, developmental and physiological stage and environmental factors. For many years these compounds were considered non-functional end products of metabolism [2]. Currently, many secondary metabolites are recognized as having important ecological functions in plants, such as protecting plants against infections by pathogens [3]. Hence, the HPLC-MS based methods were used to structure elucidation of compounds presented in the mixture of these complex secondary metabolites.

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P39
Proteomic profiling of the mammalian oocytes during the maturation process

P. Suder[1)], W. Mlodawska[2)], A. Drabik[1)], J. Ner-Kluza[1)], I. Kotsan[1)], A. Bodzon-Kulakowska[1)]

1) Department of Analytical Chemistry and Biochemistry, Faculty of Materials Science and Ceramics, AGH University of Science and Technology, Mickiewicza 30, 30-059 Krakow, Poland;
2) Department of Animal Reproduction, Anatomy and Genomics, University of Agriculture in Krakow, Al. Mickiewicza 24/28, 30-059 Kraków, Poland

Oocyte's maturation process is a crucial step, deciding about the capability of their fertilization. The maturation process of these cells is still not fully understood. There is a general agreement in the literature, that at least two crucial factors, influencing the maturation speed can be observed: composition and a balance between various carbohydrates and lipids, present in the oocyte's cytoplasm during mature cell formation [1]. A delicate balance between molecules can be reflected even in the cell's phenotype: depending on the lipids, fatty acids and carbohydrates levels and form (eg. quantity of the lipid droplets [2]) they seem to be darker or brighter in the bright-field microscopy [3]. This phenomenon can be easily observed even in cells derived from the same ovary. The investigations partially confirmed the hypothesis that oocyte's darkness level is connected to the balance between saturated, like eg. stearic and unsaturated, like arachidonic or oleic fatty acids [4]. However, this observation does not fully explains mechanism staying behind fertilization capability in the function of maturation and ageing process of the oocytes.

In our investigations we decided to search for the differences in the proteomic profiles of the oocytes, belonging to the immature, ready-for-fertilization and aged groups, which were distinguished by the microscopic techniques. We hypotesise, that the proteomic changes in the cells are crucial for keeping the susceptibility for fertilization, while the changes in the carbohydrates/lipids profiles could be the result of the proteome compounds complex activity. During investigations, we used typical proteomic set-up, consisting of Ultimate 3000 nanoLC connected via nanoESI ion source to Exploris 240 Orbitrap MS with the aid of isobaric tagging.

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P40
Surface Plasmon Resonance coupled to Mass Spectrometry to study lectin-sugar interactions

M. Diaz Hermida[1)], *S. Poyer*[2)], *R. Daniel*[1)], *W. Buchmann*[1)]

1) Université Paris-Saclay, Univ Evry, CNRS, LAMBE, 91025, Evry-Courcouronnes, France
2) Université Paris-Saclay, CNRS, Institut de Chimie des Substances Naturelles, UPR 2301, 91198, Gif-sur-Yvette, France

Biomolecular interactions are at the heart of the functioning of all living systems. The study of biointeractions is essential for understanding the global organization of the cellular machinery and their role in physiological processes. They constitute a significant challenge in analytical chemistry, diagnostics, and therapeutic research [1], [2].

Lectins are proteins that specifically recognize edible sugars. Our project aims to develop a coupling between Surface Plasmon Resonance Imaging and Mass Spectrometry (SPR_i-MS) to analyze protein-sugar interactions. The aim is to create a multiplex SPR biochip with immobilized lectins and then to use this biochip in coupling with MALDI-TOF-MS. This coupling allows the kinetics and thermodynamics of the interaction to be studied in real-time, together with the structural identification of the sugars captured from a complex mixture [1], [2], [3].

By SPR_i, this work confirmed significant interactions, between the lectin WGA and the neoglycosylated BSA grafted carrying the sugars *N*-acetylgalactosamine and *N*-acetylglucosamine, and between the lectin AIA and neoglycosylated BSA carrying galactose and its *N*-acetylated form. We attempted the MS detection of captured glycosylated BSA directly from the biochip surface; however, the lack of sensitivity in MS detection hindered the development of the coupling. The sensitivity depends, on the amount of ligands retained on biochip surface, which itself depends, among other factors, on the chemical functionalization of the biochip surface, the nature of the receptors and their immobilization on the surface [1]. Modifications of the MALDI-TOF-MS analysis conditions are being carried out with the use of MALDI imaging and the use of alternative receptors in order to evaluate their impact on the sensitivity of detection by SPR_i-MS.

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P41
Identification of LVV-H7 binding partners in response to alcohol dependence in the rat CNS

P. Mielczarek[1),2)], K. Hartman[2)], J. H. Kotlinska[3)], J. Silberring[2)]

1) Laboratory of Proteomics and Mass Spectrometry, Maj Institute of Pharmacology, Polish Academy of Sciences, Smetna 12 Str., 31-343 Krakow, Poland
2) Faculty of Materials Science and Ceramics, AGH University of Science and Technology, Mickiewicza 30 Ave., 30-059 Krakow, Poland
3) Department of Pharmacology and Pharmacodynamics, Faculty of Pharmacy With Division of Medical Analytics, Medical University of Lublin, Chodzki 4a Str., 20-093 Lublin, Poland

Hemorphins are short peptides produced during cleavage of the hemoglobin chains, with wide variety of functions in different biological systems and tissue types in both physiology and pathophysiology. LVV-hemorphin 7 (shortly called LVV-H7) is generated at high amounts in the presence of alcohol, based on *in vitro* experiments. Applying mass spectrometry we identified protein binding partners of LVV-H7 in the brain tissues of alcohol addicted and control rats. Proteins bound to this peptide immobilized on the resin were digested and then were identified by liquid chromatography combined with mass spectrometry. The most interesting proteins that were identified are: alpha-synuclein (SNCA), Glycogen synthase kinase-3 beta (GSK3B), Fatty acid synthase (FASN) and ATP synthase subunit beta, mitochondrial (ATP5B). Genetic and physical interaction networks between regulated proteins', that have been identified as binding partners of LVV-H7 peptide, characteristic for the CNS tissue in both group (the ethanol addicted rats and the control group), have been presented. The interactions were established based on the easyN from bioGRID database analysis. The presented results are very important as long as Hemorphins are considered to act as therapeutics in addiction and biomarkers in various diseases.

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How mass spectrometry can input into the drug delivery problem insight

V.A. Pashynska[1)], S.G. Stepanian[1)], M.V. Kosevich[1)], A. Gomory[2)], L. Drahos[2)]

1) B.Verkin Institute for Low Temperature Physics and Engineering of NAS of Ukraine, 47 Nauky Ave., Kharkiv, 61103, Ukraine
2) Institute of Organic Chemistry of the Research Center for Natural Sciences, Hungarian Academy of Sciences, Budapest, Hungary

Searching for the new prospective drug delivery approaches is an urgent challenge of the modern biomedical science and technology areas, including molecular medicine, molecular pharmacology, molecular biophysics and nanotechnology. Among effective ways to promote drug molecules administration into the targeting cells there is a way when penetration enhancing agents' molecules or specially created drug delivery molecular systems (liposomes, nanosomes) form noncovalent complexes with the drug to be delivered. Such complexes can penetrate the cell membranes easier due to physico-chemical properties of the delivery assisting substances: here the molecule or molecular cluster of facilitating compound works as a vehicle. In the current combined mass spectrometry and quantum chemical study, we examined the intermolecular interactions of the molecules of selected anticancer mercapto-derivatives of nucleobases with drug delivery facilitating agent dimethyl sulfoxide (DMSO known as a transdermal and transmembrane drugs penetration enhancer) or ascorbyl palmitate (AP, fat soluble form of vitamin C, known as an agent forming nanosomes called aposomes with potential applicability in the drug delivery).

Formation of stable noncovalent complexes of DMSO with the molecules of 6-mercaptopurine and 2-mercaptoadenine in the polar solvent methanol was revealed by the electrospray ionization mass spectrometry (ESI MS) probing of model binary systems containing DMSO and the anticancer drugs. The revealed complexes are similar to the supramolecular complexes of DMSO with the molecules of a number of antibiotics, formation of which was demonstrated in our previous study [1]. To evaluate the structure and describe the energetic stability of the noncovalent complexes of DMSO with the anticancer agents, the quantum chemical calculations of the complexes were performed by DFT method.

At the next stage of the current study the characterization of AP and its intermolecular interactions by a number of mass spectrometry techniques: ESI, Laser Desorption/Ionization (LDI) and Matrix-Assisted Laser Desorption/Ionization (MALDI) was carried out. Based on the obtained results the ESI MS was recommended to be used in AP nanosomes formation examining and for the study of noncovalent complexation of AP with the drugs molecules. Following ESI MS probing demonstrated that AP can also form the stable noncovalent complexes with the molecules of 6-mercaptopurine and 2-mercaptoadenine in the polar surrounding.

The results of the study as to formation of stable noncovalent complexes of DMSO or AP with the anticancer mercapto-derivatives of nucleobases allowed us to propose such noncovalent complexation as a molecular mechanism of these drug delivery facilitating agents' functional activity. At the same time the formation of noncovalent associates between the drug molecules and the delivery assisting molecules or molecular clusters (nanosomes) can be considered as a basis of for the development of molecular platforms for transdermal and transmembrane penetration facilitating of the fat insoluble drugs.

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Development of a SALDI-MS approach for the specific and sensitive detection of biomolecules: focus on Alzheimer's disease biomarkers

A. Cournut[1)], P. Moustiez[2)], Y. Coffinier[2)], C. Enjalbal[1)], C. Muracciole-Bich[1)]

1) 1Institut des Biomolécules Max Mousseron, Université de Montpellier
2) Institut d'Electronique de Microélectronique et de Nanotechnologie de Lille, Université de Lille

In SALDI-MS (Surface-Assisted Laser Desorption/Ionization Mass Spectrometry), inorganic structured surfaces directly allow desorption/ionization (D/I) phenomena by absorbing the energy of the laser beam to be transferred from the nanomaterials to the deposited analytes. The goal of this project is to develop a sensitive SALDI-MS approach for the specific detection of protein biomarkers of Alzheimer's disease, such as β-amyloid peptides 1-40 and 1-42 which can aggregate into beta-sheets depending on the operating conditions thus constituting rather complex biomolecules to monitor. According to preliminary results [1], randomly shaped nanomaterials (nanowires (NW) or vertical silicon array of nanoneedles (NN)) have been fabricated by chemical etching and functionalized by different methods such as silanization, deposition of metallic particles or TiO_2. To evaluate the designed 23 surfaces, different types of biomolecule were deposited: (i) two peptide mixtures containing a total of 10 sequences with m/z 600 – 3200 Da (Mix 1) or m/z 600 – 2100 Da (Mix 2: only containing peptides with arginine or lysine at their C-terminal position) (ii) two standard phosphopeptide mixtures and (iii) one protein mix. A RapiFlex Mass Spectrometer from Bruker was used in the positive reflectron mode to perform MS and imaging MS (MSI) experiments. Selected surfaces must have (i) physico-chemical properties allowing easy deposition (surface must be hydrophilic enough for the deposition of aqueous solutions) (ii) chemical properties providing optimal D/I of the peptides and (iii) a deposit homogeneity verified by MSI experiments. The 23 nanomaterial surfaces show different results according to D/I efficiency and deposit homogeneity depending on their surface structuration (NW or NN) and chemistry (TiO_2, SiOx, Cu, Ag or Au doping) as well as on the analyte conditioning. In particular, the aqueous solutions were supplemented with multiple salts (ammonium, sodium, potassium from 0,5 to 50 mM). In line with circular dichroism experiments, β-amyloid peptides dissolved in a 10 mM solution of dibasic ammonium citrate provided optimal signals in MS and MSI modes for 8 of the tested surfaces exhibiting homogenous deposits and an efficient D/I.

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P44
In- Vivo Tape Stripping Study with Caffeine for Comparisons on Body Sites, Age and Washing

W. Wargniez,·S. Connétable,·N. Bourokba,·O, Dufour,·S. Nouveau, S. Grégoire

L'Oréal R&I, Advanced Research, 1 avenue Eugène Schueller, 93601 Aulnay-sous-Bois, France

Purpose: Assessing the percutaneous absorption of cosmetic ingredients using in-vitro human skin reveals certain limitations, such as restricted anatomical sites and repeated exposure, and to overcome these issues, in-vivo studies are required. The aim of the study is to develop a robust non-invasive in-vivo protocol that should be applicable to a wide range of application.

Method: a robust tape stripping protocol was therefore designed according to recent recommendations, and the impact of two different washing procedures on caffeine distribution in tape strips was investigated to optimise the protocol. The optimised protocol was then used to study the effect of age and anatomical area on the percutaneous absorption of caffeine, including facial areas which are not readily available for in-vitro studies.

Results: With tape stripping, a difference between the percutaneous absorption on the face (forehead, cheek) and the volar forearm was observed. No obvious difference was observed between percutaneous absorption in young and post-menopausal women, but this could be due to the limited number of subjects.

Conclusion: This tape stripping protocol is now to be deployed to address many other factors, such as percutaneous absorption in other anatomical areas (e.g. abdomen, axilla, etc.), impact of repeated applications and effect of formulation

P45
MALDI MSI analysis of proteins with wet-interface matrix deposition

P. Kret, A. Bodzon-Kulakowska, P. Mielczarek, P.Suder

Department of Analytical Chemistry and Biochemistry, Faculty of Materials Science and Ceramics, AGH University of Science and Technology, A. Mickiewicza 30 Ave., 30-059 Krakow, Poland

Proteins are the work-horses for organism metabolism; thus, their analysis is an issue for many biochemical studies. MALDI mass spectrometry imaging (MALDI-MSI) offers a unique possibility to visualize the localization of selected proteins in the tissue sections and may facilitate proteomics studies. But as with all analytical techniques, it demands careful sample preparation.

Proteins that are present in the tissue sections have to be digested before the analysis. Then, a special matrix has to be applied. There are several ways in which a matrix could be deposited, and in our study, we used SunCollect® system, which is recognized as the wet-interface technique. Two parameters are crucial for this system – the sputtering nozzle position over the sample and the number of matrix layers

At the beginning of our study with MALDI-MSI for protein analysis, we used three types of matrices: DAN, DHB, and CHCA. They were applied on the rat cerebellum slices washed from lipids with ethanol. The trypsin digestion of proteins from those tissue sections was also performed. During our research, we checked how parameters like the kind of matrix and the parameters crucial for SunCollect device: the number of matrix layers and the sputtering nozzle height may influence the results.

We have optimized the kind of matrix, the number of layers, and nozzle settings in terms of spectra intensity and the quality of obtained ion maps. Our research shows the impact of these factors on the results and allows you to choose the appropriate sample preparation method. We hope that our research will be helpful for all scientists who start their adventure with peptide MALDI-based mass spectrometry imaging.

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P46
Desorption Electrospray Ionization – Imaging Mass Spectrometry (DESI-IMS) and Cold-Spray Ionization Mass Spectrometry (CSI-MS) in Lichenology

T. Delhaye[1)], É. Bertrand[1)], F. Lohézic-LeDevehat[2)], J. Boustie[2)], D. Rondeau[1)]

1) Univ Rennes, CNRS, IETR – UMR 6164, F-35000 Rennes, France
2) Univ Rennes, CNRS, ISCR – UMR 6226, F-35000 Rennes, France

Lichens that are symbiotic organisms resulting from the association between a fungus and a green alga or a cyanobacterium [1]. Valuation of lichens unique metabolites is generating an increasing interest whatever for therapeutic purposes or in fragrance industry. In this context, Liquid chromatography-electrospray ionization-mass spectrometry (LC-ESI-MS) remains the most common dereplication approach in lichenology, often associated with detection in MS/MS or MS^n mode [2]. However, lichens are also known for their abilities to survive in harsh conditions. This adaptability is namely attributed to the production of polyphenolic secondary metabolites characterized by UV-absorbing or antioxidant behavior. As lichens are also known for their tolerance-desiccation, it's assumed that this property would be related to water maintained in interaction in a medium constituted of large amounts of sparsely hydrophilic compounds and among them the polyphenolic secondary metabolites constituting a molecular association network also named as Natural Deep Eutectic Solvent (NADES) [3]. Deep Eutectic Solvents (DESs) are usually obtained by mixing a Hydrogen Bond Acceptor (HBA) and a Hydrogen Bond Donor (HBD). DES are characterized by a melting point lower than that of each individual component and are uninflammable and non-volatile solvents. In a first part of the presented study, DESI mass spectrometry was used as analytical tool in the dereplication of three lichens: *Pseudocyphellaria crocata*, *Xanthoparmelia pulla* and *Xanthoparmelia loxodes*. A correlation has been shown between the amounts of metabolites detected in LC-MS analysis and the intensities of the signals of the divaricatic, sténosporiv, glomellic, glomelliféric and perlatolic, disclosed in DESI-Imaging Mass Spectrometry. This indicates that DESI can be considered as a versatile tool to the wide field of lichenology, facilitating accelerated and sharp analyses of lichens and bypassing costly and tedious procedures of solvent extraction. In a second part of the study, the dereplication of *Evernia Prunastri* by DESI-MS has evidenced the formation of an unique heterodimer at *m/z* 693, formed from the association of evernic and usnic acids into a hydrated cluster. The production of such hydrated non-covalent complex was confirmed in Cold-Spray Ionization Mass Spectrometry analysis of a mixture of these two lichen metabolites and its stability evaluated by In-source Collision Induced Dissociation. This suggests the participation of the evernic and usnic acids into a NADES to the resilience to the desiccation of lichens.

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P47
May post-translational succination be involved in cardiac arrhythmia?

L. Groignet[1),2)], L. Spinosi[1),3)], P. Brocorens[2)], A.-E. Declèves[4)], J.-M. Colet[3)], J. De Winter[1)]

1) Organic Synthesis and Mass Spectrometry Laboratory, University of Mons (UMons), Place du Parc, 23, Mons, 7000, Belgium
2) Laboratory for Chemistry of Novel Materials, University of Mons (UMons), Place du Parc, 23, Mons, 7000, Belgium
3) Laboratory of Human biology and Toxicology, University of Mons (UMons), Place du Parc, 23, Mons, 7000, Belgium
4) Laboratory of Metabolic and Molecular Biochemistry, University of Mons (UMons), Place du Parc, 23, Mons, 7000, Belgium

During the Krebs cycle, the fumarate is converted into malate by the addition of a molecule of water catalysed by fumarate hydratase. But in some diseases, there is a germline mutation of the fumarase gene resulting in an enzymatic deficiency and therefore an accumulation of fumarate [1]. This molecule can undergo a Michael addition with proteins that present free thiol functions (free cysteines) and this physiologically irreversible reaction is called succination. 2-succinocystein molecules are known to be involved in some pathologies such as diabetes or cardiomyopathies when they are overexpressed [2]. An immunoassay highlighting the reaction products of succination is available. However, no information about the spontaneity of these reactions can be deducted and their efficiency remains relatively controversial in the literature [3]. In this study, an MS-based approach was selected as a potential alternative to the current assay. Indeed, using this technique could increase the knowledge about succination by determining the reaction spontaneity and the maximal number of moieties that can be grafted into the target. The first objective was to verify the existence of spontaneous reactions of succination by MS-based approach. The spontaneity of the succination reaction has been highlighted. Indeed, some peptides/proteins with free cysteines were spontaneously succinated, such as glutathione and SUMO1. SUMO1 is used in the stabilization of some target proteins, including SERCA2, a protein involved in Ca^{2+} regulation during cardiac contraction [4]. Thus, our main objective is to evaluate by Ion Mobility Mass Spectrometry and molecular dynamics whether SUMO1 succination has an impact on its 3D structure and therefore, whether it has an impact on its function and plays a role in cardiac arrhythmia.

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P48
The role of hemorphins in alcohol dependence

A. Drabik, K. Hartman, J. Silberring

Department of Analytical Chemistry and Biochemistry, AGH University of Science and Technology, Krakow, Poland

Hemorphins are a group of multifunctional peptides released from hemoglobin during physiological and pathophysiological processes, that express opioid-like activity. LVV-hemorphin 7 (LVV-H7) is released at higher concentrations in the presence of alcohol in vitro. Therefore, we predicted that alcohol intake can change the level of LVV-H7. We have accomplished behavioral tests in animal studies, along with proteomic analysis of the CNS tissues of alcohol addicted rats to verify the correlation of LVV-H7 level and ethanol dependence. We have also confirmed the BBB permeability of synthesized LVV-H7, as well as an inhibitor of its releasing enzyme using fluorescent microscopy. Utilizing mass spectrometry-based methods we identified protein the LVV-H7 degrading enzymes. The interpretation of the obtained results reveals the substantial role of LVV-H7 in the mechanism of alcohol dependence.

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P49
Insights into Aβ-Oxygen-adducts formation: A breakthrough Mass Spectrometry study in Alzheimer's disease research

A. Distefano[1)], *A. Memboeuf*[2)], *G. Grasso*[1)]

1) Chemical Sciences Department, University of Catania, Viale Andrea Doria 6, 95125 Catania, Italy;
2) Univ Brest, CNRS, CEMCA, UMR 6521, F-29200, Brest, France

Oxidative stress is a constant threat to the development of Alzheimer's disease which in combination with the increase of metals in our brain, such as copper, can lead to a dual disastrous action with an uncontrollable worsening of the pathology.[1] The generation of ROS (reactive oxygen species) can alter the sensitive redox copper equilibrium which in turn can catalyze the formation of reactive hydroxyl radicals, bringing about the oxidation and the aggregation of Aβ 1-40 peptide. [2]

The discovery of oxygen adducts is of paramount importance for the achievement of a deeper knowledge of the redox mechanism involved in AD and other neurodegenerative disorders as well. Indeed, nowadays, it is unclear the role played by O_2 molecules in the Aβ interaction, and how they could alter the awkward mechanisms occurring during the progression of the pathology.

The investigation of Aβ-oxygen adducts formed in the gas phase by the introduction of oxygen gas was achieved by the use of an *ad hoc* modified mass spectrometer.[3] Interestingly, the $CuCl_2$ addition in solution has unveiled the Aβ-Copper-oxygen adduct formation, demonstrating the capability of the oxygen molecules to interact with Aβ 1-40 peptide in the gas phase.

The pioneering results obtained with such mass spectrometer apparatus combined with the consolidated advantages of this technique may open new frontiers in the knowledge of i) oxygen adducts and, as a consequence, ii) the oxidative Aβ products achieved after the interaction with oxygen molecules in the gas phase.

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P50
Applications of mass spectrometry in oncology: diagnosis to treatment

S. Tezcan[1)], F. Arioz[2)]

1) Marmara University Faculty of Pharmacy
2) Université de Bretagne Occidentale (UBO) Faculty of Science and Technology

Despite many advances in oncology, cancer is still one of the leading health problems that threaten our lives, that remain mysterious and await solutions. For this reason, research continues rapidly to understand the problem that causes cancer and to find permanent solutions. Especially in recent years, proteomic studies provide important information in elucidating the mechanism of cancer formation with the information they reveal about changes in protein sequences in tissues or cells [1]. Proteomics is the study of the structure and function of proteins, including the way they work and interact with each other within cells [2]. In this respect, it contributes to the discovery of new cancer biomarkers. Mass spectrometric methods are one of the gold standards of proteomic studies and are used in both qualitative and quantitative analyzes of chemical substances. Mass spectrometry classifies sample isotopes, finds the chemical makeup of the unknown compound, calculates the purity of the sample, and is more sensitive than other techniques. Today, mass spectrometry is emerging as a fast and reliable method to elucidate the mechanism of cancer and detect biomarkers. Serum proteomic signatures are associated with immune responses in patients with cancer. Therefore, mass spectrometric methods are important for the development of new treatments. In addition, mass spectrometric methods are used in many clinical studies on drug resistance, response to treatment, and development of individualized treatments [3].

In this study, we prepared a mini review of studies on mass spectrometry applications in preclinical and clinical trials in oncology settings. Our aim is that this mini review is an important resource for researchers working in this field and for those interested in the subject.

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P51
Insights into the transport dynamics of prokaryotic chloride/proton antiporter

J. Portašiková[1,2], Fojtík Lukáš[1,2], Novák Petr[1] and Man Petr[1]

1) Faculty of Science, Charles University, Prague, Czech Republic
2) Institute of Microbiology of the CAS, Prague, Czech Republic

Chloride channel family includes transmembrane channels and transporters, which are present in both eukaryotic and prokaryotic organisms. They are involved in many cellular processes and their mutation can cause serious illnesses. To treat these diseases, description of their transport mechanism is needed. Bacterial chloride transporter CLC-ec1 from E. coli is often used as model protein to study protein family of chloride channels. The protein is expressed as homodimer, in which each monomer has a separate ion transport pathway. It functions as an antiporter of a single proton for two chloride anions. During transport of ions, the protein undergoes conformational changes that convert inward and outward-facing conformation. Outward-facing state is induced by protons, when Glu residues which are involved in ion transport are protonated. This state can be mimicked by a QQQ mutant, in which three key Glu residues are mutated to Gln residues [1]. So far, CLC-ec1 transport has been studied by X-ray crystallography that provided detailed but static images. Here we used to hydrogen/deuterium exchange mass spectrometry to extend the recent findings and provide more detailed insight into the transport dynamics of this protein.

Full-length wild-type and QQQ CLC-ec1 were over-expressed in bacteria, isolated via detergent solubilization and purified by affinity chromatography and gel filtration. HDX-MS based digestion was carefully optimized to provide full sequence coverage with reasonably sized peptides. Next, HDX-MS experiment was conducted at different conditions. First, we compared WT and QQQ proteins to address the role of protonation. Both proteins were followed at four pH values spanning pH range 4.4- 7.4. The deuteration rate of WT protein at pH 4.4 did not completely match the deuteration of the QQQ protein, we decided to run an experiment at even lower pH 3. These data are highlighting the step-wise protonation and the associated structural changes across the ion transport path.

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P52
The EMIE French Tour of Mass Spectrometry

T. Abar[1)], A. Badri[2)], E. Ogden[3)], F. Servant[4,5)], C. Soep[6)], A. Tsirkou[7)].

1) Université Paris Saclay, CNRS, Institut de Chimie Physique, F-91405 Orsay, France ;
2) Université Sorbonne Paris Nord, CNRS, Laboratoire de Physique des Lasers, F-93430 Villetaneuse, France ;
3) Université de Rennes, CNRS, Institut de Physique de Rennes, F-35000 Rennes, France ;
4) Synchrotron SOLEIL, L'Orme des Merisiers, Départementale 128, 91190 Saint-Aubin, France ;
5) INRAE, UAR1008, Transform Department, Rue de la Géraudière, BP 71627,44316 Nantes, France ;
6) Sorbonne Université, CNRS, Institut Parisien de Chimie Moléculaire, IPCM, F-75005 Paris, France ;
7) Institut Lumière Matière, UCBL, CNRS, F-69622 Villeurbanne, France.

Organized by the Gdr EMiE, we had the opportunity to visit several laboratories across France that specialize in mass spectrometry, with a particular focus on chemical physics. In this poster we aim to showcase our experience of the tour, and highlight the various experimental setups that we encountered.

LIST OF PARTICIPANTS

Abar, Taous FRANCE	Université Paris Saclay / CNRS Institut de Chimie Physique (ICP)	**O22** **P51**
Abdoul-Carime, Hassan FRANCE	Université de Lyon 1 Institut de Physique des 2 Infinis de Lyon	
Achanta, Nagendra Sai Kumar ITALY	National Research Council Institute of Biochemistry and Cell Biology	
Alcaraz, Christian FRANCE	CNRS / Université Paris Saclay Institut de Chimie Physique (ICP)	
Almasri, Bayan FRANCE	University of Lille MSAP	**O27**
Alves, Sandra FRANCE	Sorbonne Université, CNRS Institut Parisien de Chimie Moléculaire (IPCM)	**P25**
Arios, Filiz FRANCE	Université de Bretagne Occidentale UMR CNRS 6521, CEMCA	
Badri, Ayoub FRANCE	Université Sorbonne Paris-Nord Laboratoire de Physique des Lasers	**P51**
Bartolucci, Gianluca ITALY	University of Florence Mass Spectrometry	
Bellia, Francesco ITALY	Institute of Crystallography Mass Spectrometry Lab	
Bertrand, Émilie FRANCE	Université de Rennes 1 Institut d'Electronique et des Technologies du numéRique (IETR)	**O38** **P42**
Błaziak, Agata POLAND	Institute of Physical Chemistry Polish Academy of Sciences Environmental Chemistry Group	**P18**
Błaziak, Kacper POLAND	Faculty of Chemistry, University of Warsaw Laboratory of Biologically Active Compounds	**O26**
Bodzon-Kulakowska, Anna POLAND	AGH University of Science and Technology Department of Analytical Chemistry and Biochemistry, Faculty of Materials Science and Ceramics	**O18**
Bonose, Myriam FRANCE	Université Paris Saclay Institut de Chimie Physique (ICP) UMR 8000	**P01**
Bouhlel, Jihène FRANCE	CEA LI-MS	**P30**
Bourcier, Sophie FRANCE	Ecole Polytechnique LCM	

Bourehil, Lyna FRANCE	Sorbonne Université / SOLEIL Synchrotron / CNRS Institut Parisien de Chimie Moléculaire (IPCM) / Ligne de lumière DESIRS	**O19**
Bourgalais, Jérémy FRANCE	CNRS LRGP	
Bouvier, Caroline FRANCE	BnF Laboratoire scientifique de la bibliothèque nationale de France	**O02**
Briant, Marc FRANCE	CEA Saclay NIMBE	
Brosse, Ludovic SWITZERLAND	LNI Swiss Gas	
Brunelle, Alain FRANCE	CNRS / Sorbonne Université LAMS	
Burgers, Peter THE NETHERLANDS	Erasmus Medical Center, Rotterdam Department of Neurology, Laboratory of Neuro-Oncology	**O17**
Calabro, Kevin FRANCE	Muséum National d'Histoire Naturelle Chimie des Produits Naturels Fongiques et Bactériens	
Cao, Chenqin FRANCE	CEA Saclay Laboratoire Innovations en Spectrométrie de Masse pour la Santé (LI-MS)	**O35**
Casanova, Jean-Marie FRANCE	MS Vision	
Caselli, Alessandro ITALY	Università degli Studi di Milano Department of Chemistry	
Cellier, Benoit FRANCE	Sciex	
Chalet, Clément FRANCE	Sorbonne Université, CNRS Institut Parisien de Chimie Moléculaire (IPCM)	**P26**
Chassaing, Heather FRANCE	Sciex	
Chirot, Fabien FRANCE	Université Lyon 1, CNRS iLM	
Clodic, Gilles FRANCE	Sorbonne Université, CNRS Plateforme MS3U	

Colvin, Domnic POLAND	Nencki Institute of Experimental Biology PAS Cell Biophysics	
Cordeiro, Carlos PORTUGAL	Ciências, Universidade de Lisboa FT-ICR and Structural MS Laboratory	**P31**
Courraud, Julie GREECE	Department of Chemistry, University of Athens Laboratory of Analytical Chemistry	**O20**
Crotti, Sara ITALY	3A Laboratori - Lifeanalytics	
Damont, Annelaure FRANCE	CEA Saclay Laboratoire Innovations en Spectrométrie de Masse pour la Santé (LI-MS)	
Daniel, Régis FRANCE	CNRS LAMBE	
Dariy, Ekaterina FRANCE	Genoscope / CEA LGBM	
de Person, Marine FRANCE	Université Paris Saclay Institut Chimie Physique	
De Simone, Giulia ITALY	Istituto di Ricerche Farmacologiche Mario Negri, IRCCS Laboratory of Mass Spectrometry	
De Winter, Julien BELGIUM	University of Mons S^2MOs	
Delhaye, Thomas FRANCE	Université de Rennes Plateforme SM2	
Delsuc, Marc-André FRANCE	CNRS IGBMC	**P02**
Diaz Hermida, Marina FRANCE	Université d'Evry Val d'Essonne / Paris Saclay LAMBE UMR 8587	**P36**
Distefano, Alessia ITALY	University of Catania Laboratory of Bioinorganic Chemistry in neurodegenerative diseases	**P45**
Dossmann, Héloïse FRANCE	Sorbonne Université, CNRS Institut Parisien de Chimie Moléculaire (IPCM)	
Drabik, Anna POLAND	AGH University of Science and Technology Department of Biochemistry and Neurobiology	**P44**

Drahos, László HUNGARY	Research Centre for Natural Sciences MS Proteomics laboratory	**O03**
Drewello, Thomas GERMANY	Friedrich-Alexander-Universität Erlangen Physical Chemistry 1	
Dubocq, Florian FRANCE	ANSES UPBM	**P07**
Dumitrela, Diaconu ROMANIA	Alexandru Ioan Cuza Univesity of Iasi CERNESIM Centre	
Durand, Amélie FRANCE	CNRS Alpes ICMG	
El Omrani, Nesrine FRANCE	Université d'Evry Val d'Essonne / Paris Saclay LAMBE UMR 8587	**P13**
Elie, Nicolas FRANCE	CNRS ICSN	**P19**
Elseedy, Howida FRANCE	Université Paris Saclay Institut de Chimie Physique (ICP)	**P09**
Enjalbal, Christine FRANCE	Université de Montpellier IBMM	**P39**
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Fort, Laure FRANCE	Université Grenoble Alpes ICMG	
Freiberger, Markus GERMANY	Friedrich Alexander Universität Erlangen – Nürnberg Drewello Group	**P08**
Frison, Gilles FRANCE	Sorbonne Université, CNRS Plateforme MS3U – LCT	
Gahoual, Rabah FRANCE	Université Paris Cité Unité de Technologies Chimiques et Biologiques pour la Santé (UTCBS)	**O21**
Gatineau, David FRANCE	Université de Grenoble, CNRS Département de chimie moléculaire	

Gauthard, Florence FRANCE	Shimadzu	
Gerbaux, Pascal BELGIUM	University of Mons Organic synthesis and mass spectrometry laboratory	
Gimbert, Yves FRANCE	CNRS, Sorbonne Université, Université de Grenoble Département de Chimie Moléculaire (UGA) / Institut Parisien de Chimie Moléculaire (SU)	
Giuliani, Alexandre FRANCE	Synchrotron SOLEIL, INRAE	
Gosset-Erard, Clarisse FRANCE	CNRS / Université Paris Saclay ICSN	**P12**
Grasso, Giuseppe ITALY	University of Catania Department of Chemical Sciences	**O37**
Groignet, Louis BELGIUM	University of Mons S^2MOs	**P43**
Guérineau, Vincent FRANCE	CNRS ICSN	**P14**
Hägglund, Per DENMARK	University of Copenhagen Protein Oxidation	**O10**
Henrard, Gwendal BELGIUM	University of Mons Organic Synthesis and Mass Spectrometry	**P04**
Hueber, Amandine FRANCE	ANSES Laboratoire de sécurité des aliments	**O13**
Iacobucci, Claudio ITALY	Università degli Studi dell'Aquila Iacobucci Lab	**O16**
Jagora, Adrien FRANCE	Faculté de Pharmacie, Université Paris-Saclay BioCIS UMR-CNRS-8076	
Kervarec, Nelly FRANCE	Université de Bretagne Occidentale (UBO) SGPLAT plateforme de Masse	
Kinzelmann, Marina GERMANY	FAU Erlangen-Nürnberg Department of Chemistry and Pharmacy	**P05**
Király, Márton HUNGARY	Semmelweis University Department of Pharmaceutics	**P15**
Kjeldsen, Frank DENMARK	University of Southern Denmark Dept. Biochemistry and Molecular Biology	**O32**

Koszinowski, Konrad GERMANY	Universität Göttingen Institut für Organische und Biomolekulare Chemie	**O31**
Kret, Paulina POLAND	AGH University of Science and Technology Department of Analytical Chemistry and Biochemistry, Faculty of Materials Science and Ceramics	**P41**
Labous, Léna FRANCE	Université de Brest CEMCA, UMR UBO-CNRS 6521	**P10**
Lavanant, Hélène FRANCE	UNIROUEN COBRA UMR CNRS 6014	**O30**
Lavison-Bompard, Gwenaelle FRANCE	ANSES Laboratoire de sécurité des aliments	
Le Pogam-Alluard, Pierre FRANCE	Université Paris-Saclay BioCIS UMR CNRS 8076	
Legrand, Anaïs FRANCE	CEA Laboratoire Innovations en Spectrométrie de Masse pour la Santé (LI-MS)	**P32**
Lesage, Denis FRANCE	Sorbonne Université, CNRS Institut Parisien de Chimie Moléculaire (IPCM)	
Levasseur, Marceau FRANCE	CNRS, ICSN - UPR2301	**O23**
Le Ven, Jessica FRANCE	Shimadzu	
Liuu, Sophie FRANCE	ANSES SBCL unit	**P06**
Louarn, Essyllt FRANCE	Université Paris Saclay Institut de Chimie Physique & IRCELYON	
MacAleese, Luke FRANCE	CNRS, Université Lyon 1 Institut Lumière Matière (iLM)	
Marchand, Christophe FRANCE	CNRS, Sorbonne Université Laboratoire de biologie computationnelle et quantitative Institut de biologie Paris Seine	
Mark, Laszlo HUNGARY	University of Pecs, Medical School, Institute of Biochemistry and Medical Chemistry Analytical Biochemistry	**P27**
Marie, Arul FRANCE	MNHN PtSMBO	

Marie, Vincent FRANCE	CEA Saclay Laboratoire Innovations en Spectrométrie de Masse pour la Santé (LI-MS)	**O09**
Maroto, Alicia FRANCE	Université de Bretagne Occidentale (UBO) UMR 6521	**O33**
Martinelli, Francesca ITALY	Fondazione Edmund Mach Chimica Vitienologica e Agroalimentare	**P16**
Matheron, Lucrèce FRANCE	Sorbonne Université, CNRS Plateforme MS3U	
Memboeuf, Antony FRANCE	Université de Brest CEMCA, UMR UBO-CNRS 6521	**P04**
Menet, Marie-Claude FRANCE	Université Paris Saclay Institut de Chimie Physique (ICP)	
Mielczarek, Przemyslaw POLAND	Maj Institute of Pharmacology, Polish Academy of Sciences Laboratory of Proteomics and Mass Spectrometry	**P37**
Minasyan, Amalya FRANCE	Photonis	
Misicka-Kęsik, Aleksandra POLAND	University of Warsaw Peptide	**P11**
Molnar, Eva HUNGARY	Charles River Laboratories Hungary Kft	
Montero Bullón, Javier Fernando SPAIN	Universidad de Salamanca Metabolic Engineering Group	**O24**
Moustié, Anne FRANCE	L'Oréal ADME	
Muhieddine, Ali FRANCE	CNRS / Paris Saclay Institut des sciences moléculaires d'Orsay	**P17**
Nagy, Kinga HUNGARY	Research Centre for Natural Sciences MS Proteomics Research Group	**O14**
Ner-Kluza, Joanna POLAND	AGH University of Science and Technology Department of Analytical Chemistry and Biochemistry	
Nicol, Edith FRANCE	Ecole Polytechnique Laboratoire de Chimie Moléculaire (LCM)	**P23**
Novak, Petr CZECH REPUBLIC	Department of Biochemistry, Faculty of Science, Charles University Laboratory of biomolecule structure and function	

Ogden, Elliot FRANCE	Université de Rennes Institut de Physique de Rennes	**P51**
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Paquet, Laure SWITZERLAND	LNI Swiss Gas	
Paris, Alain FRANCE	MNHN MCAM	**P33**
Pashynska, Vlada UKRAINE	National Academy of Sciences of Ukraine B. Verkin Institute for Low temperature Physics and Engineering	**P38**
Passoni, Alice ITALY	Istituto di Ricerche Farmacologiche Mario Negri IRCCS Mass Spectrometry Laboratory	**O29**
Pittenauer, Ernst AUSTRIA	Vienna University of Technology Mass spectrometric bio- and polymer analysis	**O08**
Portašiková, Jasmína Mária CZECH REPUBLIC	Charles University in Prague Laboratory of Structural Biology and Cell Signalling	**P50**
Portela, José FRANCE	Waters	
Potier, Noelle FRANCE	CNRS / Université de Strasbourg LSMIS	
Poyer, Salomé FRANCE	CNRS ICSN	**O06**
Puyfaucher, Laetitia FRANCE	Nepita	
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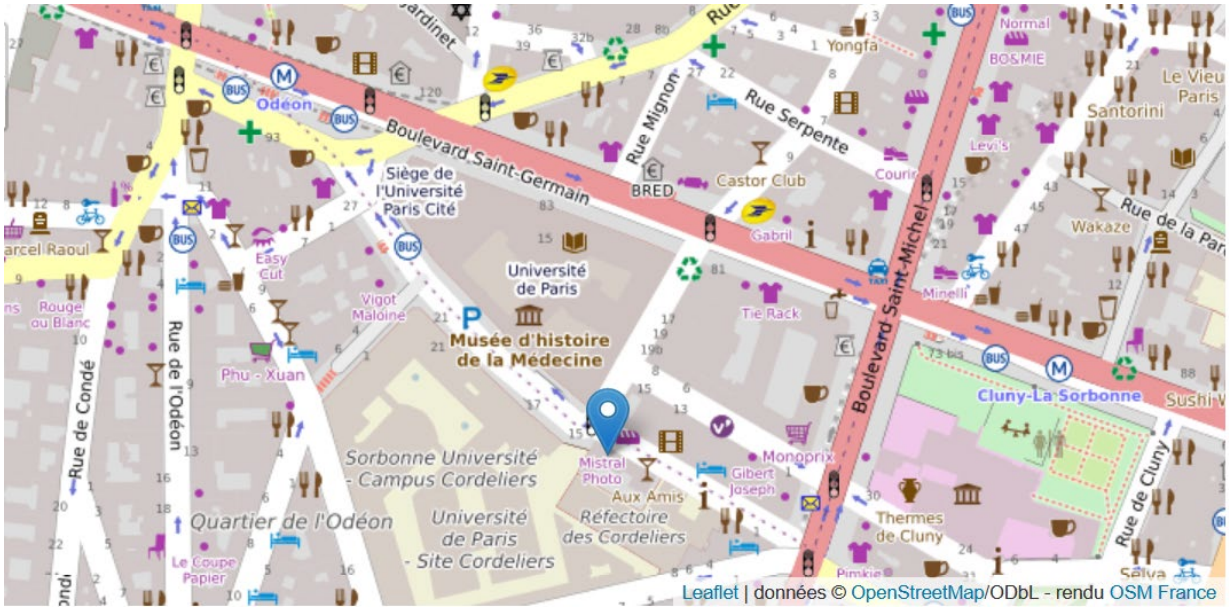
Rathahao-Paris, Estelle FRANCE	INRAE Laboratoire d'Immuno-Allergie Alimentaire	**P29**
Régnacq, Matthieu FRANCE	Sorbonne Université, CNRS Institut Parisien de Chimie Moléculaire (IPCM)	**O28**
Robert, Thomas BELGIUM	University of Mons S^2MOs	**P09**
Rondeau, David FRANCE	Université de Rennes Institut d'Electronique et des Technologies du numéRique (IETR)	

Rosé, Gauthier FRANCE	Sorbonne Université / Muséum National d'Histoire Naturelle Laboratoire d'Archéologie Moléculaire et Structurale (LAMS, UMR 8220) / Centre de Recherche sur la Conservation (CRC, USR 3224)	**P23**
Sablier, Michel FRANCE	CNRS Centre de Recherche sur la Conservation, Muséum National d'histoire Naturelle, UAR3224	
Sachon, Emmanuelle FRANCE	Sorbonne Université, CNRS Laboratoire des Biomolécules (LBM)	
Sadowski, Élodie FRANCE	Sorbonne-Université, CNRS Laboratoire des Biomolécules (LBM)	**O07**
Salpin, Jean-Yves FRANCE	CNRS LAMBE - UMR 8587	**P03**
Sanchez, Carlos FRANCE	CNRS / Sorbonne Université LISE UMR8235	
Savarino, Philippe BELGIUM	University of Mons S^2MOs	**O25**
Schmitz, Isabelle FRANCE	CNRS Laboratoire COBRA UMR 6014	
Scuderi, Debora FRANCE	CNRS / Université Paris-Saclay Institut de Chimie Physique (ICP)	
Sedlak, Eva HUNGARY	SOLVO Biotechnology | A Charles River Company Bioanalytics	
Segret, Juliette FRANCE	CNRS ICSN	**P24**
Servant, Félix FRANCE	Synchrotron SOLEIL/INRAE Ligne de lumière DESIRS	**P51**
Shafizadeh, Niloufar FRANCE	Université Paris Saclay Institut des Sciences Moléculaire (ISMO)	**O12**
Siyal, Asad Ali GERMANY	Proteome Sciences Single-cell Proteomics	
Smoluch, Marek POLAND	AGH University of Science and Technology Department of Analytical Chemistry and Biochemistry, Faculty of Materials Science and Ceramics	**P28**
Soep, Clément FRANCE	Sorbonne Université, CNRS Institut Parisien de Chimie Moléculaire (IPCM)	**P51**

Sousa Silva, Marta PORTUGAL	Ciências, Universidade de Lisboa FT-ICR and Structural MS Laboratory	**O11**
Spezia, Riccardo FRANCE	Sorbonne Université / CNRS Laboratoire de Chimie Théorique	
Suder, Piotr POLAND	AGH University of Science and Technology Biochemistry and Neurobiology Team	**P35**
Szmigielski, Rafal POLAND	Institute of Physical Chemistry Polish Academy of Sciences Environmental Chemistry Group	
Szwarc, Sarah FRANCE	Université Paris Saclay BioCIS	
Tömösi, Ferenc HUNGARY	Charles River Laboratories Hungary Bioanalytics	
Tabet, Jean-Claude FRANCE	Sorbonne Université, CNRS Institut Parisien de Chimie Moléculaire (IPCM)	**P20**
Tassignon, Benjamin BELGIUM	University of Mons Organic Synthesis and Mass Spectrometry Laboratory	**O39**
Tezcan, Songul TURKEY	Marmara University Faculty of Pharmacy	**P46**
Thissen, Roland FRANCE	UPC Institut de Chimie Physique (ICP)	**O36**
Touboul, David FRANCE	Université Paris Saclay ICSN	
Traldi, Pietro ITALY	Fondazione Istituto di Ricerca Pediatrica Città della Speranza Lab. Nano inspired biomedicine	**O40**
Tsirkou, Aikaterini FRANCE	Université Lyon 1, CNRS Spectrobio	**P51**
Valmori, Marie FRANCE	CNRS ICSN	**P29**
Van, Kim-Thanh FRANCE	CEA Saclay Laboratoire Innovations en Spectrométrie de Masse pour la Santé (LI-MS	
Van Agthoven, Marie CZECH REPUBLIC	Czech Academy of Sciences BIOCEV	**O01**
Vékey, Károly HUNGARY	Research Centre for Natural Sciences MS Proteomics	**O15**

Wargniez, William FRANCE	L'Oréal ADME	**P40**
Wilenska, Beata POLAND	University of Warsaw Laboratory of Peptide	**P34**
Witt, Matthias GERMANY	Bruker Daltonics GmbH &Co. KG MRMS	
Ye, Lei GERMANY	Department of Chemistry and Pharmacy, University of Erlangen-Nuremberg	**O04**
Zákopčaník, Marek CZECH REPUBLIC	Charles University Department of Biochemistry	**P21**

PRACTICAL INFORMATION



The **conference** will take place in the centre of Paris on the Cordeliers campus at Sorbonne University. The address of the campus is the following:

Sorbonne University - Campus des Cordeliers
15 rue de l'Ecole de médecine
75006 PARIS

The meeting will take place in the **Farabeuf amphitheater**. Once you enter the campus (main entrance), follow the signs which will direct you to the building (see the map of the campus below).

How to reach the conference site:

- **By public transportation:**

Two metro lines arrive close to the campus main entrance: lines 6 and 10 (stop at **Odéon** station). Several bus lines stop also nearby the campus (lines 38, 27, 58 (stop Rue des écoles or Odéon) and lines 63 et 86 (stop Cluny))

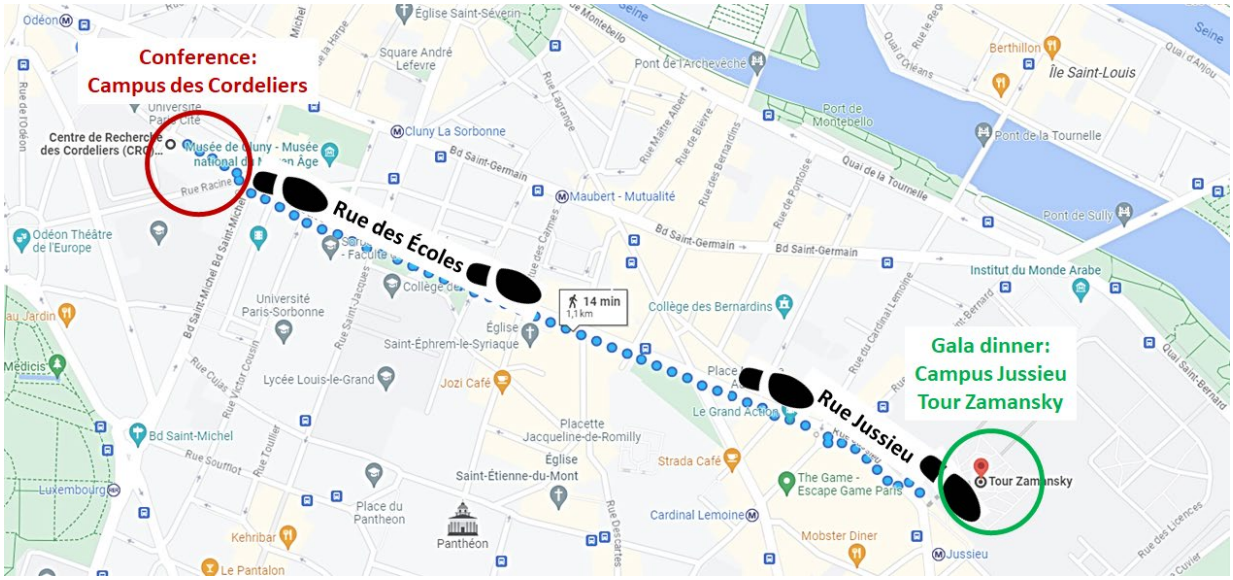
To define your journey, you can go to the **RATP public transportation site** or consult directly the **metro** or **bus** maps.

- **By car**

GPS coordinates of the campus are 48.85074234008789,2.341386079788208. No car parking is avalaible at the University.

- **By taxi**

Taxis can be reached from one of the numerous taxi stations in the city. You can also book from a taxi company. Two of the main ones are Alpha Taxis or G7 taxis.



The **Gala dinner** will take place on Tuesday, May 16th, from 7pm to 10pm.

The address is as follows:

Campus Jussieu, Tour Zamansky, 24th floor
4 place Jussieu
75005 Paris

It can be reached by foot from the conference site. A grouped departure will be organized if you want to join.

It is also accessible by metro from the conference site: line 10, stop at Jussieu station

Access to the gala dinner will be controlled. You will need to have your gala dinner ticket (given to you when you register to the conference), and your identity will be checked when you enter the Zamansky building.