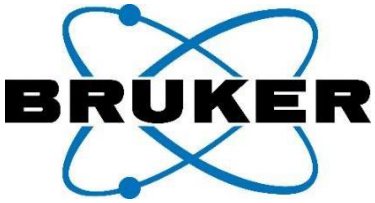
Journées Françaises de Spectrométrie de Masse

2021

En ligne du 14 au 24 juin 2021

Nos partenaires



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Contributions Orales

Lundi 14 juin

13H15-13H30	Ouverture	
13h30-14h15	**Renato Zenobi** *Temperature-programmed Native ESI Mass Spectrometry*	
14h15-14h35	Serge Della Negra	**Fondamentaux**
14h35-14h55	Marc Hagelin	
14h55-15h15	Aurélien Le Fèvre	
15H15-15H25	Pause	
15h25-15h40	Tofwerk	
15h40-16h00	**Prix de Thèse 2020 :** Jean-Arthur Amalian	
16H00-16h20	Evolène Deslignière	**Omics**
16h20-16h40	Clarisse Gosset-Erard	

O1. Mécanismes d'émission secondaire induits par nanoparticules d'or de quelques MeV

Isabelle Ribaud * [1], François Daubisse [1], Dominique Jacquet [1], Thi Than Huong Lai[2], Serge Della-Negra *[1]

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La plateforme Andromède[1] (ANR-10-EQPX-23) installée à l'IJCLab délivre des faisceaux d'agrégats et nanoparticules d'oraccélérés dans le domaine du MeV par un accélérateur électro-statique 4MV NecPelletron. Elle est dotée d'un dispositif expérimental conçu pour l'analyse de surface sous ultra-vide. Il comprend un porte cible motorisé (X, Y, Z), un ensemble de réglage et focalisation, et un détecteur d'ions secondaires multi-anodes fournissant la position des impacts et leur temps de vol. L'angle d'impact du faisceau primaire est de 45 par rapport à la normale de la surface de l'échantillon analysé.

Les rendements élevés d'ions secondaires obtenus par l'impact des nanoparticules dans la gamme du MeV sont spécifiques et liés à la taille du volume d'émission. Une valeur moyenne de l'ordre de 106 nm^3 par impact a été déterminée après irradiation de films polymères d'épaisseur de 50 et 120 nm. Un tel volume d'émission correspond à des centaines d'ions émis pour chaque impact. Le système d'acquisition et traitement de données, associé au détecteur multi-anodes permet l'identification des ions secondaires émis et la mesure de leur distribution angulaire et multi- plicité. Des sélections multiparamétriques, effectuées impact par impact, permettent d'extraire par l'analyse des coïncidences entre les différents ions secondaires la signature d'une co-émission de différents atomes et molécules et donc leur co-localisation dans un voxel (volume d'émission correspondant à un impact). Cette sélection permet aussi de déterminer les distributions de vitesses radiale et axiale pour un ion donné en fonction de son environnement sur la surface. Ce type d'analyse, effectué sur les ions positifs et négatifs provenant du dépôt organique comme sur ceux issus du substrat, met en évidence différentes séquences dans le processus d'émission secondaire induits par ces projectiles massifs. Ces différents processus seront illustrés par des exemples obtenus pour des dépôts de molécules organiques de masse variant de quelques dizaines à un millier de Da.

1.Andromede Project: Surface Analysis and Modification with Probes from Hydrogen to Nano- Particles in the MeV Energy Range, M.J. Eller, E. Cottereau, B. Rasser, E. Verzeroli, B. Agnus, G. Gaubert, X. Donzel, A. Delobbe, 2.S. Della-Negra, Nucl. Instrum. Meth. B: Beam Interactions with Materials and Atoms, 51(décembre 2015) Volume 365, Part A, Pages 367-370

O2. Super-resolution in FT-ICR MS by non-Fourier Transform genetic evolution signal processing

Marc Haegelin * [1], Ulviya Abdulkarimova [2,3], Christian Rolando [1,4], Pierre Collet[2]

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[2] University of Strasbourg, (ICUBE) – University of Strasbourg : UMR7357 CNRS, University of Strasbourg : UMR7357 CNRS – France

[3] Azerbaijan State University of Oil and Gas Industry (ASOIU) – Azerbaïdjan

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Introduction:

FTICR signal, which is a sum of damped sines, is processed by Fourier transforms (FT) to afford a frequency spectrum and then a mass spectrum. FT processing has important drawbacks as the FT of a time limited constant sine is a cardinal sine of FWHM proportional to the inverse of acquisition duration whose side-lobes can be inadvertently mistaken for real peaks. Apodization, used to solve the cardinal sine side-lobes, problem widens the peak, thus decreasing the resolving power, and modifies the isotopic mass ratios. We present here a non-Fourier Transform ICR signal processing by a genetic evolution algorithm running on GPU (Graphics Processing Unit) we named sinus_it, which searches for the damped sine in the time domain.

Methods:

Sinus_it was first tested on experimental and simulated data of substance P acquired on a Bruker FT-ICR 9.4 Tesla mass spectrometer using 2 16 cores 2.1 Ghz Intel Xeon server fitted with a gaming Nvidia RTX 2080 Ti GPU. Sinus_it has two modes, corresponding to coarse and fine distributions. In coarse mode sinus_it determines the 4 parameters of each sine amplitude, frequency, damping factor and phases. In fine mode the phases obtained in the coarse mode are used. Sinus_it execution time is linearly proportional to the size of the signal and the number of the searched sines.

Results:

On a 32k points simulated spectrum sinus_it was able to reliably find the 6 first isotopes, although the amplitude of the last one was equal to the amplitude of the white noise we added to the pure signal. On a real substance P signal we were able to find 5 first isotopes, and above all their phases. For both datasets, until the fourth isotope, accuracies better than 0.1 ppm was obtained for frequency and better than 0.1% for intensity, phase and damping. We then focused on finding the fine isotopic structures of the 1st isotopic peak. The frequencies of the closer fine isotopes are separated by 0.46 Hz. Using a sampling rate of 1M points.s-1, FT need at least 4M points, so 4 s to distinguish them. Sinus_it was able to reliably find these 3 fine isotopes bothon simulated and experimental signals on 1M point spectrum recorded in only 1 s, reaching a 4 times super-resolution close to the theoretical limit.

Conclusion:

Taking advantage of the parsimony of the FTICR signal i.e. the low number of peaks in compar- ison to points in the signal, sinus_it affords super-resolution, better mass accuracy and isotopic ratios than FT processing.

O3. Explorer le Paysage Conformationnel à l'aide de la Spectrométrie de Mobilité Ionique en Tandem

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[2] Institut Lumière Matière [Villeurbanne] – Université Claude Bernard Lyon 1, Université de Lyon, Centre National de la Recherche Scientifique : UMR5306 – France

La dynamique et la thermodynamique des changements de structures du Glu-Fibrinopeptide B isolé (GluFib) ont été étudiées par spectrométrie de mobilité ionique (IMS) en tandem. Des ions GluFib2+ doublement protonés ont d'abord été sélectionnés par IMS, puis stockés pendant une durée contrôlée dans un piège à ions thermalisé. Ensuite, les changements de conformations induits par cette thermalisation ont été suivis par une seconde IMS en fonction du temps de piégeage. À partir de ces procédés, les vitesses d'isomérisation et les populations d'équilibre des différents conformères ont pu être déterminées en fonction de la température.

Nous démontrons que les quantités thermodynamiques mesurées peuvent être directement com- parées aux observables simulées à partir d'une modélisation moléculaire d'ensembles, basée sur des paramètres d'ordre appropriés. Nous avons obtenu un bon accord qualitatif avec les simulations dites "Replica-Exchange Molecular Dynamics" (REMD), basées sur le champ de force AMOEBA et traitées à l'aide de la méthode dite "Weighted Histogram Analysis Method" (WHAM). Cela suggère que l'équilibre entre la répulsion coulombienne et l'autosolvatation optimale liée aux charges est la principale source de la bistabilité conformationnelle observée.

Nos résultats soulignent les différences entre les distributions de quasi-équilibre cinétiques, obtenues par activation par collisions, et les distributions de quasi-équilibre thermodynamiques des expériences actuelles, dues aux effets entropiques. En conséquence, nos mesures permettent non seulement de déterminer directement les énergies d'activation d'Arrhenius, mais aussi de déterminer les changements d'enthalpie et d'entropie relatifs associés à une transition de structures.

O4. Increased Collision-Induced Unfolding Experiments Throughput using Online Size Exclusion Chromatography Coupled to Native MS

Evolène Deslignière * [1], Anthony Ehkirch [1], Thomas Botzanowski [1], Alain Beck[2], Oscar Hernandez-Alba [1], Sarah Cianferani [1]

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[2] IRPF - Centre d'Immunologie Pierre-Fabre (CIPF) – Centre d'Immunologie Pierre-Fabre (CIPF), 74160 Saint-Julien-en-Genevois, France – France

Ion mobility (IM)-based collision-induced unfolding (CIU) has gained increasing attention to probe gas-phase unfolding of proteins and their noncovalent complexes, notably for biotherapeu- tics [1]. CIU detects subtle conformational changes of proteins based on their unfolding patterns, and emerges as an attractive alternative to circumvent poor IM resolution [2-4]. However, the CIU pipeline still lacks in automation for sample preparation (buffer exchange) and on-line data acquisition, precluding its wide adoption. CIU experiments are performed by raising collision voltages in the trap cell of travelling wave IM spectrometry (TWIMS) instruments before IM separation, either manually or in a semi-automatic way using sequential data acquisition through a pre-programmed sample list, resulting in a tedious and time-consuming process which ham- pers its routine use. We present here an automated workflow for CIU experiments, from sample preparation to data interpretation using online size exclusion chromatography coupled to native IM mass spectrometry (SEC–CIU). Online automated SEC–CIU experiments offer several ben- efits over nanoESI–CIU, among which are (i) improved and fast desalting compared to manual buffer exchange used for classical CIU experiments; (ii) drastic reduction of the overall data col- lection time process; and (iii) maintaining the number of unfolding transitions. We then evaluate the potential of SEC–CIU to distinguish monoclonal antibody (mAb) subclasses, illustrating the efficiency of our method for rapid mAb subclass identification at both intact and middle levels. Finally, we demonstrate that CIU data acquisition time can be further reduced either by setting up a scheduled CIU method relying on diagnostic trap collision voltages or by implementing mAb-multiplexed SEC–CIU analyses to maximize information content in a single experiment. Altogether, our results confirm the suitability of SEC–CIU to automate CIU experiments, par- ticularly for the fast characterization of next-generation mAb-based products.

1.Dixit, S. M.; Polasky, D. A.; Ruotolo, B. T., Curr. Opin. Chem. Biol. 2018, 42, 93-100. Tian, Y.; Han, L.; Buckner, 2.A. C.; Ruotolo, B. T., Anal. Chem. 2015, 87 (22), 11509-15.

3.Hernandez-Alba, O.; Wagner-Rousset, E.; Beck, A.; Cianferani, S., Anal. Chem. 2018, 90 (15), 8865-8872.

4.Botzanowski, T.; Hernandez-Alba, O.; Malissard, M.; Wagner-Rousset, E.; Deslignière, E.; Colas, O.; Haeuw, J.-F.; Beck, A.; Cianférani, S., Anal. Chem. 2020, 92 (13), 8827-8835.

O5. Characterization of post-transcriptional RNA modifications by sheathless capillary electrophoresis – mass spectrometry

Clarisse Gosset-Erard * [1,2], Antony Lechner [3], Philippe Wolff [3], Emmanuelle Leize-Wagner [1], Frédéric Aubriet [2], Yannis-Nicolas François [1], Patrick Chaimbault [2]

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RNA post-transcriptional modifications are of great interest due to their involvement in many functions and structural characteristics of RNA. Indeed, those modifications deeply in- fluence RNA structural rigidity and stability, base pairing, and protein translation. Despite its great interest, only poor information on RNA modifications is currently available. To meet the need of characterization of post-transcriptional RNA modifications, several analytical methods have been developed for their localization, the most common being the RNA sequencing tech- niques.
Capillary zone electrophoresis (CZE) is a separative technique based on the migration of analytes under an electric field according to their size and charge. This technique provides high separation efficiency and can be hyphenating to mass spectrometry (MS). Over the years, MS became a reference method for structural elucidation thanks to its specificity and sensitivity. CZE-ESI-MS hyphenation can be performed thanks to a sheathless interface generating a nano-electrospray (nanoESI) which therefore allows high detection sensitivity.

As RNA modifications generally lead to a mass shift and/or charge shift, a novel workflow based on CZE-ESI-MS/MS, in positive mode, has recently been developed by our team to characterize tRNA modifications. This workflow is based on a "bottom-up" approach with a double specific digestion using RNase T1 and RNase A, followed by a dephosphorylation using a bacterial al- kaline phosphatase. The oligonucleotides are then analyzed by CZE-ESI-MS/MS. This method enables the sequencing of RNA and mapping of modifications. Since no software is available for RNA sequencing in positive mode, data interpretation is performed manually. The proof of concept was performed on four transfer RNA from *Saccharomyces cerevisiae* and sequence coverages up to 97% were obtained. Furthermore, unambiguous identification of oligonucleotides and mapping of several modifications were achieved.
To pursue this work, we tested this method on a more complex case: a 16S ribosomal RNA of *Pseudomonas aeruginosas* which is 20-times bigger than transfer RNA and whose only canonical sequence is known.

Mardi 15 Juin

9h00-9h45	**Isabelle Compagnon** *TBA*	
9h45-10h05	Eric Largy	**Santé**
10h05-10h25	Marie-Laure Pons	
10h25-10h45	Lucas Roussell	
10H45-10H55	Pause	
10h55-11h10	Thermo *Orbitrap IQ-X Tribrid mass spectrometer and FAIMS Pro Duo interface: next generation instruments for to small molecule analysis*	
11h10-11h30	Laetitia Fougere	**Analyse ciblée et non-ciblée**
11h30-11h50	Salomé Poyer	
11h50-12H10	Posters	Vague A
12H10-12H30		

O6. Native Hydrogen/Deuterium Exchange Mass Spectrometry of Structured DNA Oligonucleotides

Eric Largy * [1], Valérie Gabelica *[1]

[1] Univ. Bordeaux, CNRS, INSERM, ARNA, UMR 5320, U1212, IECB, F-33600 Bordeaux, France (IECB) – Université de Bordeaux, Institut National de la Santé et de la Recherche Médicale – INSERM: U1212, CNRS : UMR5320 – France

Although solution hydrogen-deuterium exchange mass spectrometry (HDX/MS) is well-established for the analysis of the structure and dynamics of proteins, it is currently not exploited for nucleic acids. We recently demonstrated that structured DNA oligonucleotides are amenable to in-solution HDX/MS in native conditions, on an Exactive Orbitrap mass spectrometer (1).
In trimethylammonium acetate solutions and in soft source conditions, the protonated phosphate groups are fully back-exchanged in the source, while the exchanged nucleobases remain labelled without detectable gas-phase back-exchange. As a result, the exchange rates depend strongly on the secondary structure (hydrogen bonding status) of the oligonucleotides, but neither on their charge state nor on the presence of non-specific adducts. We show that native mass spectrometry methods can measure these exchange rates on the second-to-day time scale with high precision and accuracy. An open-source, dedicated software was developed for data treatment and mod- elling purpose.

We applied this approach on a panel of G-quadruplex forming sequences, for which we found that the exchange rates are strongly dependent on their structures. Using native MS, complexes formed with small-molecule ligands can be mass-resolved and their exchange behaviour compared to free oligonucleotides. This way, we were able to evidence changes in both conformation and dynamics upon complex formation. Adding a shape-separation step by coupling native HDX/MS with drift-tube ion-mobility spectrometry, we were able to determine the exchange rates of indi- vidual conformers in dynamic equilibrium formed by a single DNA sequence.

Coupling in-solution HDX with native MS therefore opens promising avenues for the analysis of the structural and biophysical properties of oligonucleotides and their complexes that remain challenging by traditional methods.

(1) Largy, E.; Gabelica, V. Native Hydrogen/Deuterium Exchange Mass Spectrometry of Struc- tured DNA Oligonucleotides. ***Anal. Chem. 2020****, 92 (6), 4402–4410*

O7. A multiplex targeted Mass spectrometry approach for the quantification of synuclein proteoforms in human biological fluids (CSF, plasma and saliva)

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[3] Institut de recherche en biothérapie (IRB) – CHRU Montpellier – Hôpital Saint-Eloi 34000 Montpellier, France

The major hallmark of synucleinopathies (Parkinson's disease, Lewy body dementia and Multiple system atrophy) is to retrieve intracellular inclusion called Lewy bodies in intraneuronal space [Vinnakota et al, 2018; McLean et al, 2012]. Lewy body are largely composed of alpha synuclein included phosphorylation, ubiquitination and aggregated form. Indeed, the synucleins and its proteoforms are present at different levels in brain, CSF and plasma in patient with synucleinopathies compared to control [Schmid et al, 2013; Otto et al, 2016; Zetterberg et al, 2020]. In this context, our objective is to develop a multiplexed and targeted mass spec- trometry method to quantify synucleins proteoforms in human biological fluid (CSF, plasma and saliva). For this purpose, we developed a bottom up targeted mass spectrometry approach based on protein precipitation followed by a clean-up and proteolytic digestion before Liquid Chromatography-Multiple Reaction Monitoring (LC-MRM) analysis. Results showed that we can follow 14 peptides originated from the alpha, beta and gamma synuclein. We added to our method a hemoglobin peptide to check out blood contamination in CSF and saliva. Finally, we succeeded to monitor total alpha (140aa), beta (134aa) and gamma (127aa) synuclein peptides, plus 4 alpha synuclein proteoforms (126, 112, 98 and 41) using 3 proteotypic peptides (one for 126/98, one for 112/98 and one for 41). Thanks to the multiplexed method, we obtained a protein recovery between 50-70% for alpha, between 30-45% for beta and between 35-50% for gamma synuclein depending on the biofluids analyzed. The analytical validation (LOD, LOQ, repeatability, reproducibility, matrix effect...) of synucleins LC-MRM method was performed following the EMA guidelines. The clinical validation is ongoing, and we use a cohort of more than 200 well stratified patients (plasma and CSF) with synucleinopathies.

O8. Développement d'un système robotisé d'analyse in vivo en temps-réel par spectrométrie de masse pour la chirurgie guidée des cancers

Lucas Roussel * [1]

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La qualité de la chirurgie liée à un cancer dépend de la capacité du chirurgien à identifier puis exciser l'ensemble des cellules cancéreuses afin d'éviter une récidive. Cependant, les prati- ciens sont confrontés à la difficulté de retirer tout le tissu cancéreux sans pour autant exciser inutilement du tissu sain et nuire à la qualité de vie du patient. De ce fait, en cas de doute, le chirurgien est contraint de prendre des marges chirurgicales autour de la masse tumorale lors de la chirurgie initiale voir, dans le cadre du cancer de l'ovaire, de procéder à une hystérectomie. De plus, les examens histopathologiques réalisés par le pathologiste présentent de nombreux inconvénients. Premièrement, ces résultats parviennent au chirurgien 20 à 45 min après la ré- section, rallongeant l'opération. De plus, ils sont réalisés à l'aide de colorations histologiques sans possibilité de récolter de données moléculaires ; entraînant un taux d'erreur qui peut aller jusqu'à 30% suivant les cancers. La technologie SpiderMass permettant l'analyse *in vivo*, en temps réel des tissus se basant sur le micro-échantillonnage de ceux-ci à l'aide d'un laser émettant dans le moyen IR suivi d'une analyse par spectrométrie de masse permet l'analyse de tissu de manière mini-invasive et de réaliser des classifications des types et grades de cancer.

Mon projet de thèse porte sur le développement de la technologie SpiderMass pour la chirurgie du cancer de l'ovaire via la mise en place d'un système robotisé. Dans un premier temps, je travaille à la mise en place d'une banque de spectres MS et d'une classification des différents sous-types de cancers de l'ovaire à partir d'échantillon de patientes via l'analyse de leur profil métabo-lipidique afin de pouvoir reconnaître l'état histopathologique de ce tissu en temps réel in vivo pendant la chirurgie et d'identifier les lipides permettant la discrimination de ces différentes classes.

En parallèle, je réalise une classification moléculaire de lignées de cellules du cancer de l'ovaire et de cellules saines dans l'optique de comparer les modèles cellulaires aux tissus des patients. Par la suite, j'utiliserai les modèles cellulaires pour réaliser des modèles de marge d'exérèse par la technique de bioprinting de plusieurs types cellulaires. Ce modèle sera analysé par de l'imagerie 3D SpiderMass robotisée et les données obtenues comparées à la classification réalisée, afin de déterminer la capacité du SpiderMass à trouver les cellules cancéreuses présentent dans ces mod- èles de marge. Finalement, les tests seront reproduits sur des tissus de patientes pour la mise en place d'un système robotisé et reposant sur l'AI pour la définition moléculaire par MS des marges avec une grande précision.

O9. La cartographie pour faciliter la caractérisation d'un extrait de soie de maïs pas MS et MS/MS

Laëtitia Fougere * [1], Cyril Colas [1,2], Sandrine Zubrzycki [1], Claire Elfakir [1], Emilie Destandau [1]

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[2] Centre de biophysique moléculaire – Université d'Orléans : UPR4301, Centre National de la Recherche Scientifique : UPR4301 – France

L'analyse globale d'un extrait naturel effectué par UHPLC/HRMS produit un nombre très élevé de données à interpréter, ce qui reste une tâche ardue et chronophage. Il devient nécessaire d'utiliser des outils complémentaires de cartographie des composés afin de faciliter leur interprétation et réduire sa durée.
Tout d'abord par l'apport de la masse exacte, une première cartographie peut être réalisée avec le diagramme de Kendrick qui représente le défaut en masse. La cartographie de Kendrick per- met d'observer les différences de fonction entre chaque composé proche et elle aide à lever des indéterminations de formules brutes. Puis après l'obtention des formules brutes, un diagramme de van Krevelen peut être tracé en reportant les rapports des atomes des différentes formules brutes. Ce diagramme permet de classifier les molécules carbonées, oxygénées ... Une première information de famille moléculaire peut également y être associée.

Afin d'aller plus loin dans la connaissance de l'échantillon, une carte de similarité spectrale peut être créée en utilisant les données MS/MS issues de la fragmentation des ions moléculaires. Le réseau moléculaire généré permet de regrouper les composés qui se fragmentent de la même façon en un cluster. L'identification des familles moléculaires obtenues précédemment, associées au réseau moléculaire, facilite l'annotation des clusters en vue de proposer des identifications avec un niveau de confiance plus élevé.

Les cartographies de Kendrick et de van Krevelen sont souvent utilisées dans le domaine pétrolier, elles sont peu utilisées dans la caractérisation des produits naturels, contrairement aux réseaux moléculaires, qui ont été développés pour aller plus vite dans la compréhension de la composition des extraits naturels. C'est pourquoi la combinaison de ces différentes approches apporte une complémentarité permettant de détailler au mieux un extrait complexe.
Cette méthode appliquée à un extrait alcoolique de soie de maïs a permis de mettre en évidence deux familles moléculaires principales (composés lipidiques et phénoliques). De plus, des sous familles de flavonoïdes ont pu être distinguées en fonction de leur type de liaison glycosidique (O-glycosylé ou C-glycosylé, mono C-glycosylé-O-glycosylé).

O10. Structural identification of acetogenins by tandem mass spectrometry of copper-adducted ions.

Salomé Poyer * [1], Laurent Laboureur [1], Jean-Yves Salpin [2], Pierre Champy[3], David Touboul [1]

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Annonaceous acetogenins are natural products found in the leaves and edible fruits of the *Annona* trees. These molecules have a strong cytotoxicity and are proposed as pesticides and anti-tumor agents. They have also been identified as environmental neurotoxins, probably re- sponsible for Guadeloupean parkinsonism.1 Since light has been shed on possible long-term neurotoxic effects in humans, the full characterization of acetogenins has become crucial.
Acetogenins consist of 35 or 37 carbon atoms bearing a terminal butyrolactone. They have an alkyl chain with oxygen-containing substituent and a central oxygen ring domain such as tetrahydrofuran, tetrahydropyran or epoxide whether or not flanked by hydroxyl groups. Today, nearly 500 analogues have been reported indicating the structural variability of these compounds. The NMR characterization of these analogues is difficult due to the length of the alkyl chains, which allow to determine the chemical functions but not their complete location on the molecule.

Characterization by tandem mass spectrometry (MS/MS) using collision-induced dissociation (CID) of protonated species lead to several losses of H2O molecules. It has been shown that MS/MS experiments using lithium adducts provided additional structural information compared to protonated or sodiated species.2-3 However, diagnostic ions are present at a very low intensity, which does not allow a full interpretation of the minor species. In order to improve the structural information of CID experiments, we used copper-adduct as the CuII/CuI reduction produces a free radical that induces particular dissociation pathways.4

Copper-cationized acetogenins submitted to CID lead to intense diagnostic fragment ions allowing the localization of oxygen rings and hydroxyl functions. The main fragments result from the cleavage of C-C bonds on both parts of the ring. In addition, cleavage of the C-C bond next to hydroxyl groups in the alkyl chain allows their unambiguous localization. However, copper cationized acetogenins do not have characteristic fragments when ketone functions are present. To circumvent this limitation, lithium-adducted LC-MS/MS experiments were performed in par- allel, to localize the ketone functions. Fragmentation rules were established based on acetogenin standards and dozens of analogues were identified in an extract of *Annona muricata* by LC- MS/MS using post-column infusion of LiI or CuSO4 salts.

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2. *J. Allegrand; D. Touboul, et al., Rapid Commun. Mass Spectrom.* ***2010,*** *24 (24), 3602- 3608.*
3. *J. Le Ven; I. Schmitz-Afonso, et al., J. Mass Spectrom.* ***2012,*** *47 (11), 1500-1509.*
4. *C. Afonso; A. Riu, et al., J. Mass Spectrom.* ***2005,*** *40 (3), 342-349.*

Jeudi 17 juin

13h30-14h15	**Julia Laskin** *TBA*	
14h15-14h35	Nina Ogrinc	**Imagerie**
14h35-14h55	Justine FEREY	
14h55-15h15	Caroline Bouvier	
15H15-15H25	Pause	
15h25-15h40	Jeol *Atteindre les Hautes Performances : Le nouveau spectromètre de masse JEOL à Temps de Vol : le JMS-T2000GC AccuTOFTM GC Alpha*	
15h40-16h00	Stéphanie FLAMENT	**Omics**
16H00-16h20	Julien Faugere	

O11. Robot-Assisted SpiderMass for in vivo real-time topographical molecular imaging

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Surgery of solid tumors is a difficult process and the quality of surgery is central for patient outcome. Despite important improvements in surgical practices, the completeness of tumor removal remains scarce and surgeons are still struggling in the decision making. The gold standard currently remains the collection of biopsies and examination by expert pathologist. This pro- cess is long and laborious while showing an important rate of wrong assessment. We recently introduced SpiderMass technology based on water-assisted laser desorption-ionization (WALDI) which enables to retrieve the necessary molecular information directly *in-vivo* and in real-time for point-to-point care. However, for intraoperative diagnostics and excision margins evaluation, the device must be integrated onto a robotic device to provide precise imaging of the defined area within the body.
The SpiderMass system is composed of a remote laser microprobe, a transfer line and the MS instrument. The laser microprobe is fibered and equipped with a handpiece and tuned to 2.94 μm. The desorbed material is transferred to a QTOF MS instrument through a tubing of several meters connected to the inlet of the MS instrument using a dedicated interface. For combined topological and molecular imaging, The SpiderMass system was coupled to a commer- cially available stiff 6D-axis precision MECA robotic arm (MECADEMIC, Montreal, Canada) with repeatability of 5 μm. The handpiece is attached to the robotic arm by a "home-made" 3D printed adaptor equipped with a distance sensor to capture the topographical image in real-time. The molecular data is then plotted back onto the topographic surface.
We programmed the robotic arm to generate topographical and molecular information. This is achieved in two steps. First, the investigated specimen is placed under the arm and the X, Y, and Z coordinates can be recorded point by point depending on the defined imaging area. Second, the arm was programmed to rescan the object while keeping the right focusing distance of the laser microprobe to generate the molecular data. Both acquired data were aligned in time and fused to plot the molecular distribution onto a topographic image. Preliminary tests were run at 500 μm resolution from a sponge piece withs spotted lipid standards which presents specific topography. Next the system was applied onto biological samples starting with model tissues including beef liver, apple core with seeds and then translated to skin biopsies. Finally, the system was applied inside of the body of the mouse to demonstrate the potential of future *in vivo* imaging.
The integration of the Spidermass MS-based technology onto a robotic arm of high accuracy was shown to enable imaging surfaces in 3D by MS molecular-topographic imaging. These developments give a start to *in vivo* imaging and open a new way for the technology to get it integrated within the set of conventional surgical tools.

O12. Mass Spectrometry Imaging based on FTICR: application to Pt based anticancer drugs detection in human tissue.

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[2] ToxAlim – Université Toulouse III - Paul Sabatier, Université Fédérale Toulouse Midi-Pyrénées, Institut National Polytechnique (Toulouse), Université Fédérale Toulouse Midi-Pyrénées : UMR1331, Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement – France

[3] Metatoul-AXIOM platform, National Infrastructure for Metabolomics and Fluxomics: MetaboHUB, Toxalim, INRAE, 31027 Toulouse, France – MetaToul-MetaboHUB, National infrastructure of metabolomics and Fluxomics, Toulouse, France – France

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Peritoneal carcinomatosis is a common extension of colon cancer. Platinum derivatives, such as oxaliplatin, are common metallodrugs applied during the Heated Intraoperative Chemother- apy (HIPEC), which consists in a local application of a high concentration of drug. During the HIPEC procedure, the metallodrug circulates into the peritoneal cavity and can affect other organs such as ovaries. Knowledge of penetration and distribution of this drug within the ovaries will help to better understand the effects of the drug on ovary functionality.

Mass spectrometry imaging (MSI) is a suitable tool for the evaluation of drug penetration. Pre- vious work has been published based on MALDI-TOF instrument.1 Further developments are carried out within this study with a Fourier Transform Ion Cyclotron Resonance Mass Spectrom- eter (FTICR-MS) in order to have a higher selectivity for precise image reconstruction and also for the unambiguous molecular formula attribution taking advantage of elemental fine structure. FTICR-MS is used to analyse extremely complex mixtures such as metabolome samples thanks to its high mass accuracy and ultrahigh resolution. This allows to confidently assign molecular formulas and to avoid any isobaric interference.2

Ovary tissue sections were mounted onto indium tin oxyde coated slides and coated with α-cyano-4-hydroxycinnamic acid matrix. MALDI-FTICR MSI of metabolites was performed using a 12 T SolariX XR (Bruker) in positive ion mode to search for platinum containing metabolites. Transmission parameters were optimised for the *m/z* 150-1000 range. Internal mass calibration was implemented to get accuracies below 1 ppm for each scan. Data treatment was realised with SCILS software. One platinum derived compound was localised on the 6 ovary tissue sections analyzed. Its elemental composition was confirmed thanks to the high mass accuracy and iso- topic fine structure.

1. *Bianga J. et al* Metallomics*, 2014, 6, 1382.*
2. *Kihara M. et al* JASMS*, 2017, 28, 2469.*

O13. Approfondir la connaissance de la technique d'un grand peintre du XVIIème siècle grâce à une mise en œuvre optimisée de l'imagerie TOF-SIMS

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Nicolas Poussin (1594-1665), figure majeure de la peinture du 17ème siècle, est un peintre français qui évolua à Rome une grande partie de sa vie d'artiste. Avec l'appui essentiel d'une spécialiste de ce dernier, la technique de Poussin a été au cœur de plusieurs études au LAMS par le passé, *via* des techniques invasives et non invasives (1-3).

Une peinture est construite en couches successives (du μm à quelques 100 μm) de divers mélanges de pigments dispersés dans un ou plusieurs liants, comme l'huile, l'œuf ou la colle. Une peinture ancienne est un objet unique et d'une valeur immatérielle, quand un micro-prélèvement est fait, il est préconisé de le conserver intact pour permettre des analyses ultérieures et en tirer le maxi- mum d'informations. L'échantillon expose simultanément toutes les couches de la stratigraphie, de la couche la plus profonde jusqu'au vernis.

L'analyse TOF-SIMS est une analyse de surface qui, grâce à l'extraction retardée des ions secondaires, permet d'imager à l'échelle sub-micrométrique cette succession de couches, avec les informations spectrales organique et inorganique simultanées et à haute résolution en masse (1,4,5). Cette séparation combinée en masse et en localisation permet d'accéder à la nature des liants, pigments et leurs éventuels produits de dégradation. Il est en outre possible d'accéder à une information en profondeur en intercalant entre chaque analyse une pulvérisation douce de la surface, pour analyser la matière sous-jacente intacte. En s'appuyant sur la connaissance des ions secondaires émis de façon caractéristique pour chaque ingrédient, tout en prenant en compte l'évolution temporelle de ces derniers lors du séchage et d'éventuelles altérations, il est alors possi- ble de déduire de nombreuses informations sur la structure de la stratigraphie. L'analyse permet alors de faire le lien avec les techniques traditionnelles des peintres de l'époque pour mieux com- prendre comment la peinture a été réalisée, et si besoin, comment la préserver de dégradations.

Les contraintes analytiques liées à l'inhomogénéité de ce type d'échantillon ont imposé un soin particulier à l'optimisation des conditions d'analyse pour maximiser la qualité et la détection de tous les composés en présence, malgré leur nature et localisation diverses. Dans ces conditions, il a été possible d'identifier le liant des tableaux *Bacchanales d'enfants*, d'imager et identifier au sein de la couche colorée les différentes particules de pigment constituant un mélange caractéris- tique utilisé par le peintre, ainsi qu'une succession de fines couches organiques en surface de la peinture, dont l'origine requiert l'appui des connaissances en histoire de l'art.

(1) Noun, et al. 2016; (2) Glanville, Kermes 2014; (3) Fanost, et al. 2020; (4) Vanbellingen, et al. 2015; (5) Bouvier, et al. 2021

O14. Proteomic characterization of intermediate and advanced glycation end-products in bovine serum albumin

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Introduction: Glycation, also called Maillard reaction, is a chemical reaction responsible for most yellow-to-brown colors and aromas in cooked foods. This reaction occurs between the amino group of proteins and the carbonyl group of reducing sugars (glucose, fructose...), leading to the formation of a heterogeneous and complex group of molecules called "Advanced Glycation End Products" (AGEs). These AGEs products are present in high amounts in ultra-processed foods and have been detected in kidneys in which they exhibit nephrotoxicity. Glyoxal and glyoxylic acid are two potential intermediates in the formation of these AGE structures. This work aims to study glyoxal- and glyoxylic acid-induced modifications of bovine albumin and to evaluate their contribution to the formation of AGEs.

Method: Fifty µg of BSA were digested using the eFASP method with 3 different enzymes (trypsin, GluC and Chymotrypsin). Each digestion was fractionated on High pH Reversed- Phase Peptide Fractionation Kit. The peptides were analyzed by nanoESI-LC-MS/MS on a Q-exactive plus MS. Proteins were identified using PEAKS X plus software against BSA se- quence. CML, (Carboxymethyl)arginine (CMA), glyoxal-derived hydroimidazolones (G-H1, H2 and H3) and carboxyethyllysine (CEL) modifications were searched. GODIC, GALA, GOLD, GOLA crosslinks were investigated using MassSpecStudio.

Results: A sequence coverage of 70% was obtained on highly modified BSA . CML, CEL, G-H on lysines were detected by PEAKS. Glycated peptides lost the whole or part of the Amadori prod- uct during HCD fragmentation. The optimization of NCE energy allowed identifying Amadori product reporter ions at 108.0211 Da and 78.0106 Daresulting from the loss of 3 water molecules or 3 water plus one formaldehyde molecules. 50% of AGE modifications on BSA were CML and less than 10% CEL. Twenty lysines are modified with CML and 10 with CEL some bear- ing both modifications. Some parts of the BSA sequence were not identified, indicating the presence of crosslinks either intramolecularly or between proteins. Lys–Lys crosslinks, 1,3-bis-(5- amino-5-carboxypentyl)-1H-imidazolium (GOLD) were identified for example between peptide TVMENFVAFVDKCCAADDK (position 569-587) and peptide GACLLPKIETMREK (position 198-211) of BSA.

Conclusion: Proteomic analysis allowed determining the degree of glycation of BSA as well as the different types of glycation present and the localization of AGEs on the BSA sequence.

O15. Développement d'une nouvelle méthode ciblée et hautement multiplexée pour un profilage rapide de lipidomes complexes par Scout-MRM

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Institut des Sciences Analytiques – Centre National de la Recherche Scientifique : UMR5280, Université Claude Bernard Lyon 1, UMR5280 – France

La caractérisation exhaustive de lipides chez des organismes non modèles est le plus souvent réalisée en approche globale sur des appareillages MS haute résolution. L'approche MS ciblée est la technique de choix pour une quantification précise en raison d'une meilleure sensibilité et reproductibilité. Cependant, le nombre de composés analysés est limité par le temps de cycle de l'appareil afin de maintenir un rapport signal/bruit acceptable. L'analyse par fenêtres de temps réduit l'impact du temps de cycle en focalisant l'acquisition de l'instrument sur des transitions MRM spécifiques affectées à un temps de rétention (TR) défini. L'augmentation du degré de multiplexage permet ainsi de suivre des milliers de transitions MRM. Dans ce contexte, nous avons développé une nouvelle méthode ciblée appelée Scout-MRM [1–3] pour obtenir rapidement des informations sur un lipidome complexe. Scout-MRM repose sur le suivi de transitions réparties dans des groupes qui sont déclenchés successivement lors de la détection de composés sentinelles appelés Scouts. Les Scouts choisis dans cette étude sont des lipides marqués par des isotopes stables ou des lipides avec des acides gras à chaînes impaires, répartis le long du gradient chro- matographique. Lorsque l'intensité de la transition MRM du 1er Scout dépasse un seuil défini, la surveillance du 1er groupe de transition est déclenchée. Le suivi du groupe s'arrête lorsque le 2ème Scout est détecté, induisant ainsi le déclenchement du groupe suivant, et ainsi de suite. Cette approche s'affranchit totalement du temps de rétention et est donc sans conséquence sur la détection des lipides en cas de décalage des TR et permet d'augmenter la capacité de multiplex- age. Afin de construire la méthode Scout, nous avons développé une base de données répertoriant les TR de centaines de lipides sur la base du modèle du nombre équivalent de carbones (ECN). La validation de la méthode Scout-MRM nous a permis de vérifier la linéarité, la répétabilité et la robustesse de la méthode sur différentes matrices complexes. Scout-MRM est modifiable et de nouvelles transitions de lipides peuvent être rajoutées renforçant le caractère *plug and play* de la méthode.

1.Salvador, A. et al. (2020) Scout-multiple reaction monitoring: A liquid chromatography tan- dem mass spectrometry approach for multi-residue pesticide analysis without time scheduling. ***J. Chromatogr. A***

2.Faugere, J. et al. (2020) High-multiplexed monitoring of protein biomarkers in the sentinel Gammarus fossarum by targeted scout-MRM assay, a new vision for ecotoxicoproteomics. ***J. Proteomics***

3.Rougemont, B. et al. (2017) Scout-MRM: Multiplexed Targeted Mass Spectrometry-Based Assay without Retention Time Scheduling Exemplified by Dickeya dadantii Proteomic Analysis during Plant Infection. ***Anal. Chem.***

Vendredi 18 Juin

9h00-9h45	**Jana Roithova** *Reaction monitoring by mass spectrometry*	
9h45-10h05	Elodie Logerot	
10h05-10h25	Adrien Lissarrague	**Fondamentaux**
10h25-10h45	Hélène Lavanant	
10H45-10H55	Pause	
10h55-11h10	Proteigene *Towards Standardized Omics Solution*	
11h10-11h30	Sébastien Rigaud	
11h30-11h50	Sanae Benabou Zdaou	**Fondamentaux**
11h50-12H10	Julien Maillard	
12h10-12h30	Posters	Vague B
12h30-12h50		

O16. Amidated and Carboxylated Peptides Mass Spectrometric Separation based on Survival Yield Technique

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Determination of compound purity is necessary during pharmacological development, particularly for synthetic therapeutic peptides. Indeed, to prevent proteolytic degradation, C-terminal amidation is often performed to produce peptides less sensitive to physiological pH changes compared to native carboxylated sequences. Nevertheless, from a chemical point of view, these amidated peptides can be hydrolyzed producing carboxylated forms in other words the native sequence. Hence, the quality and stability upon storage of amidated peptides must be carefully controlled. Although the recourse to a separation technique such as liquid chromatography appears to be the method of choice to monitor peptide purity, high resolution mass spectrometry (FT-ICR or Orbitrap technologies) was also investigated for its capability of separating in direct injection mode both amidated and carboxylated peptide forms, exhibiting 1 Da difference, through fine structure determination deduced from both isotopic patterns. Nevertheless, these mass spectrometers are not available in all laboratories due to their high cost. To counteract this drawback, survival yield methodology in tandem mass spectrometry was additionally undertaken to differentiate these two molecules with more affordable mass spectrometers, like ion traps. Results have shown the possible separation of these peptides from their sodiated ions which exhibit different fragmentation pathways according to the nature of the C-terminal moiety. Actually, amidated peptides cationized by alkali metals, like Na+, followed by collision-induced dissociations (CID) or post-source metastable decay (PSD) in MS/MS experiments, tend to produce more y-type ions than native peptides featuring unmodified C-termini, which in contrast, allow the gas-phase formation of [bn-1+Na+OH]+ ions issued from the C-terminal residue exclusion. Since the presence of a C-terminal amide or a carboxylic acid function triggers different fragmentations pathways, several sequences were studied showing that efficient gas-phase peptide separation is possible by varying the fragmentation energies according to survival yield methodology.

O17. Helium Charge Transfer Dissociation: an emerging fragmentation method applied for a better understanding of the structure-function relationships in carrageenans

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Carrageenans are heterogeneous polysaccharides present in the cell wall of some red seaweeds. They are made of building blocks consisting of galactose disaccharides, with various and disparate moieties (sulphation, anhydrous bridges, etc.). Carrageenans have many potential[1] and actual applications in industry, such as food or cosmetic industries[2] where they are massively used as texturing agents. The functional properties of carrageenans and thus their end-uses-are closely related to their structure (i.e., their degree of polymerization (DP), sequence and type of blocks in the chain). However, there is a large heterogeneity of natural carrageenan motifs (which can be present within the same macromolecule), and their exact structure remains partly unknown. Developing new applications of carrageenans, or optimizing existing ones, requires improving this knowledge, as well as improving our understanding of their structure-property relationship. To achieve this goal, we need to be as precise and comprehensive as possible in determining structures of carrageenans.
Structural complexity of carbohydrates has long challenged analytical sciences. But, recent advances in mass spectrometry ha considerably improved the analytical toolkit in glycosciences. Indeed, helium charge transfer dissociation (He-CTD) is an emerging useful fragmentation method[3], resulting in many informative cross-ring fragments while maintaining labile modifications. This makes it possible to localize the modifications carried by the galactoses and finally to reach a complete structure determination for oligo-carrageenans up to DP 18. Moreover, we now have access to several enzymes capable of hydrolysing the raw polymer into oligosaccharides of such DPs, with a defined specificity[4]. In this talk, we will see some characterized structures of oligo- carrageenans from genetically characterized pools of Chondrus Crispus (a genomic model for red algae) and industrial extracts from other seaweed species.

1.Pacheco-Quito E.-M., Ruiz-Caro R. & Veiga M.-D., Mar. Drugs., 18, 583, 2020
2.Zamorano J. & Canivet F., Techniques de l'ingénieur, 2018
3.Ropartz D., Li P., Jackson G. P. & Rogniaux H., Anal. Chem., 89, 3824-3828, 2017
4.Jouanneau D., Boulenguer P., Mazoyer J. & Helbert W., J. Appl. Phycol., 23, 105, 2011

O18. Sections efficaces de collision expérimentales et théoriques de polyoxométallates

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Les polyoxométallates (POM) sont des clusters anioniques constitués d'assemblages de polyè- dres de type MOx (M étant ici MoVI ou WVI) partageant des atomes d'oxygène. Les POM les plus classiques existent sous forme d'anions multichargés de structures compactes dites Lindqvist ($M6O19^{n-}$), Keggin ($XM12O\ 40^{n-}$) et Dawson ($XM18O62^{n-}$), dont les données cristal- lographiques sont bien répertoriées. A partir de ces blocs, qu'il est possible de fonctionnaliser, de multiples structures peuvent être conçues qui présentent une grande variété de propriétés physico-chimiques et de nombreuses applications potentielles (catalyse, stockage magnétique, applications bio-médicales).
Nous avons utilisé la spectrométrie de mobilité ionique en tube de dérive (DTIMS) couplée à la spectrométrie de masse pour déterminer les sections efficaces de collision (^{DT}CCS) expérimentales dans l'hélium et dans l'azote de quinze anions et agrégats anioniques issus de six sels de POM et de tetrabutylammonium (TBA) : TBA2Mo6O19 (Lindqvist), TBA4W10O32 (decatungstate), TBA3PMo12O40, TBA3PW12O40 (Keggin) et TBA6P2W18O62 et TBA9P2Nb3W15O62 (Daw- son).
A partir des géométries issues des structures cristallines des POM Lindqvist et Keggin, nous avons cherché à reproduire les valeurs de CCS des quatre anions les plus simples par calcul. En effet, les CCS peuvent être calculées par plusieurs méthodes dont la méthode dite projection approximation (PA), exact hard sphere scattering (EHSS) et la méthode des trajectoires (TM). Chacune de ces méthodes nécessite des paramètres de représentation des atomes qui sont des rayons de sphères dures pour PA et EHSS et des paramètres de Lennard Jones pour la méthode des trajectoires. Or ces paramètres n'ont pas encore été déterminés pour les atomes Mo et W. A l'aide du logiciel MOBCAL[1], nous avons déterminé pour les atomes Mo et W, les facteurs de correction à appliquer aux paramètres de Lennard Jones issus du champ de force universel (UFF)[2] pour reproduire les valeurs de ^{DT}CCS(He) et ^{DT}CCS(N2) des anions de Lindqvist. Nous montrons que ces mêmes paramètres peuvent être utilisés pour prédire les CCS d'autres struc- tures de Keggin avec une erreur de moins de 2%.

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O19. Contribution of Ion Mobility Mass Spectrometry for the Analysis of Complex Isomers Mixtures : application to Lipidyl-Cyclodextrins

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Bearing grafts based on fatty esters derivatives, Lipidyl-Cyclodextrins (L-CDs) were syn- thesized from the ring opening reaction of methyl oleate epoxide by CDs (α-CD, β-CD, γ-CD, HPα-CD, HPβ-CD, HPγ-CD) using ball milling.[1] L-CDs reaction media are thus composed of numerous isomers with different degree of substitution (DS) that need to be deciphered by extensive structural analysis. In this presentation we will focus on the benefit of ion mobility separation (IMS) for a better understanding of these complex mixtures and to illustrate our strategy, the β-CD derivatives were used as model.

First, in order to prove the effective grafting of lipidyl chains on the cyclodextrin glucose units, ESI-MS/MS experiments were performed. Then, the regio and stereoselectivity of the epoxy ring opening were investigated by UPLC-ESI-MS/MS allowing the clear separation of 12 isomers for the DS=1 derivative but without providing access to other structural information. Finally, the benefit of ion mobility separation (IMS) hyphenated with mass spectrometry (IMS-MS) was investigated [2]. The arrival time distribution (ATD) of each isomer was recorded using a travel- ling wave ion mobility cell (TWIMS) allowing the determination of their respective experimental cross section (TW CCS N2exp)[3]. Comparison with the predicted theoretical CCS (CCS N2th)[4] made it possible to propose a regioisomer assignment according to the β-CD hydroxyl po- sition (2, 3 or 6) involved in the epoxide ring opening reaction. Our attribution results were validate by NMR after MS based purification of all DS=1 isomers. This validation will allow us to investigate further molecular modeling calculation.

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O20. Characterization of DNA i-motif structures by native mass spectrometry and ion mobility spectrometry

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Cytosine-rich DNA sequences can form the i-motif structure, consisting of parallel-stranded duplexes held together by intercalated base pairs. The *in vitro* formation of i-motifs has been demonstrated by different classical spectroscopic techniques. Recently, the first direct evidence for its *in vivo* presence in human cells and control regulatory functions has been found. This structure is not only interesting from a biophysical and biomedical point of view, but also for their potential application in analytical chemistry or nanotechnology. Ion mobility spectrometry coupled to native mass spectrometry (IMS-MS) can be used to study i-motif structures because of its multiple advantages: it allows to isolate the molecules of interest from the solvent, depurinated DNA, etc. and study its intrinsic properties. Here we want to establish whether multiple coexisting conformations (i-motif, hairpin, dimers or random coil structures) can be resolved and attributed using ion mobility spectrometry.
The gas-phase structures depend on the charge state. The lowest charge states have the inconvenience of being compact whatever the starting structure and ion activation conditions[1]. In this work, electrospray supercharging agent such as m-NBA was used as additive solution in electrospray ionization IMS-MS to increase the charge states of DNA i-motif at physiological ionic strength (150 mM ammonium acetate). We studied the topology of structures of 34 mers complex i-motif (+hairpin) and its control sequences (that cannot form i-motif structure or hairpin or both of them). As i-motif formation is favored at acidic pH, we investigate the ESI-IMS-MS behavior of i-motif structures at pH ~5.6 as well pH ~7.5 for additional control.

The results show that at low collision energy, the high charge states are the responsible of solution folding of i-motif structure. Moreover, the low charge states keep a memory of the electrospray charging process and they are related to the compactness of the gas-phase structures. In addition, pre-IMS ion activation by fragmentor show high transition voltage for folded structures. Finally, the transition voltage of low charge state can also provide information about compactness stability in gas phase.

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O21. Structural analysis of petroporphyrins from asphaltene by trapped ion mobility coupled with a Fourier transform ion cyclotron resonance mass spectrometer

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Molecular characterization of compounds in highly complex mixtures such as petroleum is proving to be one of the main analytical challenges. Heavy fractions, such as asphaltenes, have immense molecular and isomeric complexity. Fourier transform ion cyclotron mass spectrometry (FTICR MS), with its unequaled resolving power, mass accuracy and dynamic range can address the isobaric complexity. Nevertheless, isomers remain largely inaccessible. Another dimension of separation is required. Recently, ion mobility mass spectrometry has revealed great potential for isomer description. In this study, the combination of trapped ion mobility and a Fourier transform mass spectrometry (TIMS-FTICR) is used to obtain information on the structural features and isomeric diversity of vanadium petroporphyrins present in heavy petroleum frac- tions. The ion mobility spectra provided information on the isomeric diversity of the different classes of porphyrins. The determination of the CCS from peak apex allows us to hypothesize about the structural aspects of the petroleum molecules. In addition, the full width at half max- imum (FWHM) was used as a measure for isomeric diversity. Finally, theoretical calculations of collision cross sections (CCS) were conducted allowing to propose putative structures in agree- ment with the experimental results. The authors believe that the adapted in-silico structural prediction will help in the future solving structure-property relationship of those unconventional petroleum molecules with heavy elements.

Lundi 21 juin

13h30-14h15	**Nicolas Sommerer** *Les polyphénols… nous les voyons, nous les buvons, nous les mangeons… nous les analysons.*	
14h15-14h35	Emilien Jamin	
14h35-14h55	Louisa BOUREL	**Omics**
14h55-15h15	Marie YAMMINE	
15h15-15H25	Pause	
15h25-15h40	Shimadzu *DPIMS : Analyses rapides LCMSMS en moins de 2 mn*	
15h40-16h00	Alexandre Guironnet	
16h00-16h20	David Touboul	**Fondamentaux**
16h20-16h40	Marine COSSET	

O22. Profiling of toxicologically relevant metabolites by DIA LC-MS/MS

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Human exposure to toxic substances, through environment or diet, is currently a central societal concern. To link this exposure to potential harmful effects to human, the most complete characterization of this chemical exposome is essential. Among these numerous compounds, some of them produce electrophilic metabolites, which are highly reactive towards biomolecules such as DNA and proteins. Metabolization through glutathione conjugation and then excretion in urine as mercapturic acids (MACs) appears as the main route of detoxification for these toxico- logically relevant compounds. Considering the particular structure of MACs, we have previously developed an untargeted profiling method, using a specific neutral loss detected during data- independent acquisition (DIA). To enable the application of this method to cohorts, we studied the reliability and performances of this DIA approach. This work was conducted during 2 studies of the colorectal cancer promotion induced by the consumption of red meat, using samples from an animal experiment or from a human cohort.

Diluted urine samples were analyzed by UPLC-HRMS using an ACQUITY I-Class UPLC sys- tem (Waters) coupled to a quadrupole time of flight mass spectrometer (Q-ToF Synapt G2-Si, Waters), using electrospray ionization in the negative mode. MACs were detected in QC samples using MSE (i.e. DIA) processed with either the UNIFI (Waters) software, or the open-source MSDIAL software, to detect the specific neutral loss of MACs produced by collision-induced dissociation. Finally, UPLC-HRMS signals of MACs were measured in all urines samples using XCMS.

The initial approach allowed the detection of hundreds of MACs without a priori, but required time consuming targeted MS/MS experiments to validate their identification, and displayed a lot of false positive detection. Therefore, injection of replicates by MSE was considered on urine samples of high red meat consumers and non-consumers from participants of Nutrinet Santé cohort study. This enabled the optimization of UNIFI detection parameters as well as the as- sessment of the reliability of MACs detection by MSE. Our results showed that replicates of MSE decrease the number of required targeted MS/MS experiments to validate DIA detections. Accuracy of deconvolution algorithm between MSDIAL and UNIFI software was compared by checking the false positive rate. It appeared that UNIFI outperformed MSDIAL in terms of detected MACs as well as the false positive rate.

Finally, we successfully applied this approach to the detection of numerous MACs in urine samples of high red meat diets of rats. Results showed an increase of MACs linked to lipid peroxidation in high red meat diets, as well as their decrease by antioxidants consumption. This novel approach allows to highlight under-studied MACs, and to characterize unknown ones.

O23. Proteomic analysis of liver tissue of patients with alcoholic hepatitis

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Introduction: Alcoholic hepatitis (AH) is the most severe form of alcoholic-associated liver disease. It is a complex life-threatening disease where therapeutics options are limited and still rely on steroids in 2021 for patients with severe alcoholic hepatitis. The prognosis is poor (40% death at 6 months) especially in non-responders and only an early liver transplantation proposed in highly selected patients can save them. Due to the selection criteria and the transplants' short- age, new therapeutics options are then essential. However, the molecular drivers and pathologic pathways of this disease are widely unknown because of the lack of animal model and the diffi- culty to access patient samples.

This work aims at studying the liver proteome of patients with AH, alcoholic cirrhosis (OH) and control using LC-MS/MS approach in order to identify proteins involved in AH pathogenesis and to identify potential therapeutic targets.

Methods: For the proteomic analysis, 5 samples of each group of interest were investigated: patients with AH, OH and control patients. The proteins were extracted with a RIPA buffer after homogenization of liver samples with a tissue homogenizer. The soluble proteins were re- covered from supernatants. The insoluble proteins were extracted from the pellets after another RIPA extraction. The proteins were then digested by trypsin with the eFASP method. The resulting peptides were analyzed by LC-MS/MS on a Q Exactive plus mass spectrometer. Proteins were quantified and identified using MaxQuant against Human UniProt Fasta dataset. The statistical analysis of identified proteins was done with Perseus, Student's tests, an ANOVA test, a PCA and a heatmap were performed. The gene ontology was determined through a String analysis.

Results: The method allowed to identify over 3,000 proteins in both fraction among which 1,900 soluble and 1,600 insoluble proteins were quantifiable.

Analyses demonstrated a specific proteomic profile of AH as compared to OH and ctrl. The OH group was heterogeneous where some samples were identical to controll or AH samples which was consistent to the heterogeneity of the clinical presentation of the OH patients. There is a unique immune response signature in AH and the gene ontology analysis allowed to identify specific over- and under-expressed biological pathways in AH such as an increase in fibrosis-associated proteins (extracellular matrix organization) and a decrease in metabolism-involved proteins.

Conclusion: This analysis highlights specific AH proteomic signatures. To validate these pre- liminary results, a larger number of samples have to be analyzed. For a more comprehensive AH signature, we are investigating its metabolome by high-resolution mass spectrometry FTICR technique in direct injection and in LC-MS/MS.

O24. A spatial proteomics approach to study yeast cell wall proteomic dynamic change through different growth phases in batch culture

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Introduction: Yeast cell wall (YCW), the outermost organelle of the yeast cell, is composed of an inner polysaccharide layer of β-glucans majorly cross-linked to a minority chitin, to which are bound mannoproteins. These latter forming the YCW outer layer are the second most abun- dant component (40%, w/w). YCW mannoproteins are proteins heavily mannosylated (50-90%, w/w), showing particular functional and health promoting properties. It is also a dynamic organelle, which composition changes in response to environmental conditions, but it has been little investigated from a proteomic point of view. This work aims to develop a subcellular fractionation method based on ultracentrifugation, in order to minimize contamination from other organelles' proteins and to prove the YCW proteomic variation during different growth phases in batch culture.
Methods: YCW lysates, obtained by mechanical disruption of S288C yeast cultured in batch culture mode in YPD medium, were subjected to an ultracentrifugation at 35 000 rpm for 19 h using an iodixanol continuous density gradient (18-48% iodixanol) diluted in 10 mM Tris- HCl buffer (pH=7.4) containing 1 mM EDTA and 12.5 % sorbitol. The resolved bands were digested by trypsin through an eFASP method application, and the peptides were analyzed by nanoESI-LC-MS/MS and allowed the identification and quantification of proteins using Proteome DiscovererTM 2.2 to query against Saccharomyces Genome Database (S288C strain) dataset. In addition, gene ontology analysis was performed to determine subcellular localization of identified proteins.
Results: Although it was so difficult to reach for cells harvested in exponential growth phase, this work showed the reliability of the applied ultracentrifugation method yeast cell wall enrichment and resolution from nucleus, just in case of stationary phase of yeast growth, where the YCW is the most developed. In the latter case, ultracentrifugation was able to increase significantly the number (up to 35 mannoproteins) and the amount (up to 25%) of identified yeast cell wall mannoproteins in the band located at a concentration of 30% iodixanol (Fraction 13). In addition, in this stationary growth phase, we saw its marker YGP1 protein as well as glucose starvation marker SUC2 enzyme.
Conclusion: This work describes the first spatial proteomic methodology allowing yeast cell wall mannoproteins' identification and quantitation with an exceptional efficiency in subcellular fractionation using a continuous gradient density of iodixanol for stationary phase harvested yeast grown in batch culture. We will also study the glycosylations of YCW mannoproteins by mass spectrometry after their chemical or enzymatic release and their chemical derivatization.

O25. In vial Ion Pairing Chromatography coupled to tandem Mass Spectrometry for Aminoglycosides analysis and application to wastewater samples

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Aminoglycosides (AGs) are used as veterinary antibiotics. In France, their consumption doubled in the last decade, owning 10% market share in animal medication in 2016. Because of antibiotic resistance risk, their analysis in water systems is necessary. Despite their growing use, few analytical methods in environmental matrices are published. Due to their high polarity (logP below -3), two techniques are usually used to enable retention: Hydrophilic interaction liquid chromatography (HILIC) and Ion-Pairing Chromatography (IPC). An IPC method coupled with tandem mass spectrometry was developed because AGs are cationic on a wide pH range and because IPC offers better robustness than HILIC.
Linear alkyl sulfonates (from C4 to C10) were used as IP reagents in the injection vial. Sepa- ration was performed with a XB-C18 column (Phenomenex), on a 1200 HPLC system (Agilent) coupled to a 5500 Qtrap mass spectrometer (Sciex). Positive electrospray ionization with Multiple Reaction Monitoring (MRM) mode detection were used. Three crucial parameters were optimized to obtain the best separation of seven AGs: organic mobile phase, IP reagents and equilibrium study.

Three organic mobile phases were tested: methanol (MeOH), acetonitrile (ACN) and 1:1 MeOH: ACN. The MeOH/ACN mixture showed the best resolution. Elution was finally performed with acidified MeOH, ACN and water.

With IP, retention increased with the alkyl chain length, as hydrophobicity increased. Retention factors (k) were plotted against IP reagent chain length and only C6 and C7 provided k between 2 and 10. Mixtures of C6 and C7 were considered. While both 50/50 and 25/75 mixtures resulted in a similar resolution, the former resulted in a faster separation, in 5 minutes. Optimization of the method led to use a 50/50 ratio of C6/C7 IP salts directly added in the vial.

Finally, column equilibration was studied. After a column cleaning to remove previous salts, 6 injections of standards were realized. Equilibrium was achieved after 5 injections. Salt effects on signal inhibition/enhancement was also studied.
The optimized method was validated and showed satisfactory resolution, performances suitable with the analysis of aminoglycosides in wastewater samples, with limits of quantifications less than 10 ng/mL for most of the compounds, low matrix effects, high accuracy (85%-115% recoveries) and reproducibility (2%-12%RSD). It was then applied successfully to raw and treated wastewater samples.

O26. Comparison of internal energy distributions generated by supercritical fluid chromatography versus liquid chromatography hyphenated with electrospray high resolution mass spectrometry

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Mass spectrometry (MS) is one of the most common analytical techniques employed in the field of chemical analysis. The internal energy (IE) distribution of ions in the gas phase have always attracted attention of MS specialists1,2,3 because this energy deposited into analytes during the ionization process directly affects the in-source fragmentation. This classical phenomenon is usually not desired because it could lead to complicate the MS data interpretation even if in-source dissociation is required for structural analysis when tandem mass spectrometry is not available. Therefore, a control of the IE from the analytical system is crucial.
Recelntly, supercritical fluid chromatography SFC using supercritical CO2 as the one of the elu- ent has attracted large attention.4 This technique is considered as a green analytical technique with various interests such as short analysis times, high efficiency, stability for samples, and safety for users. As far as we know, the IE distributions of ions formed after this separation method coupled to ESI has never been examined.

The IE distributions of thermometer ions dissolved in supercritical and liquid solvents and produced in the gas phase by electrospray (ESI) were therefore measured and compared by the survival yield method.5 The influence of different chromatographic conditions such as the nature of solvents, the composition of the mobile phase, the pressure of the backpressure regulator was studied for supercritical fluid chromatography (SFC) whereas the influence of the composition and of the flow rate of the mobile phase was investigated for liquid chromatography (LC). The MS instrumental parameters were studied in parallel for SFC and LC showing that the drying gas temperature and the fragmentor voltage affected the IE distribution, whereas the capillary voltage did not modify the IE distribution. Finally SFC led to higher internal energy, *i.e.* more in-source fragment ions, than LC when using same ESI source parameters.6

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O27. Photolabelling coupled to mass spectrometry to understand antimicrobial peptide DMS-DA6 interaction with bacterial membranes at the molecular level

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Antimicrobial peptides (AMPs) are part of the innate immune response that is effective against bacteria. AMPs cross the bacterial cell-wall, before inserting into membrane bilayers under different pore formation mechanisms. Among AMPs, DMS-DA6 is a 26 residues cationic peptide (sequence: G V W G I A K I A G K V L G N I L P H V F S S N Q S-CONH2) from the Dermaseptin family. Our work aims to understand the interactions of DMS-DA6 with mem- branes at the molecular level, using an approach coupling photolabelling to mass spectrometry (MS).
To this end, we synthesized a photoreactive DMS-DA6 containing a benzophenone (Bzp) as the photoreactive moiety and a biotin tag for purification purposes. Under UV irradiation, the photoreactive DMS-DA6 can form a covalent bond with interacting partners. We compared photolabelling yields for various photoreactive analogues of DMS-DA6. These analogues contain different linkers between the N-terminus of the peptide and the biotin tag, or between the Bzp and the peptide backbone and the Bzp moiety is introduced at different positions in the peptide sequence.

Modifications of the DMS-DA6 sequence with different substitution/insertion/deletion were also investigated, taking care to maintain an amphipathic α helix structure and the antibacterial properties of the initial DMS-DA6.

In this work, we show that the yield of photolabelling could be improved by judicious positioning of the Bzp label on the hydrophobic side of the α helix. Intact peptides/lipids photoadducts have been identified or not, depending on the position and the length of the spacer between the pep- tide backbone and the Bzp moiety. A longer and more flexible spacer led to the observation of intact photoadducts whereas shorter arms led to more constrained and less stable photoadducts which rapidly fragment *via* retro-Paterno Büchi reactions, leading to lower mass species. This spontaneous fragmentation is very informative and can be used to assess the depth of insertion of the peptide in the membrane bilayer.
Finally, MS analysis revealed a peculiar behaviour of the DMS-DA6 peptide sequence in the MALDI-TOF reflector negative ions mode in which the peptide did not appear at the expected m/z of the [M-H]- deprotonated ion but with a mass shift of +1 Da. Moreover, in source frag- mentation occurs at the peptide bond between the asparagine N15 and the isoleucine I16, leading to the so-called δ-cleavage classically observed in CID experiments of peptide anions. Takingadvantage of such fragmentation occurring directly at the MS level in the negative ions mode could be interesting in case of low amount of material impairing MS/MS analysis for further characterisation or de novo sequencing of natural antimicrobial peptides.

Mardi 22 Juin

9h00-9h45	**Marianne Fillet** *Couplage de l'électrophorèse capillaire à la spectrométrie de masse: contraintes et opportunités*	
9h45-10h05	Jean-Valery GUILLAUBEZ	**Analyse ciblée et non-ciblée**
10h05-10h25	Caterina Bordin	
10h25-10h45	Isabelle FABRIZI	
10H45-10H55	Pause	
10h55-11h10	Agilent *Quelle séparation pour la caractérisation des oligonucléotides par HRMS ?*	
11h10-11h30	Simon Ollivier	**Omics**
11h30-11h50	Téo Hébra	
11h50-12h10	Posters	Vague B
12h10-12h30		

O28. Specific Detection of Cysteine Sulphenic Acid by Coupling Mass Sectrometry with LASER Induced Dissociation

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Sous l'effet du stress oxydant, l'une des modifications induites par les espèces radicalaires oxydantes est l'oxydation des résidus cystéines (Cys) des protéines en acide sulfénique (Cys-SOH). En raison de l'importante gamme dynamique de concentration en protéines des échantillons, et parce que le phénomène d'oxydation est minoritaire et instable, la détection des Cys-SOH dans les matrices biologiques complexes est difficile.
L'utilisation de la dissociation induite par laser (LID) dans le visible (473 nm) couplée à la spectrométrie de masse (MS) ajoute une spécificité optique à sa sélectivité, et améliore ainsi la spécificité de détection des protéines à Cys oxydées faiblement concentrées. Les peptides n'absorbant pas naturellement dans le visible, l'analyse LID-MS est précédée d'un greffage spécifique des Cys-OH, avec un chromophore contenant un groupement cyclohexanedione (DabDn).

Les premiers tests ont été réalisés sur un peptide modèle à Cys oxydé à l'H2O2. Le DabDn est greffé en parallèle de l'oxydation des Cys. Après réaction, on observe la formation de peptide à Cys oxydée dérivée avec le DabDn, fragmentant en LID avec un rendement de photodissoci- ation de 97 %. Des ions b permettant le séquençage du peptide sont observés ainsi qu'un ion rapporteur issu de la fragmentation interne du chromophore. La dérivation est spécifique car seuls les Cys-OH sont dérivés, les peptides à méthionine oxydée ne réagissant pas de façon non spécifique avec le DabDn. Une étude de répétabilité et de limite de détection a été réalisée en diluant le peptide modèle oxydé et dérivé dans du plasma digéré. La LID-MS permet son analyse avec des CVs < 20 % et une limite de quantification de 2 nM. Les résultats démontrent une amélioration des performances analytiques par rapport à la CID.

La méthodologie a été appliquée à un mélange de deux protéines oxydées et dérivées avec H2O2 et DabDn, puis digérées par la trypsine. 26 peptides ciblés à Cys-OH dérivés ont été identifiés en LID avec une meilleure sensibilité qu'en CID. Enfin, ces peptides, ainsi que ceux issus de la digestion *in silico* de cinq autres protéines plasmatiques humaines, ont été suivis dans des échantillons de plasma réels afin d'identifier les protéines à Cys-SOH endogènes. La plupart d'entre eux ont été détectés en LID dans l'ensemble des échantillons, avec des différences significatives entre leurs quantités relatives.
De par sa spécificité, la LID surpasse la CID pour la détection des peptides à Cys-SOH greffés et permet d'envisager des applications cliniques dans de grandes cohortes humaines de type Alzheimer. De plus, cette méthode permettra de vérifier l'hypothèse d'un stress oxydatif massif généré par la production de cytokines lors d'une infection par la COVID19.

O29. Alkyds paint in art: identifying and unravelling their reticulation by chemical depolymerization and ultrahigh resolution mass spectrometry

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Introduction: Alkyd paints, in France glycerophtalic paints, became popular thanks to brands as Ripolin®, which introduced them in the early XXth century. Compared to traditional oil paints, they possess higher brightness and durability and thus started to appear in avant-garde artists' artworks, as Pablo Picasso's. The binder in alkyd paints is constituted of a traditional siccative oil, e.g. linseed oil, and polyester resin obtained from the reaction between an aromatic diacid and a polyol, commonly phthalic acid and pentaerythritol respectively, par- tially esterified by an unsaturated fatty acid. This work aims at characterizing the solid formed after the paint drying down to the order of few micrograms, the maximum sampling amount on a precious artwork. Being high mass polymers not amenable to mass spectrometry, we developed an original depolymerization method for characterizing the components of alkyd paints and the crosslinked structures formed during polymerization by ultrahigh resolution FTICR MS.
Methods: Both fresh and dried paints were first transamidated overnight with N,N-dimethylpropane-1,3-diamine (DMAPA) sodium amide. The reaction mixture was quenched with acidic water and extracted with dichloromethane. Alcohols were enriched by SPE then derivatized under Schotten-Baumann conditions with 4-dimethylaminobenzyl chloride. The products were dried, evaporated, then diluted with acetonitrile with 0.1% formic acid for MS analyses, performed by infusion using nanoESI ionization on a 9.4 Tesla Bruker SolariX FTICR instrument fitted with harmonized cell tuned at a resolution higher than 1 million at m/z 400.

Results: A preliminary study on present-day and historical artists' or industrial paints us- ing 1D and 2D NMR showed the great variability of alkyd paints compared to traditional oil paints. After transamidation and derivatization, (iso)phthalic acid, pentaerythritol and the com- mon fatty acids coming from triglycerides were found in both fresh and dried paints of different ageing time. In polymerized paints, signals in the high m/z region, corresponding to crosslinked structures, were classified using Kendrick and Van Krevelen plots to distinguish the nature of the crosslinking bonds. In the low m/z region, oxidation and degradation products as aldehydes and carboxylic acids were identified. The comparison between fresh, one-week and one-year polymerized paint samples showed a progressive disappearance of polyunsaturated fatty acids and the formation of oxidation products and crosslinks between unsaturated fatty acids.

Conclusions: We developed for the first time a comprehensive analysis of both acids and alcohols in alkyd paints based on chemical depolymerization and derivatization using ultrahigh resolution FTICR MS at a sensitivity compatible with precious artwork analyses currently ongoing in our laboratory, allowing to access artists' recipes.

O30. Taxonomy and classification of Upper Pleistocene bones with ultrahigh resolution MALDI-FTICR Mass Spectrometry

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Introduction: Since the beginning of the 2000s, studies of ancient molecules (DNA, proteins) contained in the archaeological bones of extinct species have opened a window on the past and have allowed us to understand the biological past of extinct species and their evolutionary process. Paleontology identifies bones based on their morphology. However, it is common to find fragmented bones in archaeological sites due to the presence of carnivores, human activity that prevents identification by paleontologists. The development of a proteomic workflow remains a challenge. The analysis of fossils must consume a low quantity of material to avoid damaging the samples. In this study, a high-throughput MALDI FT-ICR proteomic methodology was applied to 1 milligram samples of 130,000-year-old mammalian bones of from the earlier Upper Pleistocene sites in Caours and Waziers (France). This methodology will allow the characterization of biological classification and associated taxonomic ranks to each bone without protein database. Methods: Our corpus consists of bones from Caours (130,000 BP, Somme, France) and Waziers (130,000 BP, Haut-de-France, France). Type I collagen PMF were obtained by high resolution mass spectrometry using a 96-well eFASP method from 1 mg of bone powder. Bones were demineralized with TFA solution. Then insoluble bone powder was digested using a mix of trypsin/Lys C. Peptides were purified on a 96 wells SPE C18. Then, digested peptides were ana- lyzed by MALDI FT-ICR SolariX XR 9.4 Tesla. Raw data from MALDI FT-ICR were analyzed with Data Analysis 4.2 and Metaboanalyst 5.0, which classified samples by origin by comparing them to samples of known species.

Results: The methodology developed was robust and reproducible. The advantage of the use of MALDI FT-ICR is the very high resolution allowing to differentiate the peptides carrying a deamidation. The major first mono isotopic peak of a native peptide (13CONH2) relative to the monoisotopic peak of the de-amide peptide (COOH) are shifted by 0.019 Da (*m/z*). We have shown that archaeological bones from extinct taxa and bones from modern taxa can be discriminated using PCA analysis. Bones from different archaeological sites clusterized according to their geographical localization and degradation of proteins content. The methodology allowed clustering the bones from a given archeological site by family and by species. Finally, we are able to identify species by comparing the *m/z* of peptides from triptic digested bones with the *m/z* of species-specific biomarker peptides present in scientific literature.

Conclusion: This new methodology allows for analyses on low quantities of fossil bones. The classification of MALDI spectra allows grouping the samples by similarity and determining the corpus of extinct animals in an archaeological site.

O31. Molecular networking of tandem ion mobility data and its implications for glycomics

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Introduction – Data organization through molecular networking (MN) has been used in "omics" sciences over the past years as a way to efficiently mine the massive amount of spectral information produced by tandem mass spectrometry (MS). However, glycomics lags a step be- hind: carbohydrate structures involve numerous levels of isomerism, making MS and tandem MS blind to many key structural features of glycans. This roadblock can in part be alleviated with gas-phase ion mobility spectrometry (IMS), a method highly sensitive to isomerism. Notably, the last generation of high-resolution IMS-MS spectrometers now offers the possibility to perform tandem IMS or IMS/IMS. We will show how IMS/IMS can be integrated in the MN strategy. **Methods** – We analyzed a set of oligosaccharide standards, representative of plant carbohy- drates, using IMS/IMS on a Select Series Cyclic IMS mass spectrometer (Waters, Wilmslow, UK). Briefly, an IMS/IMS experiment consists of: (i) selecting a precursor ion according to its mass and ion mobility arrival time, (ii) collisionally fragmenting the precursor, and (iii) per- forming ion mobility analysis of the fragments. This generates an ion mobility spectrum, which contains ion mobility data for all the fragments. The experiment-dependent drift time values of the fragments were further calibrated to give repeatable collision cross section (CCS) values, in $Å^2$, which represent the surface of an ion that interacts with the buffer gas during IM separation. IMS/IMS spectra containing CCS and intensity values could then be exported to the GNPS platform (https://gnps.ucsd.edu) for MN analysis.

Results – We built molecular networks using our IMS/IMS-based approach and the classical MS/MS-based approach. The classical approach gave minimal information about the structure of the oligosaccharides, the clustering being based solely on the nature of the subunits (pentose or hexose). In contrast, our ion mobility-molecular networking strategy generated structurally relevant clusters that accurately reflected the structural diversity of the oligosaccharide standards and their isomerism, with regard to the nature of both the subunits and the glycosidic bond. Oligosaccharides indeed clustered according the main pattern in their backbone, regardless of lateral branching by other subunits.

Conclusions – We present a novel workflow for the organization of IMS/IMS spectra that should be of future use in the field of glycomics. We demonstrate that-when it comes to oligosaccharides and their numerous levels of isomerismsm molecular networks based on IMS/IMS spectra are widely superior to classical MS/MS-based networks to sort and organize molecules with a high degree of structural relevance.

O32. Déréplication, annotation et caractérisation de 74 azaphilones de Penicillium sclerotiorum à l'aide de réseaux moléculaires t-SNE

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Les micro-organismes associés aux insectes sociaux tels que les termites constituent une ressource originale pour l'identification putative de nouveaux squelettes chimiques ou de métabo- lites bioactifs [1]. Afin d'explorer cette chimiodiversité inusitée, 130 extraits bruts issus d'une collection de micro-organismes associés aux termites ont été analysés par chromatographie liq- uide en phase inverse couplée à une source *electrospray* et un spectromètre de masse en tandem (Q-ToF). Les données ont été acquises avec une stratégie de type données dépendantes (DDA). Après extraction des données et retraitement sous mzMine2, un réseau moléculaire a été généré [2,3] permettant d'y cartographier les concentrations minimales inhibitrices des extraits bruts contre un champignon de l'espèce *Tricophyton rubrum*, un pathogène humain. Ainsi, l'extrait de *Penicillium sclerotiorum*, souche connue pour produire des azaphilones a été sélectionné. Tout d'abord, car les azaphilones sont des composés de la famille des polycétides avec une large gamme d'activité biologique, mais aussi, car ils ont un intérêt économique dans le domaine de l'industrie alimentaire en tant que colorant [4]. De plus, en explorant le réseau moléculaire de la souche seule, il a été observé dans le métabolome de *P. sclerotiorum* une grande diversité chimique des azaphilones, diversité plus large que les données déjà rapportées dans la littérature. Le métabolome spécialisé de *P. sclerotiorum* a donc été annoté par analyse des données en MS2 ce qui a permis de proposer les voies de fragmentation des azaphilones protonées. Ces voies de fragmentation ont permis d'identifier la nature et la position de plusieurs modifications chimiques sur le squelette azaphilone tels que des acylations, des halogénations, des condensations de la lactone ou la substitution de l'oxygène du pyrane par des amines. Ainsi, 74 azaphilones, dont 49 nouvelles, ont pu être annotées. Les hypothèses de fragmentation ont également été validées par l'isolement et la caractérisation structurale complète de 8 azaphilones, dont 3 possèdent une structure nouvelle.

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Jeudi 24 juin

9h00-9h45	**Boris Macek** *Mass Spectrometry-Based Proteomics in Biomedical Research*	
9h45-10h05	KAMEL BACHIRI	**Omics**
10h05-10h25	Diego Fernando Garcia del Rio	
10h25-10h45	Lucienne Nouchikian	
10H45-10H55	Pause	
10h55-11h10	Bruker	
11h10-11h30	Mónica Gisel Arellano-Sánchez	**Analyse ciblée et non-ciblée**
11h30-11h50	Ranin Dabbousy	
11h50-12h10	Posters	Vague A
12h10-12h30		

13h30-14h15	**Nathalie Carrasco** *TBA*	
14h15-14h35	Charlotte Mase	**Analyse ciblée et non-ciblée**
14h35-14h55	Ziad Mahmoud	
14h55-15h15	Ali Zaiter	
15H15-15H25	Pause	
15h25-15h40	Waters *Development and application of the next generation of Q-ToF mass spectrometer*	
15h40-16h00	**Prix de Thèse 2021 :** Julie Guillemant	
16H00-16h20	Clôture	

O33. Identification des interactions oncogéniques du Polyomavirus à cellules de Merkel dans le Carcinome de Merkel

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A l'échelle mondiale, près d'un décès sur 6 est attribué au cancer. Cette pathologie touche plus de 18 millions de personnes chaque année. Une proportion importante de ces cancers, 15 à 20%, est associée à la présence de pathogènes parmi lesquels les onco-virus occupent une place importante. Par le passé, l'étude de ces virus a permis de découvrir des oncogènes majeurs. C'est le cas de HRAS et KRAS dont les formes humaines sont mutées dans environ 25% des cancers solides. Il est donc très intéressant d'étudier ces onco-virus pour obtenir une meilleure compréhension de l'oncogenèse.
Le carcinome à cellules de Merkel (CCM) est un cancer de la peau rare mais très agressif caractérisé par une croissance asymptomatique et rapide. L'étude de ce cancer a permis son association à un virus: le Polyomavirus à Cellules de Merkel (MCPyV). MCPyV est présent dans plus de 80% des CCM. Les CCM MCPyV+ présentent un taux de mutation nettement inférieur à celui des CCM MCPyV- ce qui suggère très fortement un rôle oncogénique pour ce virus. Il a également été montré que ses antigènes T présentaient des propriétés transformantes. Ainsi, les CCM représentent un bon modèle d'étude des cancers viro-induits, avec peu de mutations somatiques et un nombre restreint de protéines virales nécessaires et suffisantes au développement d'un cancer.

Afin d'identifier les voies oncogéniques mises en jeu, mon projet de thèse consiste à étudier les interactions entre ces protéines virales et les protéines hôtes par une approche de marquage proximal, le BioID. Il s'agit d'une technique d'étude d'interaction de proximité par fusion de la protéine d'intérêt à une biotine ligase mutée d'*E.coli* permettant la biotinylation des interacteurs de proximité en cellule vivante. Ces interacteurs sont ensuite récupérés sur billes streptavidine et identifiés par spectrométrie de masse.
L'utilisation du BioID au sein de HEK 293 Flp-In T-Rex a permis d'identifier 948 interactions avec 481 protéines hôtes dont des interactions déjà connues des antigènes T. Les modifications du protéome total suite à l'expression des antigènes T ont également été analysées. L'étude des données d'interatomique et de protéomique totale ainsi que leur comparaison ont permis l'identification de possibles axes oncogéniques impliquants les antigènes T à différents niveaux. Ces données vont être reproduites au sein de lignées MCC MCPyV + afin de mesurer l'effet de la collaboration entre les antigènes T en contexte pathologique.

O34. Studying protein complexes for assessing the function of ghost proteins in ovarian cancer.

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In eukaryotes, mRNA has been considered monocistronic. Thus, they translate a single reference protein (RefProt) from a single reference open reading frame (ORF). This, defined as the longest sequence delimited by a start and a stop codon. Mass spectrometry (MS)-based large-scale proteomic analyses rely on the interrogation of databases (DBs) for protein identification. In shotgun proteomics, it is often observed that a fraction of good quality MS/MS spectra does not match in the DBs. This can be due to proteoforms or PTMs though, they cannot be assigned. This challenges the idea of monocistronic mRNA. Using ribosome profiling, the potential of encoding a ghost proteome was displayed. OpenProt DB, predicts proteins trans- lated from alternative ORFs. These cover LncRNA and different start codons within mRNA like 5'&3'-UTR, or in frameshift (+2, +3), all named Alternative Proteins (AltProts). As there is no extensive knowledge on AltProt, crosslinking-MS (XL-MS) is an attractive technique to identify, in a large-scale approach, the protein-protein interactions (PPI) of the AltProts. For this, a crosslinker allowing the binding of two proteins close in space is used. The XL-peptides are then analyzed by nLC-MS/MS making possible to identify the 2 partners of the PPI. Finally, the PPI identified between AltProt and RefProt in cells will make possible to propose the involvement in a signaling pathway by analysis of the onthology gene of RefProt. This approach, although if it suffers of several limitations, is the only large-scale technique which allows annotation of signaling pathways for AltProt.

My work is to characterize the AltProt in Ovarian Cancer, by a shotgun approach making possible to highlight the proteome (AltProt and RefProt) specific to tumor types. Likewise, the quantitative variation of proteins describes the variations in pathology-specific signaling path- ways. In addition, the use of the XL-MS approach allows me to monitor the AltProt interactions in the proteomic landscape of the cells, and propose functions and involvement in the development of the pathology. 7512 Ref and 539 AltProt were identified by two extraction methods (RIPA & SDS 1%). For RIPA the ratio of AltProt for RefProt is 8.9% while in SDS 1% is lower but containing AltProt not identified in the RIPA extraction. With these data, we conclude that both extractions are complementary. Using principal component analysis AltProts show a clear separation between healthy and cancer cells as well as between the two kind of cancer, these results areconsistent with those obtained with the RefProt. Moreover, the heatmaps show a significant variation of AltProt and RefProt expression on cancer and healthy cells. To identify PPI a methodology using CID cleavable XLs is performed using two crosslinkers: DSSO (bifunctional), targeting the Lys of the protein and CBDPS (trifunctional) allowing an enrichment due to the integrated biotin.

O35. A simple one-step in vivo cross-linking enrichment strategy for proteome-wide interaction studies

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Within the past few years, *in vivo* cross-linking (XL) mass spectrometry (MS) has gained lots of traction to study protein-protein interactions (PPIs) in a proteome-wide manner [1]. This has been made possible mainly thanks to the technological advances with faster and more accurate mass spectrometers and the development of new algorithms for cross-linked peptide searching of large databases. For *in vivo* applications, cross-linking is performed directly in live cells, which has the great advantage of probing direct PPIs in their native environment but the drawback of dramatically increasing the complexity of the mixture. It is estimated that less than 1 % of the peptides will contain the cross-linker after digestion, making the analysis of cross-linked peptides very difficult [2]. To improve this feature, we present here a simple one-step enrichment strategy combining click-chemistry on UV-sensitive solid support with our homemade cross-linker, NNP9 [3], which allows a specific enrichment of the labeled peptides with an 85% minimum recovery. We developed our workflow on live cells of *Neisseria meningitidis*, a human pathogenic gram- negative bacterium. 10^10 bacterial cells were cross-linked with NNP9 for 3 hrs. Bacteria were then lysed, washed on a filter, and proteins digested with trypsin. Using click-chemistry, cross- linked peptides were covalently bound to agarose beads and non-labeled peptides were removed through extensive washes. Labeled peptides were then released upon exposure to UV and the mixture was analyzed using nano LC-MS/MS on a Q-Exactive HF Orbitrap. Data was pro- cessed using MassSpec Studio. We identified a total of 3342 cross-links for a single biological triplicate analysis, which is traditionally obtained with extensive fractionation. Only 83 to 108 non-labeled peptides were identified per replicate, highlighting the efficiency of our enrichment protocol. More than half of the bacterial proteome was found labeled and we obtained interesting structural data on protein complexes present in all bacterial compartments: cytosol, periplasm, and membrane. For instance, we captured interactions of the ATP and ADP bound state of the ATPase complex showing that our approach allows us to study highly dynamic proteins in their native state. We were also able to capture new PPIs such as the one with RmpM and PorB, two proteins involved in iron channeling, which is important for bacterial virulence.

Overall, our workflow allowed us to dive deep into proteome-wide analysis of live bacteria. Our unbiased approach can be applied to other biological systems and lead to capturing dynamic and novel PPIs in a native environment.

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O36. Chromium Determination in Leather: Challenges of Extraction and Speciation

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Leather industry plays an essential role in the world's economy; however, it also has a neg- ative environmental impact due to the generation of significant quantities of wastes, some of which are classified as hazardous chemicals. Moreover, hexavalent chromium, a known carcino- genic and mutagenic, can be found in leather products and cause allergic dermatitis or trigger other diseases. For this reason, it is important to quantify the total chromium in these prod- ucts, as well as the oxidation state in which this element is found due to the fact that trivalent chromium (Cr(III)) is considered an essential nutrient involved in glucose regulation, and hex- avalent chromium (Cr(VI)) represents a risk to human health. In aqueous systems, the oxidation state of chromium is determined by redox potential and pH, which poses a difficulty in the speciation of chromium, as the sample preparation protocols can lead to an interconversion be- tween Cr(III) and Cr(VI), thus distorting the results. In addition, there is no current standard describing a method to extract and quantify both species simultaneously. During this work, seven pre-treatment procedures were tested, the total chromium was determined by ICP-AES and speciation was studied by ion chromatography coupled to ICP-MS. The best extraction results were obtained with mineralization method but in such conditions the speciation is not preserved. However, EDTA extraction led to good extraction results (extraction rate between
92.5 and 108.3%) and allowed the extraction of Cr(VI) and Cr(III) as a Cr-EDTA complex. An optimization of the chromatographic conditions has been done to quantify Cr(III)-EDTA complex and Cr(VI).

O37. Toward the isolation of peroxidases using proximity-dependent biotinylation

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I**ntroduction**: Proximity-dependent biotinyaltion (PDB) is a strategy that enables in-depth understanding of subcellular proteome and other biological processes expeditiously. This technique enables protein biotinylation using peroxidases (APEX or HRP) as PDB enzymes that mediate the generation of short-lived biotin phenoxyl radicals capable of binding proteins lo- cated in the vicinity of the enzyme. The generated labelled proteins are purified by avidin-based affinity and identified by LC-MS/MS analysis. The specific dependence of this technique on peroxidases, permit to think beyond protein identification to using PDB as a tool for the first step toward the selective purification of peroxidases that are meant to play the role of the target protein and the PDB enzyme at the same time.

Methods: Self-biotinylation of horseradish peroxidase (HRP) was performed in vitro as follows. HRP (23 nmol) was incubated with biotin phenol in PBS; then hydrogen peroxide (H2O2) was added to initiate the reaction. Different concentrations of biotin phenol and H2O2 were tested in order to optimize the reaction conditions. Biotin phenol-treated samples were subjected to digestion with trypsin and labeled peptides were separated by nano LC-MS/MS. A list of identi- fied peptides and proteins were generated by data processing with Proteome DiscovererTM 2.2, searched against *Armoracia rusticana* UniProtKB database. Furthermore, in order to ascertain the efficacy of HRP to catalyze proximity biotinylatin of other proteins, we investigated the capacity of HRP to carry lysozyme biotinylation.

Results: HRP was capable of carrying the biotinylation reaction in vitro as revealed by LC- MS/MS analysis. Our data indicated that HRP is a mixture of isoforms each possessing around six tyrosine residues. Five out of thirteen isoforms expressed the modification on one tyrosine residue. Biotinylation was mostly efficient at low concentration of biotin phenol (23 nmol). As for lysozyme, two peptides expressed the modification when 10 nM HRP were used in comparison to higher amount where only one out of three tyrosine residues captured the modification.

Conclusion: Here, we proposed a new approach toward the selective purification of one of the most vastly targeted enzyme that has conquered a prevailed position in every domain of re- search. Currently, we are investigating this technique on peroxidases isolated from plant tissues and developing modified biotin-phenol for targeting specific peroxidases.

O38. Molecular cartography of a mixed plastic pyrolysis oil from municipal wastes by direct infusion Fourier Transform Ion Cyclotron Resonance

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Plastic wastes cause well known harmful effects for environment and contribute to the depletion of landfill sites. Pyrolysis oil produced from plastic waste materials is considered as an important source of monomers, fuel and chemicals that both circumvent some of the environmental concerns associated with non-renewable fossil resources and alleviate waste disposal concerns. In order to improve conversion and valorization processes, an advanced molecular description is essential. Such as crude oil, plastic oils are complex mixture including thousands of chemical species covering a wide range of mass and polarity. The most powerful technique for the analysis of this type of sample, in terms of mass accuracy and resolving power, is Fourier-Transform Ion Cyclotron Resonance Mass Spectrometry (FTICR). It allows assigning a unique molecular formula to each *m/z* signal. Besides, the use of different ionization sources ensures an extensive molecular description and enables to assess the efficiency of different catalytic chemical treatments. Electrospray source (ESI) ionizes compounds with medium to high polarity. On the contrary, atmospheric pressure photoionization (APPI) allows the characterization of less polar compounds and in particular aromatics whereas atmospheric pressure chemical ionization (APCI) affords the ionization of aliphatic species. For this purpose, this study reports the molecular characterization of plastic pyrolysis oil by the main atmospheric pressure ionization. A large predominance of hydrocarbons compounds were observed in APPI (+) and APCI (+). More- over, the use of both sources highlighted different types of molecules such as paraffins, diolefins and more particularly triolefins which had not yet been reported. Basic and neutral nitrogen containing species (N1 and N2 classes) were highlighted by ESI (+) and ESI (–) respectively. Oxygen containing species O1 to O4 were identified principally by ESI (–) but also in APPI (+) and APCI (+) and attributed as carboxylic acid and alcohol functional species. The same functionality of oxygen is founded in NxOy compounds observed in ESI (+) and ESI (–). The plastic pyrolysis oil molecular composition will be compared with petroleum fractions.

O39. Deciphering the structure of natural Amber by chemical depolymerization and analysis by ultrahigh resolution FT-ICR mass spectrometry

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Introduction: Amber and other natural fossil resins like copal are formed by the polymerization and fossilization of tree terpene extrudates. Amber's structure is based on labdatriene carboxylic acids such as communic and ozic acid, which when polymerized affords a network based on a polyisoprene backbone. As amber is largely insoluble, solid-state spectrometric techniques such as Fourier transform IR (FTIR), solid-state 13C nuclear magnetic resonance spectroscopy (NMR) were primarily used but they offer limited structural information. We present here the application of the methodology we develop for high molecular polyisoprene based on depolymer- ization by olefin metathesis [1] to the structural elucidation of amber.

Methods: Amber was depolymerized to generate end-functionalized ionizable fragments which can be analyzed by mass spectrometry. As disulfide crosslinks in amber inhibit its swelling and act as poison to Hoveyda-Grubbs catalyst, the sulfur bridges contained in amber were first cleaved by a reductive solvent, followed by desulfurization by Raney Nickel and methylation by trimethylsillyldiazomethane to preserve acid functions. Amber was then depolymerized by cross-metathesis with Hoveyda–Grubbs second-generation catalyst and *Z* -1,4-diacetoxy-2-butene chain transfer agent in dichloromethane under nitrogen and analyzed by high resolution mass spectrometry.

Results: In a first step, communic acid, described as being the major component of amber was extracted from cypress cones. Following purification, the extracted communic acid was polymerized to mimic the structure of amber and analyzed by NMR and mass spectrometry. Analysis by ultrahigh resolution FT-ICR MS produced a spectrum with 3 major peaks at *m/z* 303.2318, *m/z* 605.4564 and *m/z* 907.6810 corresponding to a monomer, dimer and trimer of communic acid respectively. In a second step, samples of amber originating from different regions (l'Oise and Archingeay in France and Madagascar) were analyzed. The mass spectra obtained for amber from Oise valley in France showed several peaks common to those obtained from poly- merized pure communic acid which is a proof that this type of amber is based on communic acid and can be attributed to amber class I. The attribution of minor peaks present in the spectra is under investigation.

Conclusion: The produced spectra provided informative fingerprints enabling greater analy- sis of the structure of amber constituents, evidence for evolutionary convergence of the skeletal chemistry of the polymers: classes I (diterpenoid labdane polymers) to V (primarily abietane and pimarane diterpenoid skeletons) as well as improvements in the identification of their origin. The new method of analysis developed for amber gives access for the first time to the bulk amber composition.

O40. Characterization and quantification of the phospholipid and polyphenol contents in Brewery Spent Grain extracts by Mass Spectrometry

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Brewery spent grains (BSG), residues of the cereal fermentation, are the main co-product of breweries (90% of the volume). Today, around 60% of the BSGs are used for animal feed but the other 40% are not valued (i.e. agricultural spreading). The use of dried BSG as raw materials for the production of new molecules of interest for the food, chemical, pharmaceutical, and cosmetic sectors could offer new fates for these co-products [1] which, despite several studies [2], remain poorly characterized. BIOVAL (2014-2021) project aims to valorize the BSG in the context of a circular economy. This approach previously requires to characterize as finely as possible the molecular content of the BSG [3].

We present here the analysis by Mass Spectrometry (MS) of the lipid and polyphenol composition of dried grains (BSG) studied during the project. They were characterized by direct infusion Electrospray Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (ESI FTICR-MS) and Ion Trap Tandem Mass Spectrometry (ESI-ITMSn). LC-MS/MS profiles were also con- ducted by (RPLC and HILIC mode) coupled with IT-MSn and Q-TOF mass spectrometry to complete these analyses and to estimate their amount (between 0.1 and 30 mg/g of dry matter). These extracts also showed the presence of phospholipids such as acylphosphatidylglycerols, acylphosphatidylethanolamine, and polyphenols such as ferrulic acid, gentisic acid, p-coumaric acid, rhamnazin, respectively. Moreover, some original lipids have been highlighted such as cyclic phosphatidic acids, known to be specific inhibitors of DNA a-polymerase [4]. Furthermore, many flavonoids have been detected like catechins and they are well-known for their antioxidant activity. The presence of these molecules indicates that the grains contain various valuable compounds of interest for the inhibition of cancer cell invasion and metastasis.

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Posters

Vague A

Mardi 15juin et Jeudi 24 Juin

P1. Comparison of 3 ionisation methods - electron ionisation, chemical ionisation and atmospheric pressure photoionisation for the characterisation of volatile organic compounds

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Gas chromatography (GC) is a reproducible, robust, selective and sensitive method to analyse volatile organic compounds (VOCs) in a wide range of applications. The separated analytes are generally characterised by mass spectrometry (MS) under vacuum conditions. The main ionisation method is the **Electron Ionisation** (EI): high energy exchanges occur, causing reproducible molecular fragmentations. **Chemical Ionisation** (CI) is another ionisation method where a reactive gas (i.e. methane or ammonia) is ionised to form reactant ions. GC-MS can also be conducted under atmospheric pressure. **Atmospheric Pressure PhotoIonisation** (APPI) is the most recent source. Emitted photons give rise to quasi-molecular ions.

In our research platform, we recently coupled a GC Trace 1310 to a High Resolution Mass Spectrometer (HRMS) Orbitrap Fusion (ThermoScientific) with the APPI source developed by Mascom (Bremen, Germany). In this work, first, we present a general overview of the technical developments carried out on 13 VOCs with the GC-APPI-HRMS hyphenated technique. Secondly, we compare the three ionisation methods listed above. For this purpose, we used 6 VOCs of different chemical classes to determine the Limit Of Detection (LOD) for each source. Positive detection in the Orbitrap allows a factor 2 to be gained in sensitivity. In MS2, High Collision Dissociation (HCD) is more informative than Collision-Induced Dissociation (CID). For the source parameters, it is better to decrease the sheath gas flow and the transfer tube temperature to 150 C in order to enhance the MS signal. In-source fragmentation occurs in MS mode for several VOCs. The LOD reached for each VOC depends on the chemical class of the molecule. EI is the most sensitive method to characterise acids (0.03 ppm), alcohols (0.006 ppm), ketones and esters (0.003 ppm) while CI with methane as reactant gas is more appropriate for aldehydes (0.008 ppm). Even if GC-APPI-HRMS is not the most sensitive method (from 0.004 to 0.3 ppm), the LOD ranges found in our experiments is generally better than in the literature. The developments in GC-APPI-HRMS are still in progress. (1) Reducing fragmentations and enhancing sensitivity by using acetone as a dopant gas, (2) conducting in vivo analyses, are our main objectives before transferring this very promising technology to aroma compounds characterisation in food extracts or in exhaled breath.

P2. Quantification of betalactams by Liquid Chromatography – cubed MS (MRM3) in Environmental Complex Matrices

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Environmental matrices such as sludge or manure are considered complex due to their heterogeneity and to the large variety of compounds that can be extracted from them. The sample preparation for this kind of matrix is then tedious, requiring multiple purification steps, where it could be easy to lose or degrade the selected analytes. This is true in particular for families with a high metabolization/degradation rate such as betalactams, composed of penicillin and cephalosporin. To reduce the sample preparation drastically and to gain time, it is necessary to develop an analytical method both selective and sensitive.

The MS/MS/MS acquisition mode, noted MS3 or MRM3, takes advantage of the hybrid triple quadrupole linear ion trap (Qtrap): Q3 is utilized as a linear ion trap which permits to select, trap and fragment MRM product ions, using radio frequency field, into the second generation of product ions. This results in a highly selective detection, reducing the signal of interfering compounds, and consequently decreasing matrix effects. By improving the signal-to-noise ratio, the detection and quantification limits are also lowered, resulting in a more sensitive method.

In this study, the presence of 6 environmentally relevant betalactams is followed in soil and sludge using LC-MRM3. After developing a LC-MS/MS method for the analysis of betalactams with a 5500 QTRAP (SCIEX), the MRM3 method has been optimised considering three parameters: excitation energy, excitation time and accumulation time in the trap. MRM3 results in an increase of peak area by a factor comprised between 10 and 80, compared to MRM, for standards solutions. The results also show the interest of this mode of detection in soils and sludge.

P3. Inorganic speciation of pine wood pyrolysis bio-oils using gel permeation chromatography - inductively coupled plasma high-resolution mass spectrometry (GPC-ICP-HRMS)

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The bio-oils (BO) are a promising renewable feedstock for the production of fuels with a lower environmental impact (CO2 neutral) than fossil fuels. Their characterization studies have increased in number in the last decades. BO contains inorganic species with elements such as Na, K, Ca, P, Fe, Si, etc., which can cause problems in processes like catalytic up-grading, and storage stability. The total analysis in BO is often performed with inductively coupled plasma optical spectrometry (ICP-OES), and other analytical techniques. From the speciation approach, the hyphenation of gel permeation chromatography (GPC) to inductively coupled plasma high-resolution mass spectrometry (ICP-HRMS) can be used as a tool to monitor the evolution of the hydrodynamic volume of the species containing the elements of interest in the BO. The present work aims for the inorganic speciation analysis of pine pyrolysis products by GPC-ICP-HRMS in order to link their composition to their respective production process. This valuable information regarding the hydrodynamic volumes of the observed species in the bio-oils could then be used in order to optimize the processes linked to the further removal of these problematic species.

To this extent, a Thermo Scientific Element XR double-focusing sector field ICP-MS (Thermo Fisher, Germany) has been hyphenated to a Dionex high-performance liquid chromatogra- phy (HPLC) system. Three Waters (Waters Corporation, USA) styrene-divinylbenzene gel permeation columns were connected in series with a Styragel guard column. Pine pyrolysis BO and distillation residue of BO have been selected. These samples were 5-fold diluted in THF, then 20 μL were injected and eluted isocratically at a flow rate of 1 mL/min of THF for about 80 min before detection using both UV and ICP-HRMS system.

The obtained size exclusion chromatograms of the inorganic species studied shown slight differences between each sample because of their nature. The pyrolysis BO showed a more dispersed distribution between high and low molecular weight regions than the distillation residue, whose chromatograms showed a more defined shape.

P4. An efficient analytical strategy to identify biomarkers of pesticide exposure by suspect screening based on UHPLC-ESI-HRMS

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Human exposure to toxic contaminants, including pesticides, is a major societal concern. Pesticides are largely used worldwide in different contexts and encompass a huge diversity of chemicals, leading to multiple exposure sources and patterns for Human. Although most of them may be considered hazardous for human health, the characterization of human exposure to pesticides still represents a challenge. Current approaches based on crossed food consumption and exposure data or biomonitoring are suitable for exposure evaluation only on a few dozen pesticides or their metabolites.

With the development of analytical techniques and exposure research towards the concept of exposome, analytical chemistry moved from targeted to non-targeted methods, which are better suited for a wide exposure assessment to mixtures of compounds.

This work presents an original workflow which was used for the analysis of 400 urine samples from a fieldwork study conducted in the frame of the EU H2020 HBM4EU project (H2020 GA N 733032). This workflow lies on non-targeted UHPLC-HRMS acquisition in both positive and negative electrospray ionization modes. Data were extracted using W4M and then annotations were made using a home-made database comprising > 3000 pesticides related markers (parent compounds and/or metabolites).

Analyses were performed by UHPLC with a Dionex RSLC-3000 LC system coupled to a Thermo LTQ-Orbitrap XL high-resolution mass spectrometer (UHPLC–HRMS, heated ESI) operating at R60000@400 (m/z 110-1100). Acquity UPLC BEH C18 (2,1x100mm, 1,7μm) columns were used with mobile phases based on formic acid / ammonium formate / MeOH and ammonium carbonate / MeOH for the positive and negative modes, respectively. Urine samples (1mL) were submitted to SPE concentration on 96-wells plates. Deuter- ated standards were added before and after SPE to have a global follow-up of both theextraction/concentration step and the HRMS analysis by checking RT, mass accuracies and signal intensities across the sequence. With this and the use of other standards / controls, a pre-established QA/QC template ensures the quality of the analyzes. Data are currently under processing and will provide information on the pesticide exposure of populations according to the age (children/adults), the season (spreading/non-spreading) as well as the proximity of the living place with pesticide spreading zones.

This methodology enables to highlight hundreds of potential biomarkers of exposure to pesticides. The interest of this approach lies on the full HRMS data acquisition which enables a posteriori screening of pesticides (and their metabolites) which were not included in the initial suspect list. In the absence of standard reference compounds in most cases, all de- tected suspect signals have to be analyzed using MSn experiments in order to support the hypotheses of identification with further structural information.

P5. In-StageTip digestion improves sample preparation prior to LC-MS/MS analysis for clinical research

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Introduction

The instrumental improvements made over the last decade allowed mass spectrometry to produce useful proteomics data for clinical research. However, clinical proteomics still faces challenges such as an increasing number of samples to analyze and working with a low amount of starting material while achieving in-depth analyses of complex samples. Tackling these issues requires a fast, robust, and reproducible sample preparation with minimal sample loss. To this end, we evaluated the in-StageTip (iST) complete solution developed by PreOmics, including peptide fractionation Add-on on three different samples.

Materials and Methods

Human synovial fluid (HSF), murine brain tissue extracts (MBT) and murine brain isolated cells extracts (MBC) have been investigated. For HSF containing highly abundant proteins such as albumin, we implemented a depletion step using Cibacron blue (Blue-SepharoseTM). The iST preparation was performed on 80 μg proteins and compared to a classical stack- ing gel preparation, in terms of peptides/proteins identified. For the low abundant MBC samples, the iST preparation was evaluated on much less protein (< 10μg). In all cases, sample preparation was completed within four hours, including the additional peptide pre- fractionation step (three fractions) based on dipole-moment and mixed-phase interactions. Peptide extracts were analysed on either a nanoLC-Q-Orbitrap (nanoAcquity, Waters, cou- pled to a Q-exactive Plus, Thermo Scientific) or on a nanoLC-TimsTOF Pro (nanoElute coupled to a TimsTOF Pro, Bruker Daltonics) platform.

Results

For HSF, the use of a double Trypsin/Lys-C digestion was needed to reduce the percentage of miscleavages below 10%. Numbers of identified proteins reached 192 (1 792 peptides), 231 (1 633 peptides) and 308 (2026 peptides) by stacking gel, iST and iST with fractionation, respectively. With the addition of an initial depletion step, 472 proteins (2 123 peptides) were identified in HSF, thus corresponding to a 2.5 fold gain compared to stacking gel. We also compared the performance of iST versus a stacking gel preparation on MBT samples leading to an average of 2 628 proteins identified (11 977 peptides) versus 1 830 proteins (8 488 peptides), respectively. Finally, the potential of using iST preparation on very low starting material amounts was demonstrated as 4 065 proteins (28 976 peptides) on average could be identified on as low as 6μg of MBC (isolated oligodendrocytes).

P6. Comparison of three data processing workflows for label-free XIC quantification on TimsTOF Pro data

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Trapped ion mobility spectrometry (Tims) and Parallel Accumulation – SErial Fragmentation (PASEF) are recent features that were introduced on the TimsTOF Pro (Bruker Daltonics) mass spectrometer notably for bottom-up proteomics analysis. Those new features imply the development of adapted data processing algorithms and software to support and take advantage of these novelties. In this context, we compared three workflows, MaxQuant, SpectroMine (Biognosys) and Peaks (Bioinformatics Solutions) to perform label-free XIC quantification.
Protein inputs from 1 to 20µg were reduced, alkylated and digested with trypsin in S-Trap cartridges (Protifi). A theoretical peptide quantity of 200ng was analysed by nLC-IMS- MS/MS (NanoElute coupled to a TimsTOF Pro, Bruker). MaxQuant (v1.6.14.0), Spec- troMine (v2.5.201125.47784) and Peaks (v 10.6) were used to perform protein XIC MS1 label-free quantification. The software parameters have been finely tuned to be as compa- rable as possible. Classical FDR filters of 1% were applied at PSMs, peptides and proteins levels depending on the software. Given our dataset, we started by working without the match between runs (MBR) options enabled. However, Peaks has an algorithm equivalent to MBR, which cannot be disabled to our knowledge. Consequently, we also performed MaxQuant searches with MBR. Peaks also differs from other software as it includes a *de novo* search step. On the other hand, SpectroMine does not have a MBR equivalent but, similar to MaxQuant's maxLFQ algorithm, it performs a normalisation of relative quantities. Therefore, we also performed MaxQuant searches with LFQ.

Comparisons were made on the number of proteins identified and quantified with and without the application of filters on missing values and CV to assess the quality of protein quantification. The highest numbers of protein groups identified were reached with Peaks with up to 5 000 on the highest starting material amount. The highest numbers of protein groups quan- tified without filters were obtained thanks to SpectroMine with up to 4 700 protein groups at highest starting material amount. After the addition of quality filters, MaxQuant (with MBR and LFQ) returns the highest numbers with up to 2 500 quantified protein groups.
Each data processing workflow has its own forces and drawbacks. Peaks provides the highest numbers of identifications helped by MBR and *de novo* search. SpectroMine provides the highest numbers of proteins quantified with almost all identified proteins that could also be quantified. Finally, MaxQuant (with MBR and LFQ) yields the highest numbers of protein groups quantified consecutively to the application of quality filters on quantitative results. While all 3 solutions are able to handle the ion mobility dimension of TimsTOF Pro data, MaxQuant is the only free software. However, SpectroMine and Peaks offer useful tools for data visualization.

P7. Développement d'une approche SALDI-MS pour la détection intracellulaire spécifiques de biomarqueurs de la maladie d'Alzheimer

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Le SALDI-MS (Surface-Assisted Laser Desorption/Ionisation Mass Spectrometry) est une approche dérivée du MALDI-MS qui permet de s'affranchir des inconvénients liés à l'utilisation de matrices organiques. En SALDI-MS, les surfaces, constituées de nanomatériaux, sont utilisées de manière à reproduire le rôle de la matrice organique. Les particules absorbent l'énergie du rayonnement laser pour la transférer aux analytes et induire les phénomènes de D/I. Ce projet a pour objectif de développer une approche SALDI-MS sensible et spécifique pour la détection intracellulaire de biomarqueurs protéiques de la maladie d'Alzheimer. Les deux axes principaux de travail sont l'élaboration de surfaces et l'analyse par spectrométrie de masse.
Les nanomatériaux étudiés ici sont de type nanoaiguilles. Plusieurs méthodes d'élaboration sont évaluées afin de déterminer quels types de nanoaiguilles permettront une D/I optimale de nos biomarqueurs d'intérêts. Cinq méthodes d'élaborations différentes ont été dans un premier temps réalisées et caractérisées par microscopie électronique. Deux d'entre-elles ont permis d'obtenir des résultats probant. La méthodologie lift-off/gravure humide per- met d'obtenir un réseau de nanoaiguilles régulier avec une taille de nanoaiguilles précise et bien définie. La méthodologie nanosphère lithographie/gravure humide permet d'obtenir un réseau de nanoaiguille moins régulier que la précédente mais permet une miniaturisation des nanoaiguilles plus simple à mettre en place.

La faisabilité de la méthode SALDI-MS est démontrée avec l'aide de surfaces d'acier texturées par un laser rouge et chimiquement modifiées. Un mélange de 9 peptides standards (m/z 600 – 2900) a été utilisé pour comparer les surfaces selon l'efficacité de D/I et l'homogénéité des dépôts. De plus, ce mélange a permis l'optimisation des paramètres intrinsèques (fluence du laser, paramètres d'imagerie...) à la spectrométrie de masse. Le solvant de dilution des échantillons est un paramètre critique pour l'analyse. En effet, la qualité de D/I des peptides sur les surfaces est dépendante du solvant dans lequel ceux-ci sont dissous. De plus, selon le solvant et l'hydrophobicité des surfaces, des angles de contacts différents sont observés lors des dépôts. Les surfaces sélectionnées doivent donc avoir des propriétés chimiques permettant une D/I des peptides du mélange optimale, un dépôt facile et un angle de contact d'environ 120 ainsi qu'une homogénéité de dépôt vérifiée par imagerie et présentant l'aspect des gouttes déposées.
Ces différents tests ont permis de déterminer le type de surfaces et leurs caractéristiques de fabrication permettant la meilleure D/I de peptides standards pour l'analyse par SALDI-MS.

P8. Chemical depolymerization of high molecular weight polyolefins for their analysis by high resolution mass spectrometry: Application on functionliazed industrial and real world polymers.

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Introduction: Polydienes, such as polybutadiene (PB) and polyisoprene (PI) are among the most commonly used polymers. However, their characterization by mass spectrometry (MS) continues to be challenging as they are highly insoluble and difficult to ionize. Develop- ing new depolymerization techniques allows the analysis and recycling of these polymers by identifying the cross-linking phenomena during their manufacturing and ageing, and chemical modifications during their use or degradation. Olefin cross-metathesis using new generation organometallic catalysts can efficiently depolymerize polyolefins and is used to generate end-functionalized ionizable oligomers from commercial polymer samples.
Methods: Native commerical PB and PI, functionlized polybutadiene supplied by Cray Valley company and synthetic and natural polyisoprene condoms were first depolymerized by cross-metathesis using Hoveyda–Grubbs second-generation catalyst and *Z* -1,4-diacetoxy- 2-butene chain transfer agent at 50 C under nitrogen. All these polymer samples were then irradiated with UV at 365 nm to mimic weathering conditions and microplastic ageing. They were later depolymerized and analyzed by infusion using nanoESI ionization on a 9.4 Tesla Bruker™ SolariX™ FTICR and atmospheric solid analysis probe on a Synapt G2 instrument (ASAP).
Results: Studies on the depolymerization of native non-functionalized PB and PI have been already published where extensive analysis by regio- and stereo-specific depolymerization and various mass spectrometry techniques allowed the identification of their fingerprint. However, after UV irradiating the same previously studied polymers, mass spectra showed the appearance of crosslinked species with 2 or more reticulated chains as major products. In the case of functionalized industrial polybutadiene with and without hydroxyl terminations, spectra revealed over ten families of peaks separated by C4H8 units. These spectra became much more complex following UV irradiation and newly formed reticulated families were identified. Finally, synthetic and natural polyisoprene condoms in native form after lubricant removal were also depolymerized by cross-metathesis and MS analysis showed the presence of families with a difference of C5H8 and up to 28 isoprene monomers in their chains. After photo-weathering, these families completely disappeared and new crosslinking products appeared with typically reticulated families of polyisoprene chains. We must point out that additives present in these types of polymers were freed by depolymerization and that they were conveniently consumed during photo-weathering. These state-of-the-art methods allow understanding manufacturing processes and environmental degradation of polymers. Their application to functional polydienes such as rubber from tires will provide a robust recycling strategy.

P9. Caractérisation de nanoparticules polydisperses par spectrométrie de masse.

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La spectrométrie de masse (MS) s'impose de plus en plus comme une technique de caractérisation de petite nanoparticules. Pour des particules très monodisperses, la MS combinée à des sources d'ionisation telles que l'électrospray (ESI) et la désorption-ionisation laser assistée par matrice (MALDI) permet une caractérisation précise de la taille du noyau métallique et de son état de charge ainsi que du nombre de ligands. Cependant pour des nanoparticules à polydispersité restreinte, qu'en est-il ?
Nous utilisons la spectrométrie de masse (MS) pour contrôler la diversité des nanopartic- ules en termes de composition et de structure, ainsi que leur stabilité. Dans ce contexte, nous utilisons des mesures MS couplé à un algorithme de corrélation multiplicative pour déterminer la masse moyenne et la distribution de masse. Ces paramètres deviennent alors des informations pertinentes.

Ce poster vise à saisir les possibilités d'analyses de nanoparticules et l'importance des informations " non isotopiquement résolues " par spectrométrie de masse (*1,2*). Celles-ci complètent le panel des techniques de caractérisation en science des matériaux.

1.Comby-Zerbino,C et al; Materials Advances 2021 , in press
2.Ziefuss, A et al; Advanced Materials, 2021 in press

P10. Microfluidics based sample preparation of microorganisms for total proteome analysis by Mass spectrometry

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Proteins support the vast majority of the metabolic functions of living organisms. This information is essential from a fundamental point of view (understanding of a system and of its components' interactions) or applied in search of a biomarker in clinical, agri-food or environmental samples. The most suitable method to study the complete proteome is using mass spectrometry (MS). Large scale protein characterization of environmental microbiota at a given point in time is known as Metaproteomics. Sample preparation in metaproteomics is challenging due to the diversity of organism / proteins.They constitute a key point and often underestimated for the success of the analyzes in terms of robustness, sensitivity, speci- ficity, analysis time and safety of manipulator from the potentially pathogenic samples. The present work demonstrates the utilization of microfluidic device that integrate all the sample preparation stages for metaproteomics: microbial protein extraction, protein chemical processing and tryptic digestion to generate peptides that will be analyzed by MS. Additionally in terms of operation, the microfluidic workflow has the advantage of automatic operation and direct peptide trapping with pre-column.Subsequently, the microfluidic sample preparation workflow was compared to existing workflow involving filter-assisted sample preparation (FASP) in terms of protein identification using orbitrap MS connected to liquid chromatography. Equal number of microbial cells was used for the two workflows and analysis was done by MS to compare the number of identified proteins. Results indicated that equivalent number of proteins was obtained by both methods, demonstrating the capacity of the novel microfluidic workflow. In future, the device will be tested for meta-samples, that is difficult to process due to diversity of microbial species.

Vague B

Vendredi 18 juin et Mardi 22 Juin

P11. Characterization of key aroma compounds in Burgundy truffle

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Truffles have a high economic value due to their gastronomic qualities appreciated in "grande cuisine". While Périgord and White Alba truffles are well-valued, Burgundy truffle (*Tuber uncinatum*) is not well-characterized in its production area. INRAE is involved to help the producers to better characterize this truffle through different research axes, espe- cially the influence of the ripeness and of the geographical origin on aromatic composition (1). For this purpose, we had first to define an analytical strategy to better characterize aroma compounds in this noble fungus.
Burgundy truffles were analyzed by Dynamic HeadSpace (DHS) (2) coupled with Gas Chromatography - Mass Spectrometry (GC-MS) for volatile organic compounds (VOC) identifi- cation. A new *in vitro* analytical method by Proton Transfer Reaction – Time of Flight-Mass Spectrometry (PTR-ToF-MS) (3) was developed to obtain an aroma mass fingerprint of all the samples. In addition, sensory analyses were performed by a trained panel.

Truffles are very aromatic, and 1 g of fungus is sufficient for GC-MS analyses. The DHS parameters were defined through a kinetic study: an incubation temperature of 36 C for 15 min was selected. The influence of the trapping and drying steps was studied to set the best parameters and enabled the identification of more than 70 compounds. Depletion of hydronium ions was observed by PTR-MS during the analysis of 25 mg of truffles. While it is not possible to weigh less without generating an error in the repeatability, a headspace dilution system with a humidified airflow was developed to permit VOC analysis. After integrating the peak areas, the data were compiled to observe the evolution of the areas according to time and incubation temperature. An analysis of variance (ANOVA) was performed to determine the significant differences between harvest places and periods. Multivariate analysis (Principal Component Analysis) was also performed. Preliminary results showed a good separation between the truffles from different places and periods. However, the data were acquired at spaced time points, and instrumental biases should consequently be considered. Further experimentations with internal standards will be conducted to control these biases and correct them for a better statistical analysis.
This study enabled the development of an analytical methodology to better characterize the Burgundy truffle. Data correlation from PTR-ToF-MS, GC-MS and sensory analyses is pos- sible to define and rank truffle qualities. In a near future, we will cross these results, with a genetic and a microbiota investigation for a scientific contribution to the constitution of an IGP (Protected Geographical Indication) request.

P12. Glycoproteomic study of Saccharomyces cerevisiae yeast cell wall mannoproteins

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Introduction: Yeast cell wall (YCW), the outermost organelle of the yeast cell, is com- posed of an inner polysaccharide layer of *β*-glucans majorly cross-linked to a minority of chitin, to which are bound mannoproteins. These latter forming the YCW outer layer are its second most abundant component (40%, w/w). In respect to their nomenclature, YCW mannoproteins are heavily mannosylated (50-90%, w/w) by both short simple *O*-linked glycans and complex *N*-linked glycans. They have functional and health promoting properties related to their particular molecular structure, but have been little investigated. This work aims to study YCW mannoproteins at the molecular level based on mass spectrometry (MS) and capillary electrophoresis (CE).

Methods: SDS-extracted YCW samples obtained by mechanical disruption of yeast sam- ples were *O* -deglycosylated using 0.1 M ammonia or Jack Bean mannosidase (20 U) and *N* -deglycosylated by PNGase F/Endo H (20 U) with an adapted eFASP method. The re- sulting peptides were analyzed by nanoESI-LC-MS/MS. Proteins were identified using Pro- teome DiscovererTM 2.2 against Saccharomyces Genome Database (S288C strain) dataset. Subcellular localization of identified proteins was determined by Gene ontology analysis. *O*- and *N*-glycans were chemically derivatized by aminative reduction reaction with butyl- 4-aminobenzoate (ABBE) and 8-aminopyrene-1,3,6-trisulfonic acid trisodium salt (APTS) analyzed by *μ*ESI-LC-MS and CE respectively. Glycosylated peptides were identified using ByonicTM.

Results: We showed the reliability of the YCW extraction method for YCW enrichment from a limited amount of dry yeast (100 mg). The *N*-deglycosylation step has the advantage to increase the sequence coverage of mannoproteins as well as the mapping of *N*-glycosylation sites on peptides when Endo H was added, while chemical *O*-deglycosylation with ammonia has its drawbacks on decreasing the number of identified peptides and thus mannoproteins (from 32 to 20) and their amount (from 16% to 11%), due to a degradative effect over- came by the use of mannosidase, allowing the identification of 37 mannoproteins composing 37% of quantified proteins. Mannoproteins *O*- and *N*-glycans were isolated simultaneously and efficiently permitting their analysis by MS and CE respectively following their chemical derivatization, although mannosidase has degraded *O*-glycans into monosaccharides limiting *O*-glycans structural studies.

Conclusion: This work describes the first one-pot glycoproteomic methodology allowing mannoproteins' structural study based on a deglycosylation protocol adapted to an eFASP method and applied on the same sample of extracted YCW. In addition, it permits *O*- *and N*-glycosylation sites mapping and a subsequent analytical study of released *O*- and *N*-glycans by mass spectrometry. It has been developed to further characterize industrial mannoproteins' samples.

P13. Development of analytical tools to study Auranofin's mechanism of action

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Auranofin (AF) is a gold-S-glucosyl-compound approved by the U.S Food and Drug Administration (FDA) for the use in rheumatoid arthritis, and it's being evaluated for anticancer activity since it is a Thioredoxin Reductase (TrxR) inhibitor. According to the proposed mechanism of action, gold is displaced from the S-glucosyl compound by the nu- cleophilic attack of the TrxR selenocysteine and then transferred to the catalytic cysteines, which are inhibited by the formation of the adduct Cys-Au-Cys. This mechanism would imply that the gold-moiety can be transferred to other less reactive protein targets, which are unknown, mainly due to the lack of appropriate analytical tools. We performed a thiol redox proteomics analysis to highlight the AF molecular effects on an ovarian cancer cell line (A2780). The identification of about 400 altered cysteine redox levels and in 650 protein expression levels allowed us to find the impact of AF on different biological pathways such as cellular respiration and endoplasmic reticulum folding activity. However, a site-centric point of view allowing us to correlate the AF protein targets with the results of our study is still missing. The development of such an analytical tool is in progress in our laboratory with promising results.

P14. Analysis of Saccharomyces cerevisiae yeast cell wall mannoproteins' O- and N-glycans by mass spectrometry coupled to liquid chromatography and capillary electrophoresis

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Introduction: Yeast cell wall (YCW), the outermost organelle of the yeast cell, is com- posed of an inner polysaccharide layer, to which are bound mannoproteins. These latter forming the YCW outer layer are its second most abundant component (40%, w/w). YCW mannoproteins are heavily mannosylated (50-90%, w/w) by both short simple *O*-linked glycans and complex *N*-linked glycans. Those latter glycans give mannoproteins exceptional functional and health promoting properties, but have been little investigated, especially from a quantitative view. This work aims to study YCW mannoproteins' glycans at the molecular level based on mass spectrometry (MS) coupled to liquid chromatography (LC) and capillary electrophoresis (CE) techniques.

Methods: SDS-extracted YCW samples obtained by mechanical disruption of yeast sam- ples were *O* -deglycosylated using NH4OH 0.1 M or Jack bean mannosidase (20 U) and *N* -deglycosylated by PNGase F/Endo H (20 U) with an adapted eFASP method. The re- sulting released *O*-glycans were chemically derivatized either by aminative reduction with butyl-4-aminobenzoate (ABBE) or by permethylation, and analyzed by a 80 min linear gradient with nanoESI-RPLC-MS. *N*-glycans were chemically derivatized by aminative reduction reaction with 8-aminopyrene-1,3,6-trisulfonic acid trisodium salt (APTS) and analyzed by CE. Small amounts of *N*-glycans were permethylated by a newly developed miniaturized in stage tip protocol and analyzed by nanoESI-LC-MS. Deglycosylated peptides were checked using Byonic.

Results: The *O*- and *N*-deglycosylation steps were proved to be efficient, through bioinformatics analysis of peptides using ByonicTM software, which has shown the absence of glycosylations upon deglycosylations' application, compared to the same but glycosylated peptides obtained through a classical eFASP method. The released *O*-glycans were detected by mass spectrometry coupled to RPLC upon their derivatization with ABBE, where the largest *O*-glycan containing 5 mannoses, eluted first at 34 min and then was followed successively by smaller *O*-glycans by 0.5 min elution time difference. CE has allowed the separation of APTS-derivatized *N*-glycans, without being able to identify them due to the lack of standards, whereas their permethylation and subsequent analysis by mass spectrometry has allowed the identification of *N*-glycans containing up to 12 mannoses.

Conclusion: This work describes a methodology allowing mannoproteins' deglycosylation adapted to an eFASP method applied on extracted YCW, and the subsequent analysis of released *O*- and *N*-glycans by mass spectrometry coupled to RPLC and CE. Currently we are developing a quantitative approach by spiking a known amount of derivatized *N*,*N*-diacetylchitobiose as a standard and identifying the heavier *N*-glycans not eluted in liquid chromatography by capillary electrophoresis coupled to mass spectrometry.

P15. Identification of N-glycan oligomannoside isomers in the diatom Phaeodactylum tricornutum

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Microalgae are emerging expression systems for the production of recombinant proteins like monoclonal antibodies useful for human immunotherapy. In this context, the characterization of the microalgae-made biopharmaceuticals, which are mainly glycoprotein-based pharmaceutical products, requires efficient analytical methodologies dedicated to the profiling of the *N*-glycosylation of the recombinant proteins. Herein, we profile the oligosaccharides *N*-linked to the proteins isolated from the diatom *Phaeodactylum tricornutum* that has already been used successfully to produce functional monoclonal antibodies. The combination of ion mobility spectrometry–mass spectrometry and electrospray ionization-multistage tandem mass spectrometry allows us to decipher the detailed structure of the oligomannoside isomers synthesized by this diatom and to demonstrate that the processing of the oligomannosides *N*-linked to proteins occurs in this diatom as reported in mammals. There- fore, *P. tricornutum* synthesizes a canonical Man5GlcNAc2 (Man-5) that is substrate for *N*-acetylglucosaminyltransferase I that initiates the synthesis of the complex *N*-glycans.

P16. Overcoming process issues in Refining & Petrochemical assets with bio and plastic based feedstocks: developing tailormade analytical tools to tackle new challenges

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Mass Spectrometry provides either universal detection and structural identification or compound-specific detection (SIM mode). However, such selective SIM mode is highly limited to screen for specific families (i.e. S and N compounds) in complex samples. Additionally, ionization is highly compound-dependent so specific standards for each analyte are needed. Given the relevance of N- and S-containing compounds in environmental and industrial samples, selective detectors exclusive for each element (NCD and SCD respectively) have been developed. The GC-combustion-MS system presented in 20101 was able to provide both qualitative information and generic universal quantification of organic compounds. The combustion interface converted the entire C contained into the different compounds eluting from the column into CO2. It is expected that other volatile species, H2O, SOx and NOx (if S and N are present) could potentially been detected. The new set-up presented here consists of a ceramic tube (400x3x0.5mm) containing 2 Pt wires. Combustion is carried out online at temperatures 850-1150 C while adding a flow of O2 diluted in He (0.3%) online mixed with the eluting flow from the column using a capillary flow "T". The six-way valve installed allows operation either in conventional GC-MSD or GC-combustion-MS modes. The online conversion in a combustion furnace of all the eluting compounds into the same volatile species (CO2, H2O, NO and SOx) makes their detection compound-independent opening the door to their generic quantification using simple element-containing standards. Detection limits achievable for S and N (1 ppb) are lower than those obtained with the standard dedicated SCD and NCD. In the case of H, (detection limit 8 ppb) it is worth stressing that even no adequate alternatives exist. The proof of concept has been optimized, demonstrated and
validated with the quantification of model mixtures containing different types of organic compounds.2 Applicability to real samples has been also proved with the successful identification and quantification of different N and S-containing compounds in diesel samples. The total N and S content obtained perfectly matched those obtained using well-established (ASTM) methods. Finally, the H/C molar ratio could be determined in a cooker gas oil sample and its respective aromatic and aliphatic fractions to assess unsaturation.

1.S. C. D´ıaz; J. R. Encinar; A. Sanz-Medel; J. I. G. Alonso, Anal Chem., 82, (2010), 6862.
2.L. Freije-Carrelo; J. Garc´ıa-Bellido; L. A. Sobrado; M. Moldovan; B. Bouyssiere; P. Giusti;
3.J. R. Encinar, Chem. Commun., DOI: 10.1039/c9cc09842a (2020)

P17. Exploring the mechanism of native electrospray of nucleic acids

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Mass spectrometry (MS) is one of the most utilized analytical techniques in chemistry and biochemistry, and electrospray ionization (ESI) happens to be the preferred ionization method associated with MS today. We use the least possibly energetic instrumental conditions to preserve both covalent bonds and weak non-covalent bonds present in solution in "native" ESI-MS and then probe the secondary, tertiary and/or quaternary structures in the gas phase of nucleic acids. This is very important to understand how the structures of the analytes are affected during the charging and desolvation process in ESI. We have tried to probe the structural change of structured and nonstructured oligonucleotides by ion mobility mass spectrometry. The changes in the charge state distribution with different ionic strength and supercharging agent are examined in detail. The collision cross-section distribution of ions in the gas phase helped to understand the ionization mechanism during ESI.

P18. AltProt Vision, the hidden kingdom of proteomic

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It was conventionally admitted that eukaryotes mature messenger ribonucleic acids (mR- NAs) are monocistronic leading to the translation of a single protein. However, large-scale proteomics has led to a massive identification of proteins from alternative open reading frames (AltORFs) translated from mRNAs in addition to the predicted proteins issued from the reference open reading frame (RefORF) or from non-coding RNAs (ncRNAs). These al- ternative proteins (AltProts) are not represented in the conventional protein databases and this ”Ghost proteome” was never considered until recently. However, these proteins were shown to be functional and there are growing evidences that they are involved in central functions such as cellular regulation both in the physiological and physiopathological con- text. Ghost proteins, therefore, represent a novel world, filling the gap in the understanding of signaling pathways, establishing as new markers of pathologies and therapeutic targets. In glioblastoma (GBM), variations of ncRNA expression have been demonstrated in tumor processes, especially in the regulation of major signaling pathways. Moreover, a large number of ncRNAs present in their sequences an ORF allowing their translations into AltProt and so constituting the ”ghost proteome”. In GBM, AltProts have been shown to be implicated in protein-protein interaction (PPI) with reference proteins (RefProt) reflecting involvement in signaling pathways linked to cellular mobility and transfer RNA regulation. More recently, clinical studies have revealed that AltProt are also involved in the patient’s survival and bad prognosis.

P19. Thiol Redox Proteomics Compatible Subcellular Fractionation

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Cysteine thiol oxidation, together with phosphorylation is one of the most studied pro- tein post-translational modifications. Classical proteomics workflows promote the detection of cytoplasm proteins which are characterized by the presence of a high number of reduced cysteines because of the higher reduction potential of this subcellular compartment. To improve the detectability of reversibly oxidized cysteines, many protocols based on their labelling and enrichment have been developed. Despite this, the detection of catalytic cysteines in more oxidizing subcellular compartments such as the endoplasmic reticulum is still a challenge. Subcellular fractionation is a powerful tool to improve the efficiency of the proteomics analyses, allowing to increase the identification rate of proteins belonging to the less represented compartments. The adaptation of this strategy to thiol redoxome analysis needs challenging optimization steps to ensure the accomplishment of the reduced thiol saturation and the prevention of artefactual oxidations. In this study we adapted a commercially available subcellular fractionation kit to the thiol redoxome analysis.

P20. Search for a high-mass calibrant for NALIM (Native Liquid MALDI MS): the case of mouse IgA

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Native mass spectrometry (native MS) is a set of methods to analyze biomolecular assemblies directly in an instrument. To this end, biomolecular objects or complexes are exposed only to non-denaturing conditions to preserve their three-dimensional structure intact from the sample solution to the gas phase.
In pharmacology, membrane proteins represent two-thirds of therapeutic targets, yet only 10% have been targeted so far. The characterization of membrane proteins by native MS can open many paths for biology and pre-clinical studies.

To this day, the go-to method for native MS is based on electrospray ionization (ESI). However, membrane protein analysis by native ESI-MS is still a challenge. This is because these proteins necessitate detergents for solubilization in MS-compatible buffers. Detergents in turn can give rise to ion suppression, adduction, and degraded instrument performance. These effects can be somewhat mitigated by working above the detergent's critical micellar concentration [1].

MALDI (Matrix-assisted laser desorption-ionization) ionization presents numerous advantages in this context. It is highly tolerant to contaminants and consumes little quantities of sample, making it *de facto* attractive for the native MS analysis of membrane proteins. Using these unique properties, we established a new native MS MALDI method using liquid spots called Native Liquid MALDI (NALIM) [2]. The success of this method relies on: (i) a matrix mixture that can be used in native conditions (pH ~ 5), (ii) a liquid matrix that enables to gently transfer complexes in the gas phase while avoiding the transition through the solid state, which is the basis of classical solid-spot deposition methods.

We are currently extending applications of the NALIM method to the observation of membrane protein complexes. In NALIM, we mainly observe low charge states (1+ and 2+), the monocharged state being largely predominant. Thus, the application of NALIM to large membrane protein-containing assemblies requires calibrant standards at high *m/z* ratios. In search of such calibrants, we investigated the use of an immunoglobulin A.

Here we report on the suitability and usability of an IgA for calibration over a wide mass range. Optimizations include biochemical preparation, control of the degree of oligomerization and instrumental setup for NaLiM.

1.Barrera, N.P., Bartolo, N.D., Booth, P.J., and Robinson, C.V. (2008). Micelles Protect Membrane Complexes from Solution to Vacuum. Science 321, 243–246.
2.Beaufour, M., Ginguené, D., Le Meur, R., Castaing, B., and Cadene, M. (2018). Liquid Native MALDI Mass Spectrometry for the Detection of Protein-Protein Complexes. J. Am. Soc. Mass Spectrom. 29, 1981–1994.

P21. SpiderMass un nouvel outil diagnostic pour le cancer œsogastrique

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Avec près de 10 millions de décès, le cancer est la deuxième cause de décès dans le monde. Le cancer œsogastrique (EC) est le 4ème cancer le plus diagnostiqué avec un nombre de cas en constante augmentation. L'EC est malheureusement très souvent asymptomatique et donc diagnostiqué tardivement. La chirurgie reste parmi les premiers traitements mais les chirurgiens sont confrontés à divers problèmes, en particulier, la délimitation précise de l'étendue de la tumeur. L'évaluation des tissus à réséquer est habituellement obtenue par une intervention chirurgicale exploratoire et des analyses histologiques, afin d'assurer l'exérèse complète de la tumeur. Cependant, cette évaluation histopathologique est réalisée ex vivo et rallonge le temps de la chirurgie sans mentionner le taux d'erreur important. Afin d'améliorer la chirurgie des cancers, une technologie basée sur la MS (SpiderMass) a été développée pour permettre un diagnostic rapide et précis in vivo et en temps réel basées sur les informations moléculaires.

Le SpiderMass est une technique de micro-échantillonnage constituée d'un laser fibré, d'un tube de transfert et d'un spectromètre de masse. La technologie utilise l'eau comme matrice endogène pour obtenir une ablation par excitation résonnante. Les analyses SpiderMass sont réalisées sur un instrument Q-TOF et les spectres MS générés servent pour construire des modèles de classification via le logiciel « Abstract Model Builder ». Enfin, les analyses MALDI MSI ont été réalisées sur un MALDI-TOF après dépôt de la matrice (Norharmane 7mg / mL).

Des spectres MS ont été obtenus en mode positif et négatif via le SpiderMass à partir de sections minces de biopsies d'EC. Les spectres générés ont été soumis à une analyse non supervisée par ACP puis une analyse supervisée par LDA pour construire un modèle de classification. Les résultats obtenus montrent que la technologie est capable de distinguer les tissus cancéreux et sains. De plus, nous avons pu séparer différents types d'EC tels que le Poorly Cohesive Carcinoma (PCC) de l'adénocarcinome. Il a également été possible de discriminer les 2 sous-types de PCC à savoir, les Signet Ring Cell (SRC) et les Non Otherwise Specified (NOS). Les marqueurs discriminants des différents types et sous-types ont pu être identifiés par MS/MS et une validation croisée a été réalisée par comparaison aux données d'imagerie MALDI-MS (MALDI-MS) à partir de sections consécutives de tissus. Ainsi, le SpiderMass a démontré sa capacité à analyser des biopsies ex vivo et à classer correctement les types et sous-types de tumeurs. Dans un proche avenir, nous testerons la technologie à l'hôpital, en effectuant un diagnostic ex vivo peropératoire pour obtenir une comparaison avec l'examen pathologique de référence.

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