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A new tool for multi-block PLS discriminant analysis of metabolomic data: application to systems epidemiology

Marion Brandolini-Bunlon, Mélanie Pétéra, Pierrette Gaudreau, Blandine Comte, Stephanie Bougeard, Estelle Pujos-Guillot

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12èmes Journées Scientifiques du Réseau Francophone de Métabolomique et Fluxomique

RFMF
Réseau Francophone de Métabolomique et Fluxomique

21 – 23 Mai 2019 Clermont-Ferrand

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PROGRAMMES

ATELIERS ET 12 JS

RÉSEAU FRANCOPHONE DE MÉTABOLOMIQUE ET FLUXOMIQUE

Programme des ateliers des 12èmes Journées

Lundi 20 mai 2019

Université Clermont Auvergne, IAE School of Management

11h-13h : Metabo MOOC (usemetabo) au service des enseignants (Rolin Dominique & Alain Bouchereau).

11h-13h : Juniors (RFMF Junior).

14h-16h : Intégration de données en métabolomique (Marion Brandolini & Julien Boccard).

14h-18h : Les réseaux moléculaires, découverte et prise en main (Amina Bouslimani, Pieter Dorrestein, Nicolas Elie, Yann Guitton, Louis Felix Nothias & David Touboul).

16h30-19h30 : MIMOSA, a mass spectrometry application for processing and analysis of metabolomics data – Application to lipidomics (Djomangan Adama Ouattara, Joséphine Abi-Ghanem).

Programme des 12èmes Journées Scientifiques

MARDI 21 MAI

8h00-9h00 Accueil des participants

9h00-9h30 Bienvenue

9h00-9h20 Allocution de Bienvenue

9h20-9h30 Introduction aux 12èmes journées Scientifiques du RFMF **Fabien Jourdan**, Président du RFMF et **Blandine Comte**, **Cyril Jousse** et **Estelle Pujos-Guillot**, Coordinateurs du Comité Local d'Organisation

9h30-12h05 **Session Santé/Pharmacologie**
Modérateurs : Estelle Pujos-Guillot – Sylvain Dechaumet

9h30-10h15 Metabolomics: recent developments in the analytical process - **Coral Barbas** Université San Pablo, Madrid, Espagne

10h15-10h35 Stable isotope labeling highlights enhanced fatty acid and lipid metabolism in human acute myeloid leukemia - **Justine Bertrand Michel**. METATOUL, Inserm, Toulouse, France

10h35-11h05 Pause, visite de l'exposition

Modérateurs : Blandine Comte – Justine Leenders

11h05-11h35 Methodological developments in Ultra-High-Resolution NMR for a deeper understanding of metabolic processes in relapsed/refractory diffuse large B-cell lymphoma - **Nicolas Giraud**. Laboratoire de Chimie et Biochimie Toxicologiques et Pharmacologiques, Université de Paris, Sorbonne Paris Cité, Université Paris Descartes - Paris V, France

11h35-11h50 The microbiome and drug metabolism - **Marine Letertre**. Imperial College London, Royaume-Uni

11h50-12h10 Suivi longitudinal du métabolisme cérébral par imagerie spectroscopique de Résonance Magnétique Nucléaire chez des modèles murins progressifs de la maladie de Parkinson **Carine Chassain**. CHU Gabriel Montpied, Clermont-Ferrand, France

12h10-12h25 **Session constructeur :** Waters - MetaboQuan et LipidQuan - Méthodes clés en main pour l'analyse ciblée des lipides et des métabolites - **Christophe Siroit**. Waters SAS, Saint-Quentin en Yvelines, France

12h25-13h15 Déjeuner

13h15-14h25 **Session posters :** Présence des auteurs devant leurs posters

13h40-14h10 **Session parallèle constructeur :** Thermo Fisher Scientific - Improved metabolome coverage and increased confidence in unknown identification through novel automated acquisition strategy combining sequential injections and MSn - **Marie Pierre Pavageau**. Thermo Fisher Scientific, San Jose, CA, USA

14h25-16h45 **Session Microbiologie/Biotechnologie**
Modérateurs : Fabien Jourdan – Binta Dieme

14h25-15h10 Using mass spectrometry to connect the worlds chemistry of life - a big data problem for natural products **Peter Dorrestein** Université de Californie, USA

15h10-15h30 Expanding the BCAA pathway of E. coli reveals the molecular basis of its robustness and homeostasis **Mickael Dinclaux**. Laboratoire d'Ingénierie des Systèmes Biologiques et des Procédés (LISBP), Université de Toulouse, CNRS, INRA, INSA, Toulouse, France

15h30-15h45 Y flux: A high throughput fluxomic workflow for exploration of metabolic phenotypes - **Cécilia Berges**. Laboratoire d'Ingénierie des Systèmes Biologiques et des Procédés (LISBP), Université de Toulouse, CNRS, INRA, INSA, Toulouse, France

15h45-16h00 **Session constructeur :** Accelerate your lab capabilities combining a metabolomic workbench and the new 6546 LC-QTOF Agilent - **Jérémy Jeudy**. Agilent Technologies France, Les Ulis, France

16h00-16h10 **Flash posters session 1:**
Modérateurs : Floriant Bellvert – Blandine Comte

- **Noémie Butin** : A combined metabolomics and lipidomics approach enables the stratification of acute-on- chronic liver failure patients according to their severity. CEA, INRA, Université Paris Saclay, Laboratoire d'Etude du Métabolisme des Médicaments, MetaboHUB-Paris, Gif-Sur-Yvette, France
- **David Mallet** : Metabolic characterization of different phases of Parkinson's disease. Grenoble Institut des Neurosciences - CEA, CHU Grenoble, Inserm U1216, Université Grenoble Alpes, France
- **Sergio Polakof** : Impaired postprandial skeletal muscle metabolism in a minipig model of insulin resistance: insights from arteriovenous and biopsy-based metabolomics analyses. Université Clermont Auvergne, INRA, UMR1019 Unité de Nutrition Humaine, Clermont-Ferrand, France

16h10-16h40 Pause, visite de l'exposition

16h40-17h40 **Session Microbiologie/Biotechnologie**
Modérateurs : Floriant Bellvert – Grégory Genta-Jouve

16h40-17h10 Improved riboflavin production with Ashbya gossypii from vegetable oil based on 13C metabolic network analysis with combined labeling analysis by NMR and mass spectrometry - **Lindsay Peyriga**. Laboratoire d'Ingénierie des Systèmes Biologiques et des Procédés (LISBP), Université

17h10-17h25 Session constructeur : Shimadzu - Applications en omics du nouveau QTOF Ultra-Fast MS LCMS-9030 - **Etienne Maout**. Shimadzu France, Marne-la-Vallée, France.

17h25-17h40 Feature-based molecular networking of untargeted mass spectrometry data: Bridging MS-DIAL, MZmine2, OpenMS, and XC-MS, with the GNPS web-platform - **Louis Félix Nothias**. University of California, San Diego, USA

17h40-17h50 Flash posters session 2 :
Modérateurs : Floriant Bellvert – Cyril Jousse

- **Nicolas Elie** : MetGem Software for the Generation of Molecular Networks Based on the t-SNE Algorithm. Institut de Chimie des Substances Naturelles, CNRS, UPR2301, France
- **Stéphanie Monnerie** : Analytic correlation filtration: A new tool to reduce analytical complexity of meta bolomic datasets. Université Clermont Auvergne, INRA, UMR1019 UNH, Mapping, Clermont-Ferrand, France
- **Matthieu Schoumacher** : New insight in Metabolomics based study of Age Related Macular Degeneration (AMD): Lipoprotein profile and subclass analysis. Centre Indisciplinaire de Recherche sur le Médicament (CIRM), Belgique

17h50-19h50 Assemblée générale RFMF

19h50-21h30 Apéritif et session posters

MERCREDI 22 MAI

9h00-10h25 Session développements analytiques
Modérateurs : Patrick Giraudeau – Justine Leenders

9h00-9h45 Pros and cons of fingerprinting and profiling in NMR based metabolomics - **Claudio Luchinat**, Université de Florence, Italie

09h45-10h05 Supercritical fluid chromatography coupled to high resolution mass spectrometry for dereplication and quantification of natural products - **David Touboul**. Institut de Chimie des Substances Naturelles (ICSN), CNRS UPR2301, Université Paris-Sud, Université Paris-Saclay, Gif-sur-Yvette, France

10h05-10h25 Apport de la spectrométrie de masse à haute résolution et à mobilité ionique dans les analyses méta bo lomiques à haut débit et à large échelle - **Aurélie Delvaux**. Institut Parisien de Chimie Moléculaire IPCM, Université Pierre et Marie Curie (UPMC) - Paris VI, France

10h25-10h55 Pause, visite de l'exposition

10h55-12h15 **Session développements analytiques**

10h55-11h10 Session constructeur : Proteomic solution Proteigene - Assessing gut microbiota-host crosstalk: Development of a standardized and quantitative targeted metabolomics assay - **Barbara Wolf**. BIOCRATES Life Sciences AG, Innsbruck, Austria

11h10-11h30 13C-labeled yeast extracts: a powerful tool for normalization and quantification in metabolomics and lipidomics? - **Thaïs Hautbergue**. Service de Pharmacologie et d'Immunoanalyse, Laboratoire d'Etude du Métabolisme des Médicaments, CEA, INRA, Université Paris Saclay, MetaboHUB, Gif-sur-Yvette, France

1h30-11h45 Advanced high-field and benchtop NMR methods for lipid profiling of microalgae

- **Dylan Bouillaud**. Génie des Procédés-Environnement-Agro-alimentaire - Université de Nantes, CNRS : UMR6144, France

11h45-12h15 Illuminating lifestyles using metabolomics of personal objects - **Amina Bouslimani**. University of California, San Diego, USA

12h15-13h15 Déjeuner

13h15-14h15 **Session posters :** Présence des auteurs devant leurs posters

14h15-15h00 **Session Environnement/Plantes**

Modérateurs : Cyril Jousse – Guillaume Meiffren

14h15-15h00 Metabolomics in the research and development of plant and protection products: current state and future perspectives - **Konstantinos A. Aliferis** - Université d'Athènes, Grèce

15h00-15h30 Large scale micropollutants screening in a wetland ecosystem using a non-targeted high resolution mass spectrometry approach - **Loïc Maurer**. Laboratoire des sciences de l'ingénieur, de l'informatique et de l'imagerie, Université de Strasbourg, UMR7357, CNRS FR3627, Ecole Nationale du Génie de l'Eau et de l'Environnement de Strasbourg, INSA Strasbourg, France

15h30-15h50 Anti-leishmaniasis and Metabolomic approach from the leaves extract of Psidium guajava L. from Lao PDR **Chiobouaphong Phakeovilay**. UMR 152 Pharma Dev, Université de Toulouse, IRD, UPS, Toulouse, France ; Université Paul Sabatier - Toulouse III, France

15h50-16h00 **Flash posters session 3 :**

Modérateurs : Floriant Bellvert – Estelle Pujos-Guillot

- **Christelle Ghaffar** : Étude de la réponse métabolique de Pseudomonas

syringae 32b-74 isolée des nuages à une exposition sub-létale au mercure. Université Clermont-Auvergne, CNRS, Institut de Chimie de Clermont-Ferrand (ICCF), Clermont-Ferrand, France

- **Ines Rémy-Larsonnier** : Profilage métabolique de molécules d'intérêt du piment (Capsicum) en nutrition animale. Laboratoire d'Ecologie Microbienne/CESN, Villeurbanne, France

Roland Wedekind : Syringol metabolites as biomarkers of smoked meat intake. Centre International de Recherche contre le Cancer - International Agency for Research on Cancer, Organisation Mondiale de la Santé/World Health Organization

16h00-16h30 Pause, visite de l'exposition

16h30-16h45 Session constructeur : Bruker - Analyses du plasma par RMN chez les adultes phenylcétonuriques sous prise en charge nutritionnelle montrent des déséquilibres métabolomiques - **Claire Cannet**. Bruker BioSpin, Wissembourg, France

16h45-17h25 Session Bioinformatique/Base de données

Modérateurs : Franck Giacomoni – Grégory Genta-Jouve

16h45-17h05 The Exposome-Explorer database in 2019: recent development on dietary biomarkers and their association with cancer risk - **Vanessa Neveu**. Centre International de Recherche contre le Cancer - Inter national Agency for Research on Cancer, Organisation Mondiale de la Santé/World Health Organization Office, France

17h05-17h25 Dynamic retention time databases for steroidomics - **Santiago Codesido**. Analytical Sciences, School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Suisse

17h25-17h45 RFMF d'Honneur - **Anne-Marie Delort** - Cloud microorganisms: what does metabolomics tell us? Université Clermont-Auvergne, CNRS, Institut de Chimie de Clermont-Ferrand (ICCF), Clermont-Ferrand, France

17h45-19h45 Assemblée générale RFMF Junior

20h00 Dîner de gala

JEUDI 23 MAI

9h30-10h40 Session Statistiques

Modérateurs : Julien Boccard – Mélanie Pétéra

9h30-9h50 Fusion de données de transcriptomique et de métabolomique pour une meilleure compréhension des effets d'un contaminant alimentaire - **Marie Tremblay-Franco**. UMR1331 Toxalim, Université de Toulouse, INRA, ENVT, INP-Purpan, UPS, Toulouse, France

9h50-10h10 A new tool for multi-block PLS discriminant analysis of metabolomic data: application to systems epidemiology - **Marion Brandolini-Bunlon.** Université Clermont Auvergne, INRA, UNH, Plateforme d'Exploration du Métabolisme, MetaboHUB Clermont, Clermont-Ferrand, France

10h10-10h25 Metabolomics and proteomics data integration and feature selection for high-throughput phenotyping - **Alyssa Imbert**. CEA, LIST, Laboratoire Analyse de Données et Intelligence des Systèmes, MetaboHUB, Gif-Sur-Yvette, France

10h25-10h40 **Session constructeur:** Sciex - Industrializing Flux Capabilities - **Yoann Fillâtre**. AB Sciex, Les Ulis, France

10h40-11h10 Pause, visite de l'exposition

11h10-12h35 **Session Alimentation/Qualité des aliments**
Modérateurs : Erwan Engel – Binta Dieme

11h10-11h40 Discovery and validation of food intake biomarkers using untargeted metabolomics in human intervention and cross-sectional studies - **Claudine Manach**. Université Clermont Auvergne, INRA, Unité de Nutrition Humaine, UMR1019, Clermont-Ferrand, France

11h40-12h00 Pratiques culturales, qualité des graines de colza et santé du consommateur: une étude preuve de principe « multi-omique » chez la souris au métabolisme dérégulé - **Jean-Charles Martin**. Centre recherche en CardioVasculaire et Nutrition, INSERM 1263, INRA 1260, Université Aix Marseille, Marseille, France

12h00-12h15 Découverte de marqueurs précoces de l'altération microbiologique des ovoproduits par des approches de métabolomique croisée (RMN-spectrométrie de masse) - **Rémy Coat**. Laboratoire de génie des Procédés-Environnement-Agro-alimentaire, Université Bretagne Loire, IMT Atlantique Bretagne-Pays de la Loire, Oniris, CNRS UMR6144, Université de Nantes, France

12h15-12h35 Analyse non ciblée de contaminants dans les aliments : validation d'une approche de type métabolomique sur deux technologies UHPLC-HRMS et deux scenarii de contamination « en aveugle » **Mathieu Cladière**. UMR Ingénierie Procédés Aliments (GENIAL), INRA UMR1145, AgroParisTech, Université Paris-Saclay, Massy, France

12h35-12h55 Prix de thèse - **Florent Olivon** - Nouvelle stratégie de priorisation pour l'étude des produits naturels par l'ap proche des réseaux moléculaires multi-informatifs. Institut de Chimie des Substances Naturelles, CNRS-ICSN, UPR 2301, Université Paris-Saclay, Gif-sur-Yvette, France

13h00 Clôture

RESUMES DES ATELIERS

Atelier 1 - metabo MOOC (usemetabo) au service des enseignants

Animateur(s) : Rolin Dominique (dominique.rolin@inra.fr) & Alain Bouchereau alain.bouchereau@univ-rennes1.fr)

Plus les autres contributeurs du MOOC que vous retrouverez sur le site de FUN à cette adresse : https://www.fun-mooc.fr/courses/course-v1:cnrs+136001+session01/about

Public envisagé et prérequis :

- Public : Enseignants-chercheurs, vacataires d'enseignements, conférenciers auprès d'étudiants, en quête de ressources pour leurs enseignements/interventions dans le domaine de la métabolomique dans leurs établissements.

- Prérequis : enseigner, intervenir auprès d'étudiants, dans un cursus universitaire, niveaux LM

Contexte et objectifs de l'atelier :

Les communautés bio-informatique et métabolomique,fédérées autour d'infrastructures nationales (Institut Français de Bioinformatique (IFB) et MetaboHUB), ont notamment pour mission de contribuer à la formation aux nouveaux usages technologiques et aux champs de leurs applications.
Les MOOC (Massive Open Online Courses) ont récemment fait une apparition dans le paysage de l'enseignement supérieur mais aussi de la recherche, suscitant de forts intérêts. C'est dans ce contexte et grâce aux soutiens de l'IFB, de MetaboHUB, du Réseau Francophone de Métabolomique et Fluxomique (RFMF) et de l'Université de Rennes 1 qu'il a été décidé de créer un MOOC dédié à la métabolomique : Usemetabo.
Usemetabo , dont la première sortie a eu lieu en octobre dernier a eu pour principal objectif de présenter en français pour des étudiants de niveau licence les concepts et méthodologies appliqués au profilage métabolique et à la métabolomique, en révéler les principaux usages et en illustrer les principaux champs d'applications. Il a connu un franc succès.
Les ressources vidéo sont d'ores et déjà disponibles sur un site WEB.
Cet atelier est conçu sous la forme d'une séance d'échanges entre ceux qui ont participé à l'élaboration de ce MOOC (20 contributeurs) et ceux qui ont ou auraient la volonté d'enseigner la métabolomique dans leur établissement. Ce MOOC est appelé à vivre, à évoluer et à fournir d'autres formes de ressources pédagogiques facilement accessibles aux enseignants. Il nous semble essentiel de pouvoir partager ces projets avec la communauté enseignante, dans le cadre du RFMF, et recueillir les besoins/les envies en matière d'outils pédagogiques et de contenus valorisables sous différents formats pour faciliter l'enseignement de la métabolomique et de la fluxomique à l'échelle nationale.
Références (publications, sites, outils, etc.) :
Voir le site de FUN pour le MOOC de la métabolomique :

https://www.fun-mooc.fr/courses/course-v1:cnrs+136001+session01/about

Voir la vidéo introduisant usemetabo : https://www.dailymotion.com/video/x6mt32w

Atelier 2 - MIMOSA: A mass spectrometry application for processing and analysis of metabolomics data – Application to lipidomics

Animateur(s) : Djomangan Adama Ouattara (Djomangan.OUATTARA@bioaster.org), Joséphine Abi-Ghanem (Josephine.ABIGHANEM@bioaster.org)

Public envisagé et prérequis :

- Public : Bioinformaticians and biochemists in mass spectrometry

- Prérequis : Mass spectrometry in metabolomics / Basic knowledge of MS data processing (peak picking, alignment and annotation)

Objectif de l'atelier :

The objective of this workshop is to introduce the main fundamentals of the bioinformatics tools developed at BIOASTER for the processing and analysis of mass spectrometry data in metabolomics, with a focus on lipidomics. MIMOSA (Mass spectrometry processIng of MetabOlomicS DatA) is an application module for BIOCODE, which is a transversal computational framework for metabolomics. MIMOSA allows building simple to complex pipelines to process and analyze metabolomics data with a high reproducibility and traceability. MIMOSA is ready-to-use, fast and extendable. It is specially designed to handle large metabolomics data (up to hundreds of raw files) in a reasonable time laps on a classical laptop. It allows interfacing available software like ProteoWizard, OpenMS and built-in functions for data normalization, statistical analysis and the identification of metabolites using Sirius, Metfrag and LipidMatch. As well, other tools can easily be interfaced into the MIMOSA to build customized workflows. MIMOSA is available as open Matlab scripts and a standalone application to non-developer users. Its extension to more open programming languages is planned.

Compétences acquises à la sortie de l'atelier :

- Processing of raw MS metabolomics data (with custom metadata), feature annotation, feature identification, basic MS data analyses (unsupervised, supervised analyses)

- Use of MIMOSA using Matlab scripts

- Use of MIMOSA using the standalone application (for non-developer users)

- Use of OpenMS, ProteoWizard, LipidMatch tools for untargeted metabolomics and lipidomics

- Use of the BIOCODE framework under Matlab environments

Références (publications, sites, outils, etc.) :

Abi-Ghanem et al, MIMOSA: A computational application for mass spectrometry preprocessing of metabolomics data, to submit to BMC Bioinformatics February 2019

Ouattara et al, BIOCODE: A transversal framework for computational workflow standardization – Fundamental concepts of the PRISM architecture, to submit to BMC Bioinformatics February 2019

Röst HL, Sachsenberg T, Aiche S, Bielow C, Weisser H, Aicheler F, et al. OpenMS: a flexible open-source software platform for mass spectrometry data analysis. Nat Methods. 2016;13:741–8

Koelmel JP, Kroeger NM, Patterson RE, Cochran JA, Beecher CWW, Garrett TJ, et al. LipidMatch: an automated workflow for rule-based lipid identification using untargeted high-resolution tandem mass spectrometry data. BMC Bioinformatics. 2017;18:331.

Ruttkies C, Schymanski EL, Wolf S, Hollender J, Neumann S. MetFrag relaunched: incorporating strategies beyond in silico fragmentation. J Cheminform. 2016;8:3

Pré-requis pour les participants : afin de profiter pleinement de cet atelier, merci de bien prendre en compte les configurations matérielles requises (si besoin, merci de contacter les animateurs pour confirmation).

- Personnal computers
- Free Matlab (2017, 2018 or 2019) pre-installed (Warning: the installation time is long)
    - At least the Bioinformatics Toolbox is required
    - It is recommended to install all the toolboxes by default
- ProteoWizard 3.0.x pre-installed (will be provided otherwise)
- OpenMS 2.3 preinstalled (will be provided otherwise)

Atelier 3 - Intégration de données en métabolomique

Animateur(s) : Marion Brandolini - marion.brandolini-bunlon@inra.fr et Julien Boccard - julien.boccard@unige.ch

Public envisagé et les prérequis :

- Public : Chercheurs, ingénieurs, étudiants travaillant en métabolomique et en quête d'informations sur les concepts, méthodes et outils d'intégration de données.

- Prérequis : Notions de base en traitement de données métabolomiques

Objectif de l'atelier :

L'objectif de cet atelier est, dans un premier temps, d'introduire les concepts clefs (structure des données, niveaux de fusion) et les stratégies expérimentales d'intégration (bioinformatique ou chimiométrique) des données métabolomiques, et d'en illustrer quelques-unes à travers différents exemples d'applications.

Il est, dans un deuxième temps, de créer des interactions entre participants, avec des échanges sur les différentes stratégies au regard de la nature des données et du type de question de recherche posée.

Compétences acquises à la sortie de l'atelier :

- Compréhension globale des différentes stratégies d'intégration des données métabolomiques

- Capacité de s'orienter vers une approche adéquate pour intégrer ses données expérimentales

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Atelier 4 - Les réseaux moléculaires, découverte et prise en main

Animateur(s) : Amina Bouslimani (abouslimani@ucsd.edu), Pieter Dorrestein (pdorrestein@ucsd.edu), Nicolas Elie (nicolas.elie@cnrs.fr), Yann Guitton (yann.guitton@oniris-nantes.fr), Louis Felix Nothias (nothias@ucsd.edu), David Touboul (david.touboul@cnrs.fr)

Public envisagé et les prérequis :

- Public : Utilisateurs de spectrométrie de masse (débutants ou expérimentés) appliquée à la métabolomique.

- Prérequis : Spectrométrie de masse et métabolomique, Notions de base en spectrométrie de masse en tandem (MS/MS), acquisition automatique de spectres MS/MS, annotation de spectres MS/MS.

Contexte et objectifs de l'atelier :

L'atelier 'Réseaux Moléculaires' (Molecular Network) a pour but d'introduire une approche bioinformatique permettant d'organiser, visualiser et interpréter des données de spectrométrie de masse en tandem (MS/MS) obtenues en mode non ciblé, utilisé communément en métabolomique. La méthode des réseaux moléculaires est basée sur l'évaluation du degré de similarité spectrale et permet de comparer, aligner et grouper des spectres MS/MS collectés à partir de différents échantillons afin de mettre en évidence des familles de molécules ayant des structures chimiques similaires. A travers l'analyse de 'Réseaux Moléculaires', il est également possible d'annoter les spectres MS/MS en les comparant aux spectres de référence des banques publiques. Durant cet atelier, nous présenterons les concepts de bases, et les étapes clés requises pour créer un réseau moléculaire avec la plateforme GNPS (http://gnps.ucsd.edu, Global Natural Products Social Molecular networking) ainsi que le logiciel MetGem (https://metgem.github.io/, pour la génération de réseaux moléculaires avec l'outil t-SNE). Notamment, vous apprendrez à créer et visualiser un réseau moléculaire à partir de données MS/MS. Cet atelier sera également l'occasion de vous familiariser avec les outils permettant la conversion de vos données aux différents formats de fichiers (mzML, mgf...). Vous utiliserez par la suite l'outil 'Library Search' qui permet d'annoter des spectres MS/MS grace aux banques publiques de spectres de références. Au cours de cet atelier, des fichiers de démonstrations seront mis à disposition, toutefois il est possible d'utiliser vos propres fichiers MS/MS. L'atelier se terminera par une présentation des autres outils disponibles sur la plateforme GNPS, tels que le Molecular Blast (MASST) permettant de rechercher un spectre MS/MS dans toutes les données publiques déposées sur GNPS/MassIVE, et le feature based molecular networking qui permet l'intégration d'OpenMS, MZmine2, MS-DIAL, XC-MS et MetaboScape, avec GNPS, ou d'autres logiciels d'analyses de données MS/MS.

Compétences acquises à la sortie de l'atelier :

Les participants apprendront à :

i) Gérer les formats de données

ii) Créer et visualiser un réseau moléculaire sur la plateforme GNPS.

iii) Naviguer sur la plateforme GNPS afin d'accéder aux résultats d'analyses et de les interpréter.

iv) Chercher un spectre MS/MS dans les données publiques déposées sur GNPS/MassIVE.

v) Accéder à d'autres outils et ressources disponibles sur GNPS.

vi) Créer, visualiser et interpréter des réseaux avec le logiciel MetGem.

Références (publications, sites, outils, etc.) :

Wang, M. et al. Sharing and community curation of mass spectrometry data with Global Natural Products Social Molecular Networking, Nature Biotechnology 34, 828-837 (2016) - https://www.nature.com/articles/nbt.3597

Olivon et al. MetGem Software for the Generation of Molecular Networks Based on the t-SNE Algorithm, Analytical Chemistry 90(23), 13900-13908 (2018) - https://pubs.acs.org/doi/10.1021/acs.analchem.8b03099

GNPS website- https://gnps.ucsd.edu/ProteoSAFe/static/gnps-splash2.jsp

GNPS documentation - https://ccms-ucsd.github.io/GNPSDocumentation/

GNPS YouTube channel - https://www.youtube.com/channel/UCufTdDIUPjfoN604Igv_29g/ videos

MetGem - https://metgem.github.io/

Pré-requis pour les participants : afin de profiter pleinement de cet atelier, merci de bien prendre en compte les configurations matérielles requises (si besoin, merci de contacter les animateurs pour confirmation).

Les participants doivent disposer d'ordinateurs personnels (Windows, MacOS, linux) et de connaissances de base sur la spectrométrie de masse en mode tandem. Le logiciel MetGem est téléchargeable à l'adresse suivante: https://metgem.github.io/ et installable sous Windows.

Atelier 5 - Juniors

Animateur(s) : RFMF Junior (rfmf.junior@gmail.com)

Public envisagé et les prérequis :

Public : Cet atelier vise plus particulièrement la communauté junior mais reste ouvert à tous !

Prérequis : Aucuns

Objectif de l'atelier : Créer des échanges par groupes autour des outils analytiques, statistiques et méthodologiques utilisés en métabolomique et fluxomique.

Compétences acquises à la sortie de l'atelier : Une vision plus éclairée des différents outils disponibles et utilisés dans le domaine de la métabolomique, fluxomique ainsi que des nouveautés en cours de développement qui viendront compléter et améliorer nos ressources.

Références (publications, sites, outils, etc.) : http://www.rfmf.fr/rfmf-junior.html

COMMUNICATIONS INVITEES

Présentation des Invités

Pr Coral Barbas Universidad San Pablo Madrid, CEMBIO

Prof Coral BARBAS is currently FullProfessor of Analytical Chemistry at Pharmacy Faculty, Universidad CEU San Pablo, Madrid (Spain) and Director for the "Centre for Metabolomics and Bioanalysis" (CEMBIO) at this Faculty. She is also Director for CEU International School of Doctorate (CEINDO); Visiting Professor at Imperial College London, Department of Surgery and Cancer and at Bialystok Medical University. As previous appointments she was Vice-Chancellor for Research at Universidad CEU San Pablo and Marie Curie Fellow at Kings College London. She is the author of more than 200 papers, with current research interests in all the steps in metabolomics workflow: experimental design, sample pretreatment, analytical methods for targeted and untargeted metabolomics, method validation, data treatment, compound identification and interpretation. Her researcher is focused on multiplatform analysis with GC-MS, LC-MS and CE-MS of all kind of biological samples searching for disease biomarkers, prognostic biomarkers, mechanisms of action of a drug, diet, etc. Her awards include the medal of Bialystok Medical University and she was named to the 2016 Power List, the 50 Most Influential Women in Analytical Chemistry in the World, *The Analytical Scientis* and recently she has received the award of the Belgian Society of Pharmaceutical Sciences (BSPS 2018).

Pieter Dorrestein University of California - San Diego, Collaborative Mass Spectrometry Innovation Center

Dorrestein is Professor at the University of California - San Diego. He is the Director of the Collaborative Mass Spectrometry Innovation Center and a Co-Director, Institute for Metabolomics Medicine in the Skaggs School of Pharmacy & Pharmaceutical Sciences, and Departments of Pharmacology and Pediatrics. Since his arrival to UCSD in 2006, Prof. Dorrestein has been pioneering the development of mass spectrometry methods to study the chemical ecological crosstalk between populations of microorganisms, including host interactions for agricultural, diagnostic and therapeutic applications. He participated in panels for the white house science and technology office of president on the launch of a national microbiome initiative and has been on panels for the National Academy of Sciences on the Chemistry of the Microbiome. He has co-authored over 220 publications and his work has been featured by the wall street journal, CNN, NYTimes, Fox, BBC and hundreds of other news outlets. He has been recognized with several awards, among them are awards from the Beckman foundation, V-foundation in cancer research, EUREKA award for unconventional and enabling research, Hearst Foundation, Pharmaceutical Research and Manufacturing Association research award and the Abel award in pharmacology. For a more detailed biography see http://www.nature.com/news/the-man-who-can-map-the-chemicals-all-over-your-body-1.20035

Claudio Luchinat University of Florence,CERM

Claudio Luchinat is full Professor of Chemistry at the University of Florence, Director of CERM (Center of Magnetic Resonance) and of CIRMMP (Interuniversity Consortium on Magnetic Resonance of MetalloProteins). His research interests include development of NMR-based structural methodologies, electron and nuclear relaxation, NMR of paramagnetic species, relaxometry, bioinorganic chemistry and metabolomics. His h-index is 79 (Scholar), and his papers have been quoted more than 25.000 times.H has held seminars in many prestigious universities and research institutions worldwide, and plenary lectures in International Workshops, Symposia and Conferences. He has been awarded the 1989 "Raffaello Nasini" gold medal, the 1994 Federchimica Award "For an Intelligent Future", the 1996 SBIC European Medal for Biological Inorganic Chemistry, the 2001 GDRM gold medal, the 2017 Sapio Senior Award and the 2018 Richard R. Ernst Prize in Magnetic Resonance. http://www.cerm.unifi.it/people/claudio-luchinat

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Konstantinos A. Aliferis Agricultural University of Athens, PMG

Konstantinos A. Aliferis is Professor of Pesticide Science at the Agricultural University of Athens (Greece), head of the Pesticide Metabolomics Group (Dept of Plant Science). Since late 2017, Dr Aliferis is also Adjunct Professor at McGill University in Plant Science Dept. His skills are covering plant functional genomics (metabolomics), advanced crop protection, pesticide research and development, research on bioactive plant and fungal metabolites-natural products (bioactivity, MoA, and toxicological risk assessment), cannabis R&D, fungal resistance to fungicides and fungal metabolomics (GC/MS, FT-ICR/MS, Orbitrap MS/MS, & NMR analyzers), GM plant risk assessment, metabolic networking, shotgun proteomics, RNA sequencing data analyses, multi-level “omics” data integration, biomarker discovery, quantitative structure-activity relationship (QSAR) modeling, and food chemistry (chemoinformatics, pesticide residues). Dr Aliferis is also an expert in the state-of-the-art mega-variate analyses (PCA, O2PLS, PLS-DA, OPLS-DA, SIMCA, HCA, Heatmaps), “omics” data fusion, bioinformatics analysis, metabolic networking, and biological interpretation of large-scale “omics” data. For further information : Pesticide Metabolomics Group (PMG): http://www.aua.gr/pesticide-metabolomicsgroup/

Oral Invité 1– OI 1

METABOLOMICS: RECENT DEVELOPMENTS IN THE ANALYTICAL PROCESS

Coral Barbas

[1] Center for Metabolomics and Bioanalysis (CEMBIO), Faculty of Pharmacy, San Pablo CEU University, Madrid, Spain

Metabolomics is the omics with a closer relationship with Analytical Chemistry due to the analytical difficulties to detect such broad range of compounds in terms of physic-chemical properties and concentrations. Even more, I would say that Metabolomics is producing an evolution in analytical terms and concepts. Metabolomics workflow mimics the classical steps in the "Analytical Process" and our group has been developing tools to increase analytical quality and confidence at every step of the workflow.

Some researchers tend to think that working without a priori hypothesis means working in a blind way where everything is valid. Indeed a proper design and development of a metabolomics assay requires a tight control of all factors to avoid artifactual findings. Technological advances in nuclear magnetic resonance and mass spectrometry are significantly improving our capacity to obtain more data from each biological sample; however meaningful results can be only obtained if all the steps are carefully considered.

The final success of untargeted metabolomics depends on applying the principles of the Analytical Process to every step in the workflow: i) Clear definition of the objective of the analysis. Ii)Sampling and sample storage to guarantee a representative and homogeneous sample. Iii) Sample pre-treatment to obtain a sample as complete and non-biased as possible while being compatible with the instrumental techniques. Iv) Analytical methods with a proper quality control. V) Use of appropriate statistics and data treatment. Vi) Identification of statistically significant metabolites. Vii) Interpretation of results

Our group has been working in improving the methodology in many of these areas while at the same time applying our developments in real world studies because the path is made by walking.

Examples of different studies to avoid black boxes in untargeted metabolomics will be presented.

1.-Sampling and sample collection: Brain tissue obtained from autopsy is practically the only source of normal brain in humans. The post-mortem time (PT)-induced changes that may occur at both the metabolomics and anatomical levels in the brains will affect the conclusions obtained. and work in better known environments such as post-mortem brain sample evolution (1). In another study a

popular method of cell isolation (fluorescence-activated cell sorting (FACS)) was considered. The impact of FACS on the cell metabolome was deeply investigated (2).

2.-Identification. CEU Mass Mediator (CMM) is an on-line open source tool developed at CEMBIO (3). It aims aiding researchers when performing metabolite annotation. Its database integrates several metabolomic databases including Human Metabolome Database (HMDB), KEGG and LipidMaps and 672,042 simulated compounds from MINE. In addition, CMM scores the annotations which matched the query parameters using 122 rules based on expert knowledge.

Since this last major revision, CMM has continued to grow, expanding the knowledge base of its expert system and including new services to support researchers in the metabolite annotation and identification process (4). The information from external databases has been refreshed, and an in-house library with oxidized lipids not present in other sources has been added. This has increased the number of experimental metabolites up 332,665 and the number of predicted metabolites to 681,198. Furthermore, new taxonomy and ontology metadata have been included. CMM has expanded its functionalities with a service for the annotation of oxidized glycerophosphocholines, a service for spectral comparison from MS2 data, and a spectral quality-assessment service.

References

1.- C. Gonzalez-Riano et al. Metabolomics and neuroanatomical evaluation of post-mortem changes in the hippocampus. Brain Structure and Function 2017. 222, 6, 2831–2853.

2.- Binek A et al. Flow Cytometry Has a Significant Impact on the Cellular Metabolome. J Proteom Res 2018 (on line publication).

3.-Gil de la Fuente et al. Knowledge-based metabolite annotation tool: CEU Mass Mediator. J. Pharm. Biomed. Anal. 2018 154, 138-149.

4.-Gil-De-La Fuente, et al. CEU Mass Mediator 3.0: a metabolite annotation tool. J. Proteome Res 2019, 18 (2), 797–802.

Oral Invité 2– OI 2

Using Mass Spectrometry to Connect The Worlds Chemistry of Life – a Big Data Problem For Natural Products

Peter Dorrestein

Imagine if we could figure out the chemical composition of any natural sample and understand the relationships of the molecules to all other samples in seconds – akin to a Google search but instead of using a text we use mass spectrometry information. This would transform the life sciences. The foundation for such infrastructure is being created through community wide knowledge capture and analysis tool development. In this lecture I will describe the steps we have taken with GNPS, MASST, ReDU and other tools to achieve that goal.

Oral Invité 3– OI 3

Pros and cons of fingerprinting and profiling in NMR-based metabolomics

Claudio Luchinat

Magnetic Resonance Center (CERM) and Department of Chemistry – University of Florence - Italy

The two main approaches to metabolomics are fingerprinting and profiling. Their characteristics have been recently reviewed by us[1]. Fingerprinting by NMR is the global evaluation of the whole spectrum, viewed as a snap-shot of all (assigned or unassigned) detectable metabolites present in the sample. This can be done by transforming NMR spectra in a data matrix through bucketing, or using full resolution spectra. In the latter case, specific algorithms for peak alignment are needed. Fingerprinting is essentially used to provide fast sample classification by multivariate statistical techniques. However, due the need of finely comparing different sets of data, fingerprinting is not robust to bias introduced by non-perfectly standardized pre-analytical and analytical procedures. Profiling, in contrast, deals with the determination of the concentrations of all quantifiable metabolites in the sample. Profiling provides more meaningful data from a biochemical perspective, as it also enables the identification of metabolites and metabolic pathways associated with a specific pathology and is more robust to (non-critical) sample variations. However, the analysis required to assign and deconvolute the NMR signals for quantification is non-trivial and not yet completely automated. Also, the molecules quantifiable via profiling are presently significantly less numerous than those contributing to the fingerprint: the latter, therefore, is still the best tool for sample classification and statistical model building. However, if progress in automated peak assignment and quantitation continues, the gap in information content between profiling and fingerprinting will be progressively reduced. Promising approaches to this end will be discussed[2].

[1] A. Vignoli, V. Ghini, G. Meoni, C. Licari, P. G. Takis, L. Tenori, P. Turano, C. Luchinat, Angew. Chem. Int. Ed Engl. 2019, 58, 968–994.

[2] P. G. Takis, H. Schäfer, M. Spraul, C. Luchinat, Nat. Commun. 2017, 8, 1662.

Oral Invité 4– OI 4

Metabolomics in the research and development of plant protection products: current state and future perspectives

Konstantinos A. Aliferis

Pesticide Science Laboratory, Agriculture University of Athens, Iera Odos 75, 18855 Athens, Greece

Department of Plant Science, McGill University, 21111 Lakeshore Road, Sainte-Anne-de-Bellevue, Quebec, Canada, H9X 3V9

konstantinos.aliferis@aua.gr, konstantinos.aliferis@mcgill.ca

Plant protection products (PPPs) represent the backbone of the agri-food sector worldwide in the effort to secure food quality and supply. Nonetheless, the sector is facing major challenges such as, among others, the development of resistance to PPPs pest and pathogens, the presence of residues of PPPs in the food and the environment, toxicity issues to non-target organisms, and the increasing concerns of the public and international organizations on food safety. In order to address such challenges, the implementation of advanced bioanalytical tools for the discovery of new sources of bioactivity, which will ideally exhibit new or alternative mode(s)-of-action (MoA), improved efficacy and toxicological profiles, is necessary. Based on its unique capacities, metabolomics could serve as an ideal tool towards this direction, and additionally could accelerate the research and development (R&D) of PPPs, which is a time consuming and costly process. Within this context, my research program is focusing on the in-depth study of the mechanism by which plant pathogens develop resistance to PPPs, the discovery and assessment of new sources of bioactivity as PPPs, in vitro and in planta (e.g., endophytes, natural products, bioelicitors, nanomaterials), and the development of advanced metabolomics for the discovery of the MoA of bioactive compounds. Here, the current state and future perspectives of the application of metabolomics in the R&D of PPPs will be presented.

COMMUNICATIONS ORALES

Oral 1 – O1

Stable Isotope Labeling Highlights Enhanced Fatty Acid and Lipid Metabolism in Human Acute Myeloid Leukemia

Lucille Stuani [1], Fabien Riols [2], Pierre Millard [3], Aurélie Batut[2] , Fanny Viars [2], Marie Sabatier [2], Estelle Saland[2], Jean-Charles Portais[3,4], Jean-Emmanuel Sarry [1], Justine Bertrand-Michel [2]

[1] Team ResistAML, Drug resistance oncometabolism in acute myeloid leukemia, CRCT – Inserm : UMR1037, CNRS : UMR5294, Université Paul Sabatier-Toulouse III - UPS – 2 avenue Hubert Curien TOULOUSE

[2] Plateforme MetaToul Lipidomique, I2MC – Inserm : UMR1048 –Toulouse

[3] LISBP UMR5504, 135 Avenue de rangueil 31077 Toulouse cedex 04, France

[4] Plateforme MetaToul Réseaux métabolique, LISBP Toulouse

Background: In Acute Myeloid Leukemia (AML), a complete response to chemotherapy is usually obtained after conventional chemotherapy but overall patient survival is poor due to highly frequent relapses. As opposed to chronic myeloid leukemia, B lymphoma or multiple myeloma, AML is one of the rare malignant hemopathies the therapy of which has not significantly improved during the past 30 years despite intense research efforts. One promising approach is to determine metabolic dependencies in AML cells. Moreover, two key metabolic enzymes, isocitrate dehydrogenases (IDH1/2), are mutated in more than 15% of AML patient, reinforcing the interest in studying metabolic reprogramming, in particular in this subgroup of patients. Methods: Using a multi-omics approach combining proteomics, lipidomics, and isotopic profiling of [U-13C] glucose and [U-13C] glutamine cultures with more classical biochemical analyses, we studied the impact of the IDH1 R132H mutation in AML cells on lipid biosynthesis. Results: Global proteomic and lipidomic approaches showed a dysregulation of lipid metabolism, especially an increase of phosphatidylinositol, sphingolipids (especially few species of ceramide, sphingosine, and sphinganine), free cholesterol and monounsaturated fatty acids in IDH1 mutant cells. Isotopic profiling of fatty acids revealed that higher lipid anabolism in IDH1 mutant cells corroborated with an increase in lipogenesis fluxes. Conclusions: This integrative approach was efficient to gain insight into metabolism and dynamics of lipid species in leukemic cells. Therefore, we have determined that lipid anabolism is strongly reprogrammed in IDH1 mutant
AML cells with a crucial dysregulation of fatty acid metabolism and fluxes, both being mediated by 2-HG (2-Hydroxyglutarate) production1. 1Stuani *et al*, Int. J. of Molecular Sciences, 2018

Mots-Clés: lipidomique, marquage isotopique, cancer

Oral Keynote 1 – OK1

Methodological Developments in Ultra-High-Resolution NMR For a Deeper Understanding of Metabolic Processes in Relapsed/Refractory Diffuse Large B-Cell Lymphoma

Gildas Bertho [1], Leonardo Lordello [2], Stéphanie Nuan-Aliman [2], Cédric Caradeuc [1], Catherine Thieblemont [3,2], Véronique Baud [2], Nicolas Giraud [1]

[1] Laboratoire de Chimie et Biochimie Toxicologiques et Pharmacologiques, Université de Paris, Sorbonne Paris Cité – Université Paris Descartes - Paris 5 : UMR8601 – France

[2] NF-kappaB, Différenciation et Cancer, Université de Paris, Sorbonne Paris Cité – Université Paris Descartes - Paris 5 : EA7324 – France

[3] Assistance Publique–Hopitaux de Paris – Hopital-Saint-Louis, Hematooncology–Universite Paris Diderot, Sorbonne Paris-Cite, Paris, France – France

Lymphoma refers to the group of blood cancers that develop from lymphocytes, which are key infection-fighting cells of the immune system. Among the many different forms of lymphomas, Diffuse Large B-Cell Lymphoma (DLBCL) is the most common aggressive non-Hodgkin lymphoma that accounts for around 40% of cases. Although DLBCL treatment outcome has significantly improved, to date up to 40% of patients will either be refractory or relapse and die. Over the last years, metabolism has become a major topic in the field of cancer research, unveiling new approaches to decipher the mechanism of the disease, as well as tools for developing new treatments.[1] In this context, we are carrying out a collaboration to overcome drug resistance in relapsed/refractory diffuse large B-cell lymphoma (DLBCL). Prof. C. Thieblemont (Hospital Saint-Louis, Paris) has recently successfully set up a phase 1 clinical trialusing a combination of three metabolic inhibitors that was shown to induce a global remission in 75% of relapsed/refractory DLBCL patients included in the trial.[2] These results were validated by Dr. V. Baud (INSERM, Univ. Paris Descartes) who showed that combining anti-metabolic drugs induces massive DLBCL cell death.

Here we will show how 1H NMR can be used to get a deeper insight into the metabolism of DLBCL and decipher the mechanism of action of metabolic inhibitors. We will describe how standard protocols have been applied on the cellular endo- and exo-metabolomes studied on DLBCL cell lines and shed light on how these antimetabolic drugs impact on DLBCL metabolomes. We will also present the methodological developments that are in progress in our group to acquire broadband homonuclear decoupled spectra on these complex biofluids. Despite their intrinsic lower sensitivity, the most recent methods in the field of "pure shift" NMR [3] yield significant resolution enhancements when they are implemented in standard pulse sequences designed for NMR-based metabolomics, which paves the way to a higher level of information and to new protocols for statistical analyses.

Mots-Clés: Lymphoma, Metabolism, NMR, Pure Shift

Oral Junior 1 – OJ1

The microbiome and drug metabolism

Marine Letertre [1], Nyasha Munjoma [2], Adele Costabile [3], Lesley Hoyles[4], Aadra Bhatt [5], Matthew Redinbo [5], Jeremy Nicholson [6], Jonathan Swann [7], Ian Wilson [1]

[1] Imperial College London – Royaume-Uni
[2] Waters Corporation – Royaume-Uni
[3] University of Roehampton – Royaume-Uni
[4] Nottingham Trent University – Royaume-Uni
[5] University of North Carolina – États-Unis
[6] Murdoch University – Australie

Understanding drug-microbiome-host interactions should now be considered as a key point to determine the pharmacological profile of a drug as well as its toxicological profile. On one side, the gut microbiota can, directly and indirectly, affect the pharmacological profile of orally administered drugs (or metabolites excreted into the gut via e.g. the bile), and on the other, the drug itself can perturb the microbiome structure and its functionality with potential ramifications for host health. Pharmaco-metabonomics, a useful tool to patient stratification, was applied to a multifaceted approach in order to cover the wide range of gut microbial-drug interactions, and to develop an appropriate in vitro tool. The simultaneous use of targeted and untargeted UPLC-MS metabolic profiling allowed (1) to monitor the detoxification of methotrexate by the bacterial enzyme FGCP and (2), to observed dose-related effects of the drug on the urine and faecal metabolic profile and microbial community in rats. As it was reported that the microbial metabolite *p*-cresol, coming from the microbial degradation of tyrosine, can negatively impact paracetamol metabolism and sulfate-conjugation pathway in general, a targeted UPLC-MS method to quantify *p*-cresol and catabolites has been developed and it will soon be applied in a fit-for-purpose method on mice samples which received two weeks of high-tyrosine diet to manipulate their sulfate metabolism prior to receiving a sub-lethal dose of acetaminophen. Understand the interactions occurring between a given drug and the gut microbiota can also help to therapeutically target the gut microbiota and limits adverse-drug reactions of an existing drug. Indeed, in order to enhance irinotecan efficacy and limits it toxicity, a bacterial β-glucuronidase inhibitor is being developed. Its administration in mice model seems promising without significantly influence the metabolome of the animals. Finally, preliminary results of an in vitro model based on miniaturized batch cultures and semi-targeted UPLC-MS showed drug and donor-specific effects on compound metabolism. We are currently working on scaling-down the model to move toward a high-throughput method to easily automate and to implant it in pharmaceutical and clinical laboratories.

Mots-Clés: Pharmaco, metabonomics, microbiome, UPLC, MS, methotrexate, acetaminophen, p, cresol, B, glucuronidase

Oral 2 – O2

Suivi longitudinal du métabolisme cérébral par imagerie spectroscopique de Résonance Magnétique Nucléaire chez des modèles murins progressifs de la maladie de Parkinson

Carine Chassain [1], Christophe Melon [2], Guilhem Pages [3], Yann Le Fur [4], Paolo Gubellini [5], Pascal Salin [5], Lydia Kerkerian-Le Goff [2], Franck Durif [6]

[1] CHU Gabriel Montpied (CHU) – CHU Clermont-Ferrand – 58 rue Montalembert 63000 Clermont-Ferrand, France
[2] IBDM, UMR 7288 CNRS / Aix-Marseille Université, Marseille CNRS – France
[3] AgroResonance-UR370 QuaPA, Saint-Genes Champanelle – INRA Clermont-Ferrand-Theix – France
[4] CRMBM, UMR 7339 CNRS / Aix-Marseille Université Marseille – CNRS – France
[5] IBDM, UMR 7288 CNRS / Aix-Marseille Université, Marseille – CNRS – France
[6] Université Clermont Auvergne (UCA), EA7280 NPSY-Sydo, Clermont-Ferrand – Université Clermont Auvergne (UCA) – France

Les modèles animaux de la maladie de Parkinson (MP), reproduisant la dégénération progressive des neurones dopaminergiques de la substance noire pars compacta (SN), ouvrent de nouvelles opportunités pour en étudier l'évolution. L'imagerie spectroscopique de Résonance Magnétique Nucléaire (MRSI) est utilisée pour suivre au cours du temps l'évolution des profils métaboliques dans les composants clés des ganglions de la base (striatum dorsal, cortex moteur, noyaux sous-thalamique (NST) et SN).
Les acquisitions MRSI sont réalisées à 11,7T. Des images anatomiques permettent de visualiser les régions d'intérêt et de positionner la MRSI. Cette technique permet de collecter des spectres provenant de volumes d'intérêt de tout un plan de coupe avec une résolution de 1,6x1,6x2 mm3. Les données MRSI sont analysées sous CSIAPO puis la quantification est effectuée avec le logiciel LCModel. Des rats reçoivent une injection stéréotaxique unilatérale d'un substrat inhibiteur des transporteurs au glutamate, le PDC (n=7) ou du véhicule (n=5) dans la SNc gauche. Pour le modèle α-synucléine (n=7), des vecteurs viraux adéno-associés recombinants sont injectés aux mêmes sites. L'imagerie spectroscopique a été réalisée 30, 60 et 120 jours après la chirurgie. A chaque point, l'activité motrice des rats est mesurée et à la fin de l'étude les rats sont sacrifiés pour quantifier la dénervation dopaminergique nigrale.

Les taux de glutamate sont plus élevés au niveau du striatum chez les deux modèles. La quantification du glutamate au niveau du NST montre une augmentation dans le NST ipsilatéral à la lésion, mais aussi du côté controlatéral. Les deux modèles ont des atteintes motrices et la dénervation DA est vérifiée.
La MRSI est une technique utile pour la caractérisation longitudinale des profils de métabolites sur des modèles animaux de MP. Elle souligne l'implication de la réactivité glutamatergique du NST controlatéral à l'atteinte primaire des neurones dopaminergiques de la SN dans la progression de la MP. Comprendre les circuits et les mécanismes interhémisphériques impliqués pourraient permettre de proposer des nouvelles stratégies thérapeutiques pour ralentir cette progression.

Mots-Clés: Imagerie spectroscopique, RMN, maladie de Parkinson, modèles murins

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Re-inventing the Untargeted Metabolomics Workflow with the Thermo Scientific™ Orbitrap ID-X™ Tribrid™ MS

Marie Pierre Pavageau, ThermoFisher Scientific

Compound identification is a current bottleneck in the broad implementation of metabolomics. The development of an automated acquisition strategy implemented on a Tribrid™ mass spectrometer, enabling multistage fragmentation (MS^n) associated with an integrated software and library has increased the number of detected metabolites in samples and enables confident biological interpretation of the results.

Oral 3 – O3

Expanding the BCAA pathway of E. coli reveals the molecular basis of its robustness and homeostasis

Mickael Dinclaux[1], Pierre Millard [1], Fabien Létisse[1]

[1] Laboratoire d'Ingénierie des Systèmes Biologiques et des Procédés (LISBP) – LISBP, Université de Toulouse, CNRS, INRA, INSA, Toulouse, France – France

Escherichia coli K-12 MG1655 strain (F- lambda- ilvG- rfb-50 rph-1) is a model organism for scientific research and a workhorse for biotechnology, which carries frameshift mutations in its genome. Among them, a reported frameshift mutation concerns *ilvG* gene (ilvG-) which encodes the acetohydroxy-acid synthase II (AHAS; EC 2.2.1.6), leading an inactive gene product and a valine sensitivity characterized by a growth defect in minimal medium supplemented with valine. Here, we wondered if the lack of AHAS II enzyme induced other disorders at the metabolic level despite of the presence of the two isoenzymes, AHAS I and AHAS III. By using an exometabolome comparative analysis using 1D-H NMR between the wild-type strain and a strain repaired for this specific mutation, we identified the accumulation of three metabolites, namely propionate, DHIV (2,3-dihydroxy-isovalerate) and DHMP (2,3-dihydroxy-3-methylpentanoate). The two latter metabolites are intermediates of the branched chain amino acids (BCAA) pathway, both being the substrate of dihydroxy acid dehydratase, for the formation of valine and leucine, and for the formation of isoleucine respectively. Surprisingly, the reaction catalyzed by the dihydroxy-acid dehydratase takes place downstream of that catalyzed by the AHAS, making the accumulation of DHIV and DHMP not obvious to explain. Propionate is reported to be produced from ketobutyrate in anaerobiosis by pyruvate formate lyase. An investigation of propionate accumulation observed in this study in aerobic condition show the existence of a new metabolic process in connection with the absence of AHAS II. Combining metabolomics approaches based on both MS and NMR, molecular biology tools and *in silico* models, we pinpointed local metabolic reorganizations explaining the accumulation of these metabolites in culture supernatants. This works demonstrates the molecular basis of the robustness and homeostasis of this metabolic node in *E. coli* which contributes to the adaptative ability of metabolism to genetic modifications.

Mots-Clés: E. coli, BCAA, homeostasis, robustness

Oral Junior 2 – OJ2

Y flux: A high throughput fluxomic workflow for exploration of metabolic phenotypes

Cécilia Berges[1,2], Edern Cahoreau [1,2], Lindsay Peyriga [1,2], Stephanie Heux [1], Pierre Millard [1], Floriant Bellvert [1,2], Jean-Charles Portais [1,2,3]

[1] LISBP – LISBP, Université de Toulouse, CNRS, INRA, INSA, Toulouse, France – France
[2] MetaToul - MetaboHub – ANR-11-INBS-0010 – France
[3] Stromalab – Université de Toulouse, CNRS ERL5311, EFS, INP-ENVT, Inserm U1031, UPS – France

Despite the progress that has been made in sequencing technology and bioinformatic methods, a non-negligible percentage of genes are still not characterized. Even for an organism very well known as *E. coli*, we can find more than 40% of genes with unknown or putative function. Highthroughput (HT) omics approaches enable the large-scale analysis of biological molecules and try to get a comprehensive understanding of living organism and their regulation. Among those, fluxomics aims at measuring the actual rates of biochemical reactions in metabolic networks. However, a 13C fluxomic experiment requires several complex experimental and computational steps as weel as large input of manual steps. These makes classical fluxomics a low throughput approach, which limits the number of organisms, strains and conditions which can be investigated at once. To increase fluxomics throughput and robustness while reducing experimental costs and human efforts, we designed a complete workflow in order to automatize, parallelize, integrate and optimize each step of fluxomics experiments. This includes (1) experimental design, (2) automated labelling experiment, (3) isotopic and metabolomics analyses, (4) Data processing (5) Flux calculation and Statistical analysis. We then decided to challenge our workflow and evaluate the impact of unknown function genes mutation on central metabolic fluxes of *E. coli*. For this purpose, we selected 182 mutant strains of the Keio collection for which the function was not characterized. Flux maps were obtained for every strain on labelled glucose condition; they were statistically analyzed and compared to reference flux maps of known strains. Among these 182 strains investigated, two strains (ydcS and ybjP) showed significant modifications of central metabolism with metabolic fluxes changes compared to the wild type strain without any significant variation of physiology. Thanks to this development, we are able to quantify flux responses to environmental or genetic perturbations in particular for screening of genes functions in complement to other omics approaches. It opens the way to very large-scale fluxomics screening experiment and it can be applied in many fields : biotechnology, biomarker, drug development, medical science, toxicology or pharmacology.

Mots-Clés: Flux analysis, High throughput, Fluxomic workflow, Biotechnology, y genes

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Oral Flash 1 – FP1

A combined metabolomics and lipidomics approach enables the stratification of acute-on-chronic liver failure patients according to their severity

Noemie Butin [1], **François Fenaille**[1], **Benoit Colsch** [1], **Christophe Junot** [1], **Joan Clària** [2,3], **Richard Moreau** [4,5], **Vicente Arroyo** [5]

[1] CEA Saclay, Laboratoire d'Etude du Métabolisme des Médicaments, MetaboHUB-Paris, DRF/IBITEC-S/SPI, 91191 Gif-sur-Yvette Cedex, France. – CEA, CNRS, Université Paris-Saclay, CEA Saclay 91191 Gif sur Yvette France – France

[2] European Foundation for the Study of Chronic Liver Failure (EF-CLIF), Barcelona, Spain – Travessera de Gràcia, 11, Planta 7ª, 08021 Barcelona, Spain, Espagne

[3] Department of Biochemistry and Molecular Genetics, Hospital Clinic, Institut d'Investigacions Biomèdiques August-Pi-Sunyer (IDIBAPS), University of Barcelona, Barcelona, Spain – Carrer del Rosselló, 149, 08036 Barcelona, Spain, Espagne

[4] INSERM and Université Paris Diderot-Paris 7, Centre de Recherche sur l'Inflammation (CRI), Paris and Clichy, France – Institut National de la Santé et de la Recherche Médicale - INSERM – France

[5] European Foundation for the Study of Chronic Liver Failure (EF-CLIF), Barcelona, Spain – Espagne

Acute-on-Chronic Liver Failure (ACLF) is a recently recognized syndrome characterized by acute decompensation (AD) of cirrhosis, an organ/system failure(s) and extremely poor survival. ACLF can be triggered by a precipitating event (e.g. bacterial infection) and is invariably associated with exacerbated systemic inflammation. According to the European Foundation for the study of Chronic Liver Failure (EF-CLIF), patients with ACLF can be classified into three groups, essentially according to the number of impaired organs. In the present project, we investigated whether metabolomics and lipidomics can identify potential new diagnostic biomarkers of ACLF.
In our study, a cohort of more of 800 serum samples from decompensated cirrhotic patients with and without ACLF were analyzed and compared to healthy subjects by LC-HRMS. Data mining procedures using multivariate and univariate analyses were then performed to highlight discriminant metabolites with a preliminary work on data processing to normalize data and to overcome analytical biases occurring in the analysis of a large biological cohort. Our data confirmed the metabolic and lipidomic cirrhosis signatures obtained in previous studies, especially regarding to the levels of glycerophosphatidylcholines, amino acids and energy metabolites. Furthermore, our approach enabled to discriminate between decompensated cirrhotic patients with ACLF and those without ACLF, and a specific metabolite signature associated with the ACLF grade was obtained. Moreover, we found that Kynurenine pathway is activated in patients with acute decompensation, culminate in patients with ACLF and may be involved in the pathogenesis of ACLF, clinical course and mortality.

Mots-Clés: Metabolomics, ACLF, LC/MS

Oral Flash 2 – FP2

Metabolic characterization of different phases of Parkinson's disease

David Mallet [1], Thibault Duffourd [1], Carole Carcenac [1], Sébastien Carnicella [1], Emmanuel Barbier [1], Sabrina Boulet [1], Florence Fauvelle [1]

[1] Grenoble Institut des Neurosciences – CEA, CHU Grenoble, Inserm : U1216, Université Grenoble Alpes – France

Context: Parkinson's disease (PD) is the second most common neurodegenerative disorder, affecting almost 1% of the population beyond the age of 60. Currently, its diagnosis relies on the expression of the well-known motor symptoms (akinesia, rigidity, and tremor) which appear in the late stage of the disease. Detecting the disease earlier represents a key step to develop curative treatments which are so far only symptomatic. Long considered as a purely motor disease, PD is nevertheless also characterized by neuropsychiatric disorders (apathy, depression, anxiety...) that can develop during the early stages of the disease as well as later on.

Objectives: Our aim is to find specific molecular markers of early phases of PD, when only the neuropsychiatric

symptoms are expressed, using 1HNMR-based metabolomics on serum samples and on brain tissues of a rodent model allowing investigation of different phases of PD.

Methods: The animal model is based on a specific, partial, bilateral 6-OHDA-induced lesion in dopaminergic neurons. For each rat, motor functions and apathetic-like behaviors were assessed using a stepping test and operant sucrose self-administration, respectively.

Both serum and intact tissue samples were analyzed using a CPMG pulse sequence by respectively liquid NMR at 950 MHz (IBS Grenoble) and HRMAS-NMR at 500 MHz (IRMaGE, CEA Grenoble). Spectra were submitted to multivariate statistics (SIMCA-v14) in order to investigate if metabolic profile is correlated to behavioral and histological data.

Results/conclusion: In our animal cohort we observed a gradation in the symptoms, well in line with PD progression, from only neuropsychiatric, to the expression of neuropsychiatric associated with motor symptoms. For more precise evaluation, a score based on behavioral performances and on the striatal dopaminergic denervation was built. Serum and tissue spectra showed a good correlation of this score with the gradation of symptoms at different phases of PD.

In both samples, the energetic pathways seems to be modified with the progression of the disease, while some amino acids, like alanine and serine, are also dysregulated prior to the appearance of motors symptoms. We will further use the same methodology in serum with *De Novo* patients.

Mots-Clés: Parkinson, Biomarkers, liquid 1HNMR, Tissu, HRMAS NMR (High resolution magic angle spinning NMR)

Oral Flash 3 – FP3

Impaired postprandial skeletal muscle metabolism in a minipig model of insulin resistance: insights from arteriovenous and biopsy-based metabolomics analyses

Marie Tremblay-Franco [1,2], Nathalie Poupin [1], Florence Castelli , Jérémie David , Marianne Jarzaguet , Didier Rémond , François Fenaille [3], Dominique Dardevet [4], Sergio Polakof [4]

[1] UMR 1331 Toxalim - INRA-ENVT-INP-EIP-UPS – INRA [Toulouse] – France
[2] Metatoul Axiom platform - Metabohub national Infrastructure – Toulouse – France
[3] CEA-INRA UMR 0496/DRF/Institut Joliot/SPI/LEMM, Université Paris Saclay, MetaboHUB – CEA – Centre d'Etudes Nucléaires de Saclay, 91191 Gif-sur-Yvette, France, France
[4] INRA UMR1019 Unité de Nutrition Humaine – Université Clermont Auvergne, INRA, UNH – France

The postprandial phase (PP) represents one of the most challenging phenomena in wholebody metabolism, as the metabolism must adapt to major changes in blood composition following meal intake. This capacity of adaptation is strongly dependent on the metabolic flexibility of the individual, which is compromised in insulin resistant (IR) conditions, particularly at the skeletal muscle level (1). We applied an untargeted LC/MS-based metabolomics approach to skeletal muscle biopsies obtained before (0) and 1.5, 3 and 6 h after a regular meal in a minipig (dexamethasone treated) model of IR (2). Biopsies analyses were complemented by the assessment of the incoming (arterial) and outgoing (venous) blood metabolomes. As the consequence of the anabolic effect of the meal, in healthy animals we observed increased PP muscle levels of metabolites related to glucose (lactate, glucose-1,6biP, lactoylglutathione), energy (citrate, malate), amino acids (AA; methionine, glutamate, aspartate) and nucleotide (GTP, inosine, guanosine) metabolisms. In contrast, these metabolites showed a blunted PP profile in IR minipigs, which illustrates the loss of response to the meal induced by such condition. These include the inability to uptake and use glucose through glycolysis, stimulate protein synthesis due to the lack of AA entry into the muscle and net loss of other nitrogen resources (nucleotides). For some of these metabolites (lactate, AA) the observed changes in the biopsies were in line with the arteriovenous differences across the muscle, which confirms that they were actively uptaken, while for other (nucleotides) the altered profiles in the muscle were the consequence of intramuscular metabolic changes. As a whole, our high-throughput arteriovenous metabolomics approach across the muscle allowed us to further explore the PP metabolic changes observed in the muscle biopsies from IR minipigs. This strategy could be therefore useful to determine in the systemic circulation metabolites (potential biomarkers) able to sign the IR condition without having access to the skeletal muscle biopsies.

(1) Galgani et al. Am J Physiol. 2008;295(5):E1009-17.
(2) Revel et al. PLoS ONE. 2017;12(10):e0186204.

Mots-Clés: insulin resistance, human nutrition, minipig, arteriovenous metabolome, biopsy, postprandial

Oral Keynote 2 – OK2

Improved riboflavin production with Ashbya gossypii from vegetable oil based on 13C metabolic network analysis with combined labeling analysis by NMR and mass spectrometry

Lindsay Peyriga [1], Susanne Katharina Schwechheimer [3], Judith Becker [3], Jean-Charles Portais [2,4,5], Christoph Wittmann [3]

[1] Metatoul - Metabohub – Institut national de la recherche agronomique (INRA) : UMR792, CNRS : UMR5504, INSA - Institut National des Sciences Appliquées – France

[3] Institute of Systems Biotechnology, Saarland University – Allemagne

[4] STROMALAB – Institut National de la Santé et de la Recherche Médicale - INSERM – France

[5] Laboratoire dÍngénierie des Systèmes Biologiques et des Procédés – Institut National de la Recherche Agronomique : UMR0792, Institut National des Sciences Appliquées - Toulouse, Centre National de la Recherche Scientifique : UMR5504 – France

The fungus *Ashbya gossypii* is an important industrial producer of riboflavin, i.e. vitamin B2. In order to meet the constantly increasing demands for improved production processes, it appears essential to better understand the underlying metabolic pathways of the vitamin. Here, we used a highly sophisticated set-up of parallel 13C tracer studies with labeling analysis by NMR and mass spectrometry to resolve carbon fluxes in the overproducing strain *A. gossypii B2* during growth and subsequent riboflavin production from vegetable oil as carbon source, yeast extract, and supplemented glycine. The studies provided a detailed picture of the underlying metabolism. Glycine was exclusively used as carbon-two donor of the vitamin's pyrimidine ring, which is part of its isoalloxazine ring structure, but did not contribute to the carbon-one metabolism due to the proven absence of a functional glycine cleavage system. The pools of serine and glycine were closely connected due to a highly reversible serine hydroxymethyltransferase. Transmembrane formate flux simulations revealed that the onecarbon metabolism displayed a severe bottleneck during initial riboflavin production, which was overcome in later phases of the cultivation by intrinsic formate accumulation. The transiently limiting carbon-one pool was successfully replenished by time-resolved feeding of small amounts of formate and serine, respectively. This increased the intracellular availability of glycine, serine, and formate and resulted in a final riboflavin titer increase of 45%.

Mots-Clés: Ashbya gossypii, Riboflavin, 13C tracer, Metabolic flux, Carbon, one metabolism, NMR, MS

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Oral Junior 3 – OJ3

Feature-Based Molecular Networking of Untargeted Mass Spectrometry Data: Bridging MS-DIAL, MZmine2, OpenMS, and XC-MS, with the GNPS web-platform

Louis-Félix Nothias [1], Daniel Petras [1], Mingxun Wang [1], Robin Schmid [1,2], Abinesh Sarvepalli [1], Zheng Zhang [1], Melissa Esposito [1], Ricardo Da Silva [1], Alexander Aksenov [1], Pieter Dorrestein [1]

1 University of California [San Diego] – États-Unis
2 [2] University of Münster – Allemagne

Molecular networking has become an essential bioinformatic tool to annotate non-targeted tandem mass spectrometry data (MS/MS). Available on the GNPS web-platform (http://gnps.ucsd.edu), molecular networking accelerates the annotation of molecular/spectral families by propagating spectral library matches across the networks. Based on the MS-Cluster algorithm, molecular networking enables the large scale metabolomics meta-analysis, up to thousands of files. However, as MS-Cluster uses the MS/MS spectral counts as a proxy for the ion distribution between samples, other methods are needed to estimate the relative ion abundance, especially when analysing a single study.

In the present work, we introduce feature-based molecular networking, a collection of computational tools integrated together into a seamless LC-MS data processing pipeline combined with
molecular networking on the GNPS web-platform (https://ccms-ucsd.github.io/GNPSDocumentation/featureba Utilizing software with advanced graphical user interface (MS-DIAL, MZmine2, MetaboScape) or advanced computational libraries (OpenMS, and XC-MS), the workflows serves both experimentalists, bioinformaticians, and software developers.

We benchmarked feature-based molecular networking on dilutions of a reference serum samples (NIST 1950 SRM), along with various mixtures of fecal and plant extracts, on both Orbitrap and QTOF mass spectrometers. The benchmarking of feature-based molecular networking showed that: 1) it outperformed MS-Cluster based molecular networking for the estimation of the ion relative distribution between samples, 2) it reduced the number of spectral artefacts, and 3) it prevented the merging of isomeric MS/MS spectra. These results were observed for both Orbitrap and QTOF instruments, and different sample types, indicating that feature-based molecular networking has significant advantages over MS-Cluster based molecular networking. The feature-based molecular networking is proposed to serve as an essential complementary tool to the "classical" MS-Cluster-based molecular networking which is a robust and nearly parameterless method, and is the only method capable of performing large scale meta-analysis of untargeted LC-MS/MS datasets.

Mots-Clés: molecular networking, GNPS, mass spectrometry, feature, based molecular networking

Oral Flash 4 – FP4

MetGem Software for the Generation of Molecular Networks Based on the t-SNE Algorithm

Nicolas Elie [1], Florent Olivon [1], Gwendal Grelier [1], Fanny Roussi [1], Marc Litaudon [1], David Touboul [1]

[1] Institut de Chimie des Substances Naturelles – Centre National de la Recherche Scientifique : UPR2301 – France

Metabolomic studies based on MS produce large amount of data requiring dedicated chemo/bioinformatics tools to explore them. Whereas many algorithms are available for the data treatment at MS1level, methodologies for MS2 data are scarce. One of the most popular is the generation of molecular networks (MNs) through the calculation of cosine score on a collection of MS2 spectra available on the online GNPS platform. We introduce here dedicated software, called MetGem (https://metgem.github.io), allowing the generation of GNPS-like MNs together with a t-distributed stochastic neighbor embedding (t-SNE) based visualization of the cosine score matrix. Starting from the .mgf file, all spectra detected are compared to each other using the GNPSbased cosine score calculation system.
One way to represent these results is to conceive a square matrix gathering together highdimensional objects, i.e. MS2 spectra, whose dimensions contain the similarities (cosine score values), taken pairwise, between all the spectra of the dataset. As it is difficult to apprehend these high-dimensional objects and visualize them in a meaningful manner, several manifold learning algorithms have been developed for dimensionality reduction purposes and pattern recognition. The idea developed herein was to feed the t-SNE algorithm with the pairwise similarity matrix. Considering that only small distances are reliable in high-dimensional spaces, t-SNE aggregates local data points closer in the lower-dimensional space. By focusing preferentially on the local structure of the data, t-SNE tends to extract better clustered local groups of point. Thus, it allows distinguishing easily patterns lying in different manifolds by simple visual analysis and in an unsupervised way.

Performances and advantages of the t-SNE output have been evaluated on two datasets, i.e. LC-MS2 analyses of fractions from a supercritical CO2 extraction of S. lineata leaves and a bark extract of C. peltatum. In both cases, t-SNE allows us to annotate more nodes compared to the GNPS-based cosine score calculation system and thus a deeper exploration of the experimental datasets was demonstrated.
Finally, in terms of network calculation and visualization, our interface running on laptop computer is up to 20 times faster for small and medium datasets (< 1000 MS2 spectra) and competes equally with GNPS for larger files (up to 35000 MS2 spectra).

Mots-Clés: cosine, similarity, MS2, t, SNE, molecular networking, python, desktop, chemo, informatics, bioinformatics, mass spectrometry, algorithm, metgem, mgf, GNPS, manifold, machine learning

Oral Flash 5 – FP5

Analytic correlation filtration: A new tool to reduce analytical complexity of metabolomic datasets

Stéphanie Monnerie[1], Mélanie Pétéra [2], Bernard Lyan [2], Pierrette Gaudreau [3], Blandine Comte [1], Estelle Pujos-Guillot [2,1]

[1] Université Clermont Auvergne, INRA, UNH, Mapping – (INRA) : UMR1019 – France

[2] Université Clermont Auvergne, INRA, UNH, Plateforme d'Exploration du Métabolisme, MetaboHUB Clermont – INRA Clermont-Ferrand-Theix : UMR1019 – France

[3] Centre de Recherche du Centre hospitalier de l'Université de Montréal ; Département de médecine, Université de Montréal – Canada

Metabolomics generates massive and complex data that need dedicated workflows to extract the meaningful information and to enrich our knowledge of biological systems. For biological interpretation, experts are mainly focusing on metabolites rather than on the redundant different analytical species. Moreover, the high degree of correlation in datasets is a constraint for the use of data mining and statistical methods.
In this context, we developed a new tool to detect analytical correlation into datasets without confounding them with biological correlations that may exist within samples. The algorithm principle is to group features coming from the same analyte and to propose one single representative per group. The user can define grouping criteria with various options including correlation coefficient, retention time, mass information from a reference list of isotopes, adducts and fragments. Thresholds for all these criteria can be fixed and the representative feature can be determined following four methods according to the user needs and the analytical technology.

We chose to compare the present tool to one of the most commonly used free package proposing a grouping method: 'CAMERA', using its Galaxy version 'CAMERA.annotate' also available in Workflow4Metabolomics (W4M; http://workflow4metabolomics.org). To illustrate the 'Analytic correlation filtration tool' functionalities and the results obtainable on typical experimental data, a published dataset available on W4M was used as an example. This dataset named 'Sacurine' was obtained from human urine samples analysed by LC-HRMS in negative ionization mode (Thevenot *et al*., 2015).

Within the 3,120 ions of the urine dataset, the tool allowed creating 2,651 groups, meaning that 15% of ions are proposed to be filtered because of analytical redundancies. While CAMERA generated more than 20 groups of more than 10 ions, the proposed tool subdivided them into smaller ones corresponding to individual annotated metabolites, thus demonstrating the efficiency and relevance of the present approach.

As a key element in metabolomics data analysis, the tool will be available *via* the web-based galaxy platform W4M with different output files for vizualisation for further data analysis within workflows.

Mots-Clés: analytical redundancies, filtration, workflow

Oral Flash 6 – FP6

New insight in Metabolomics based study of Age Related Macular Degeneration (AMD): Lipoprotein profile and subclass analysis.

Matthieu Schoumacher[1] , Vincent Lambert , Justine Leenders [1], Bernadette Govaerts [2], Sylvain Hansen , Jean-Marie Rakic , Agnès Noël , Pascal De Tullio [1]

[1] Centre Indisciplinaire de Recherche sur le Médicament (CIRM) – Institut de Pharmacie Quartier HOPITAL - Av. Hippocrate, 15 (Bât. B36) Sart-Tilman B-4000 Liège, Belgique
[2] Institut de Statistique, Biostatistique et Sciences Actuarielles (ISBA) – Université Catholique de Louvain, Voie du Roman Pays 20, 1348 Louvain-La-Neuve, Belgique

Age-related macular degeneration (AMD) is the leading cause of blindness among the elderly population in developed countries. 90% of all vision loss due to AMD result from the exudative form of this pathology, which is characterized by a choroidal neovascularization (CNV). Currently, diagnosis of AMD relies on ophthalmologic exams and treatments of the exudative form are based on the use of anti-angiogenic drug targeting vascular endothelial growth factors. Despite these advance, several clinical challenges have to be overcome. Among those, the identification of biomarkers that could allow to refine patient stratification, to follow disease progression and evaluate responses to treatment are mandatory.For this purpose, we decide to apply NMRbased metabolomics approach on both AMD patients and on a laser-induced murine choroidal neovascularization experimental model.
In the clinical study, the metabolomics approach does not allow a complete differentiation between control and AMD patients. However, focusing only on AMD group, a clear-cut separation between active and non-active phases could be highlighted.
In the mice model, discrimination between laser-induced and control mice occurs only when CNV is install ed.In both human and animal studies, lactate and lipoprotein profile were identified as the main biomarkers. Mechanistically, we demonstrated that lactate, plays a critical role in the onset of the inflammatory and angiogenic phases and could be correlated with the CNV development. Then, controlling lactate level appears as a new therapeutic approach of AMD.
On the other hand, lipoprotein profile is of particular interest for patient follow-up. Indeed, evaluation of lipoprotein profile change trough a simple methods allowed us to establish clear modification of profiles according to the active or non-active status of the patient and to the induced or non-induced status of the mice.
This work focuses on the lipoprotein profile and on the development of a methodology that could be used to characterize and compared the different profiles in the human and the mice studies.

Mots-Clés: NMR, metabolomics, AMD, Lipoprotein

Oral 4 – O4

Supercritical fluid chromatography coupled to high resolution mass spectrometry for dereplication and quantification of natural products

Laurent Laboureur [1], Thi Phuong Thuy Hoang , Morgane Barthélemy [1], Pierre Champy [3], Véronique Eparvier[1], David Touboul[1]

[1] Institut de Chimie des Substances Naturelles (CNRS-ICSN) – CNRS : UPR2301 – ICSN, CNRS UPR2301, Université Paris-Sud, Université Paris-Saclay, Avenue de la Terrasse, 91198 Gif-sur-Yvette, France,

[2] CNRS UMR 8076 BioCIS - BIOmolécules : Conception, Isolement et Synthèse (BioCIS) – CNRS : UMR8076 – CNRS UMR 8076 BioCIS - BIOmolécules : Conception, Isolement et Synthèse Bureau D5 309 - Bâtiment D5 - 5, rue J.B. Clément 92296 Châtenay-Malabry, France, France

The development of the most valuable analytical strategies need to include optimization of analytical parameters such as accuracy, sensitivity, reproducibility, speed, while operator safety and environmental impacts are never considered at the same level. An absurd situation has emerged, due to the side effects of analytical methods, especially those developed to investigate hydrophobic compounds: a large amount of chemical waste is generated. Taking into account growing public concerns on environmental questions, the development of environmentally friendly analytical methods is mandatory. Supercritical Fluids (SF), such as SF-CO2, can be considered as a green solvent for chromatography showing solvation properties closed to the ones of hexane. The advantages of SFC coupled to high resolution tandem mass spectrometry (SFC-HRMS/MS) will be exemplified with the dereplication of complex mixtures of secondary metabolites, *i.e.* acetogenins (environmental toxins) and N-Acyl homoserine lactones (AHLs) implied in bacterial quorum sensing.

For the dereplication of Annonaceous acetogenins from *Annona muricata* L., SFC-HRMS/MS method was developed and showed shorter retention times, higher number of separated isomers and sensitivity compared to LC-HRMS/MS. In order to improve the quality of structural information by modifying the fragmentation pathways, a post-column cationization by lithium salts has been implemented for the first time. Due to the CO2 depressurization before the entrance of the mass spectrometer, the lithium salt was directly added in the make-up solvent leading an efficient exchange of sodium or proton with lithium for the cationization under ESI. For the quantification of AHLs playing crucial roles in microorganism communication, SFCHRMS offers the unique advantage to make possible the sample injection in pure organic solvents such as ethyl acetate or chloroform. In fact, acetonitrile is often used to prepare samples containing AHLs due to their fast hydrolysis in alcohol or water. Nevertheless, long chain AHLs (C16 and higher) are not soluble in acetonitrile making the identification of long chain AHLs not possible by classical LC-MS/MS approach. SFC-HRMS is therefore improving the quantitative analysis of such long chain AHLs within retention times lower than 10 min.

Mots clefs : supercritical fluid chromatography, acetogenin, N, Acyl homoserine lactone, mass spectrometry

Oral 5 – O5

Apport de la spectrométrie de masse à haute résolution et à mobilité ionique dans les analyses métabolomiques à haut débit et à large échelle

Aurélie Delvaux[1], Sandra Alves [1], Estelle Rathahao - Paris [2]

[1] Institut Parisien de Chimie Moléculaire IPCM – Université Pierre et Marie Curie [UPMC] - Paris VI – France
[2] UMR Service de Pharmacologie et Immunoanalyse – CEA INRA Saclay – France

A l'heure actuelle, la RMN et la MS sont les deux techniques analytiques majoritairement employées pour conduire des études métabolomiques. La MS, grâce à sa sensibilité et à sa sélectivité permet de détecter un grand nombre de métabolites dans des matrices biologiques complexes. En outre, le couplage avec des méthodes séparatives permet d'augmenter sa couverture métabolomique. Néanmoins cette approche augmente drastiquement le temps d'analyse et reste limitée pour la distinction de beaucoup d'isomères.
La mobilité ionique (IM) est une technique permettant de séparer des composés ionisés en phase gazeuse sous l'action d'un champ électrique. La mobilité ionique couplée à la spectrométrie de masse présente une grande capacité de séparation des isomères comparés aux autres techniques en couplage avec la MS. De plus, les analyses en mobilité relevant du domaine des millisecondes, le couplage de l'IM – à la MS présente également l'avantage de réaliser des analyses rapides (de l'ordre de la minute contre des dizaines de minute pour la LC) ouvrant l'application à l'analyse d'un grand nombre d'échantillons.
Ainsi, notre projet vise à développer une méthode d'analyse métabolomique à haut débit et à large échelle utilisant la technique de mobilité ionique de type TIMS (Trapped Ion Mobility Spectrometer) couplée à un analyseur à temps de vol. Nous avons tout d'abord évalué l'influence des paramètres instrumentaux sur la détection des métabolites de faibles masses moléculaires. D'autres conditions analytiques telles que la concentration des métabolites ont également été étudiées. Des composés isomères standards ont été analysés dans le but d'évaluer la capacité du TIMS pour la séparation de différents types d'isomères (énantiomères, isomères de fonction, position etc). Enfin, nous avons évalué le potentiel de l'instrument TIMS pour l'analyse directe d'un mélange complexe, tel que l'urine, ainsi que sa capacité à séparer des métabolites isomères dans ce mélange. Nous avons pu détecter et séparer les métabolites isomères d'un xénobiotique et fragmenter spécifiquement un couple.

Mots-Clés: mobilité ionique, spectrométrie de masse, isomères, haut débit

Oral Sponsor 5 – OS5

Platinium

Assessing Gut Microbiota-Host Crosstalk: Development of a Standardized and Quantitative Targeted Metabolomics Assay

PHAM, Tuan Hai[1], SOMMER, Ulf[1], HEISCHMANN, Svenja[1], KIRCHBERG, Doreen[1], IWANOWA, Xenia[1], WOLF, Barbara[1], BURATTI, Martin[1], ROEHRING, Cornelia[1], KOAL, Therese[1]

[1]BIOCRATES Life Sciences AG, Innsbruck, Austria, barbara.wolf@biocrates.com

Introduction

Targeted metabolomics provides a snapshot of the metabolic phenotype and is increasingly acknowledged across scientific fields. Here, we developed a standardized, quantitative targeted assay for multiplexed analysis of central host and gut bacteria-derived metabolites by mass spectrometry.

Material & Methods

10 µL of plasma and fecal samples were pipetted on a 96 well-plate, preloaded with internal standards. After derivatization and extraction, LC-MS and FIA-MS analyses were performed (Agilent 1290 Infinity UHPLC – SCIEX QTRAP® 5500). MetIDQ™ software was used for the entire automated workflow, from sample registration to quality-controlled results.

Results

In total, over 500 metabolites from 14 small molecule and 11 lipid classes were analyzed by the newly developed standardized, quantitative targeted assay. The LC-MS analysis provided quantitative results of small molecules covering bile acids, indole derivatives, amino acids and related compounds, amongst other classes. Acylcarnitines, lipids, and monosaccharides were analyzed by FIA-MS. Using the 96-well plate format, 80 samples, together with blank, quality controls, and calibrators, can be analyzed within 35 hours.

Conclusion

The newly developed standardized, quantitative targeted assay enables multiplexed analysis of over 500 host and gut microbiota-derived metabolites. Future applications will demonstrate the power of this solution for metabolic phenotyping specifically assessing gut microbiota-host crosstalk.

Oral 6 – O6

13C Labeled Yeast extracts: a powerful tool for normalization and quantification in metabolomics and lipidomics?

Thaïs Hautbergue[1], Cécilia Bergès [2], Emeline Chu-Van [1], A. Tournadre[3], Annelaure Damont [1], Florence Castelli [1], Justine Bertrand-Michel [3],François Fenaille [1], Floriant Bellvert [2], Benoit Colsch[1]

[1] Service de Pharmacologie et d'Immunoanalyse, Laboratoire d'Etude du Métabolisme des Médicaments, CEA, INRA, Université Paris Saclay, MetaboHUB, F-91191 Gif-sur-Yvette France
[2] MetaToul- MetaboHUB, Toulouse – INSA - Institut National des Sciences Appliquées – France
[3] MetaToulLipidomics Facility, INSERM UMR1048, MetaboHUB, Toulouse INSERM – France

Batch correction and compound quantification are two major challenges in metabolomic and lipidomic studies by mass spectrometry. Absolute quantification of molecules requires labeled standards often difficult to synthesized or not available. In addition, due to the emergence of a large cohort analysis, batch normalization must be applied in order to correct differences in signal intensities between analyses of samples.
To overcome these limitations, we evaluate an original approach using extracts of fully 13C labeled *Saccharomyces cerevisiae* as a pool of internal standards for the normalization and quantification of hundreds of metabolites and lipids in human biofluids.

Unlabeled and 13C-labeled *Saccharomyces cerevisiae* were produced via fermentation in medium enriched with unlabeled or labeled glucose respectively. Yeast metabolites were extracted using a Methanolic solution, while lipids were extracted using a modified Folch method. Extracts were analyzed by LC-HRMS.

Comparison of unlabeled and labeled extracts allowed validation of 150 robust metabolites whose equivalent ions were found in both extracts with a mass difference consistent with their number of carbon atoms. These metabolites corresponded to the following major metabolic pathways: amino acid metabolism, citrate cycle, purine and pyrimidine metabolism, pentose phosphate pathway, glycolysis and glutathione metabolism. Untargeted lipidomic analysis of labeled and unlabeled extracts allowed detection of several lipid classes such as glycerophospholipids, glycerolipids, sphingolipids and fatty acids. However, some sterols and glycolipids were different between yeast and mammal due to incorporation of ergosterol instead of cholesterol for example. To go further in the evaluation of this approach, yeast extract will be spiked in NIST plasma for which concentration of several metabolites are known. Finally, labeled yeast extracts will allow normalizing analytical batches considering the specific chemical behavior of each class of molecules and will allow identifying and quantifying hundreds of compounds. This also will help in structural characterization of unknown molecules commonly detected in samples and yeast extracts by indicating the number of carbon atoms of each ion in full scan or tandem mass spectrometry analyses.

Mots-Clés: Labeled Yeast Extract, Quantification, Batch correction, Mass spectrometry

Oral Junior 4 – OJ4

Advanced high-field and benchtop NMR methods for lipid profiling of microalgae

Dylan Bouillaud [1,2], Arturo Vladimir Heredia Marquez [1], Thomas Castaing Cordier [2], Delphine Drouin [1], Olivier Gonçalves [1], Jonathan Farjon [2], Patrick Giraudeau [2,3]

[1] Génie des Procédés–Environnement–Agro-alimentaire – Université de Nantes, CNRS : UMR6144,

[2] Chimie Et Interdisciplinarité : Synthèse, Analyse, Modélisation – Université de Nantes, Centre National de la Recherche Scientifique : UMR6230 – France

[3] Institut Universitaire de France – Ministère de l'Education nationale, de l'Enseignement supérieur et de la Recherche, Ministère de lÉducation nationale, de l'Enseignement supérieur et de la Recherche, Ministère de lÉducation nationale, de l'Enseignement supérieur et de la Recherche – France

Microalgae are increasingly studied in both academy and industry owing to their broad applicative potential. Indeed, they are able to create biomass with significant productivities compared to traditional crops. Besides, there is a great biodiversity in microalgae and some species can produce valuable compounds in an ecological way, since microalgae consume CO2, a highly emitted gas largely involved in the greenhouse effect.
Lipids are important molecules in the list of valuable compounds produced by microalgae. They can be used in food industry or for biofuel production. Some species of microalgae have the ability to produce reserve lipid –mainly triglycerides– under nitrogen starvation conditions: microalgae are then disrupted and a metabolic shift is provoked. In order to better understand this phenomena and find the best way to produce lipids, biological and process research works are carried out by a substantial research community.

NMR has a great potential to study the lipidic metabolism of microalgae in a non-destructive and reproducible fashion. In this context, we aim at bringing some of the most recent NMR developments into the bioprocess community, in order to maximize the observable information. On the one hand, advanced high-field NMR methods offering the best performance in sensitivity and resolution are investigated for the lipid characterization of microalgae extracts. Different quantitative pulse sequences (1H NMR, 13C NMR and our recently developed quantitative 2D NMR methods [1]) are evaluated in this study in terms of analytical performance and available information on lipids.

On the other hand, we investigate the potential of recently developed benchtop NMR spectroscopy [2] as an online lipid sensor. We demonstrate the potential of this transportable setting for the real-time and *in vivo* monitoring of microalgae cultivations. Thanks to the implementation of advanced solvent suppression NMR pulse sequences [3], a lipidic profile can be obtained and quantified in order to monitor the lipid concentration in real time, in a flow-compatible and non-invasive way.

Mots-Clés: NMR, benchtop NMR, microalgae, lipid, profiling, monitoring

Oral Keynote 3 – OK3

Illuminating lifestyles using metabolomics of personal objects

Amina Bouslimani[1], Louis-Félix Nothias [1], Kathleen Dorrestein [1], Chris Callewaert [1], Ricardo R. Da Silva [1], Alan Jarmusch [1], Rob Knight [1], Pieter C. Dorrestein[1]

[1] University of California [San Diego] – États-Unis

Due to the exchange of molecules between humans and the external environment, an individual's personal belongings can yield a large amount of meaningful chemical information. Over 1200 samples collected from hands and personal items (phone, keys, computer, wallet) of 120 individuals were analyzed using an untargeted high resolution UPLC-QTOF MS/MS based workflow. Molecular networking based on spectral similarities and the GNPS knowledgebase platform were applied for rapid characterization of chemistries recovered from objects and for translating them into lifestyle readouts. Data connecting hands of individuals to their personal objects provides insights into unambiguous lifestyle characteristics such as health, diet, preference for cosmetics, frequented locations and even environmental exposures. Many skin-associated molecules were detected on several everyday items. Particularly, food derived molecules indicative of a person's diet were found alongside signatures associated to an individual's health such as antidepressants, antihistamines and medications used to treat high blood pressure and skin disorders. Hygienic status such as beauty products, sunscreens in addition to insect repellents, pesticides and fungicides were also revealed. The chemical information from objects also allowed narrowing down pools of individuals that share the same lifestyle characteristics. Specifically, 30 individuals used sunscreen, 9 went on camping or hiking trips, while 22 used medications. Lifestyle profiles were constructed using chemical traces recovered from personal items. For example, two prescribed medications were found on objects of individual 58: a muscle relaxant Cyclobenzaprine and an antidepressant Lorazepam. Additionally, Viagra was also detected which suggests that these objects belong to a male individual; and the illicit drug cocaine was also found on objects of this same individual 58. The ability of lifestyle profiling to accurately connect items to individual lifestyles makes this method amenable to understanding the habits of suspects within a criminal investigation based on trace evidence. This work and the methods used could also be applied in clinics by inspiring non-invasive approaches to measure drug metabolism, patient compliance and environmental exposures.

Mots-Clés: lifestyle, mass spectrometry, metabolomics, skin, objects.

Oral 7 – O7

Large scale micropollutants screening in a wetland ecosystem using a non targeted high resolution mass spectrometry approach

Loïc Maurer [1,2], Claire Villette [1,2], Adrien Wanko [1], Dimitri Heintz [2]

[1] Laboratoire des sciences de língénieur, de línformatique et de límagerie – université de Strasbourg : UMR7357, Centre National de la Recherche Scientifique : FR3627, Ecole Nationale du Génie de l'Eau et de l'Environnement de Strasbourg, INSA Strasbourg – France

[2] Institut de biologie moléculaire des plantes – université de Strasbourg, Centre National de la Recherche Scientifique : UPR2357 – France

Many studies have focused on micropollutants and their potential environmental impacts. The release of micropollutants to the environment has several origins; wastewater (WW) is one of the major sources of the release of diversified micropollutants. Indeed, wastewater treatment plants (WWTP) were initially not designed to remove micropollutants. Most of the studies investigated targeted micropollutants in WWTP (ex, Hijosa-Valsero et al, 2010). The development of metabolomics databases and high-resolution mass spectrometry (HRMS) helps performing nontargeted analyses and to obtain a global view of this pollution.

Constructed wetlands (CW) are common WWTP in rural areas in France. Micropollutants distribution in CW was obtained using a nontargeted analysis in three compartments of the ecosystem (water, plants, sludge). To this end, an extraction method based on a double extraction and freeze-drying was developed. Then the investigation was performed in LC -HRMS using the TargetScreener method (Bruker). The identification was performed using Metaboscape 4.0 software (Bruker). The Schymanski classification (Schymanski et al, 2015) was used to annotate the obtained molecular features in each compartment. Molecular formula (4th level) were generated using SmartFormula and putative identifications (3rd level) were achieved by comparing experimental data with public databases (plants, toxics ...).

This approach highlighted emerging substances that are not routinely monitored yet. A trend in the distribution of micropollutants could be shown: around 50% of the tentative identifications were anthropogenic molecules (drugs, ...). These classes of molecules were found in each compartment. These results shed new light on the diffusion of toxic molecules in the environment. This approach coupled with the study of environmental risk, could help the management of sludge generated by CW.

Mots-Clés: micropollutants, wetland ecosystem, non targeted analyses, high resolution mass spectrometry (HRMS)

Oral 8 – O8

Anti-leishmaniasis and Metabolomic approach from the leaves extract of Psidium guajava L. from Lao PDR.

Chiobouaphong Phakeovilay [1,2], **Sandra Bourgeade-Delmas** *[1], **Pierre Perio**[1], **Karine Reybier** [1], **Françoise Nepveu**[1], **Guillaume Marti**[1]

1 UMR 152 Pharma Dev, Université de Toulouse, IRD, UPS, Toulouse, France – Université Paul Sabatier - Toulouse III – France
2 Faculty of Pharmacy, University of Health Sciences, Ministry of Health, Lao PDR – Laos

Introduction Leishmaniasis disease is the in the top five of communicable diseases via parasites and vectors. The available treatments involve many side effects, including toxicity, non-specific targeting and resistance development. Thus, new antileishmanial chemical entities are of utmost interest to fight against this disease.

Objectives To identify potential antileishmanial components from Psidium guajava leaves by applying a metabolomic approach and correlation analysis.

Method Crude extracts Psidium guajava leaves were submitted to liquid-liquid separation between polar and apolar compounds and analyzed by liquid chromatography coupled to high resolution mass spectrometry and together with screening of antileishmanial activity. The putative active compounds were highlighted by multivariate correlation analysis between the biological response and LC-MS profiles of several P. guajava samples.

Results Our results showed that apolar phase from the P. guajava extracts were the most actives (IC50 = 2.886±0.02446 µg/mL). Multivariate data analysis of polar extracts highlighted a family of triterpenes compounds like Jacoumaric acid (IC50 = 1.318±0.05497 µg/mL) and its derivatives.

Conclusion Our approach allowed the identification of antileishmanial potential compounds from the complex crude extracts in few steps and can be easily implemented to several other natural product discovery workflow.

Mots-Clés: Metabolomic, Antileishmanial activity, LC_MS, Psidium guajava, Jacoumaric acid

Oral Flash 7 – FP7

Étude de la réponse métabolique de Pseudomonas syringae 32b-74 isolée des nuages à une exposition sub – létale au mercure

Christelle Ghaffar , Martine Sancelme[1], Binta Diémé[2], Marie Lagrée[3], Cyril Jousse[4,5], Pierre Amato [3]

[1] Institut de Chimie de Clermont Ferrand (ICCF) – Université Clermont Auvergne, CNRS, SIGMA Clermont – France
[2] Université François-Rabelais, INSERM U930 "Imagerie et Cerveau", CHRU de Tours, 10 Bv Tonnellé, 37044 Tours, France –
[3] Institut de Chimie de Clermont-Ferrand - Clermont Auvergne (ICCF) – SIGMA Clermont, Université Clermont Auvergne : UMR6296, Centre National de la Recherche Scientifique : UMR6296 – 24 Avenue des Landais / 63177 Aubière Cedex, France
[4] Institut de Chimie de Clermont-Ferrand (ICCF) – SIGMA Clermont, Université Clermont Auvergne : UMR6296, Centre National de la Recherche Scientifique : UMR6296 – 24 Avenue des Landais / 63177 Aubière Cedex, France
[5] Plateforme d'Exploration du Métabolisme, MetaboHUB Clermont-Ferrand, Clermont-Ferrand, France (PFEM) – Université Clermont Auvergne, INRA, Université Clermont Auvergne – France

Ces dernières décennies, les fortes activités industrielles ont conduit à une augmentation de la pollution aux métaux lourds de l'atmosphère, et notamment au mercure (1,7 ng d'Hg / m3 d'air) 1. Celui-ci est présent sous différentes formes dans l'environnement : élémentaire (Hg0), ionique (Hg2+) et méthylé (Me – Hg). Ce dernier agit sur les groupes thiols des protéines, siège de sa toxicité. Certaines bactéries présentent un mécanisme de résistance codé par l'opéron *mer* et sous le contrôle du régulateur transcriptionnel MerR 2.
Des études montrent que des microorganismes subsistent dans l'atmosphère et les nuages, avec des conséquences possibles sur les processus physico-chimiques atmosphériques 3 4. Ces derniers sont donc exposés à la pollution atmosphérique du mercure, et notamment à la forme ionique Hg2+.
Nous étudions ici l'impact du mercure à dose sub – létale sur le métabolisme d'une souche bactérienne précédemment isolée d'eau de nuage et dépourvue de l'opéron *mer* : *Pseudomonas syringae* 32b74 5. L'objectif est d'identifier des marqueurs métaboliques spécifiques de l'exposition au mercure, dans l'optique d'une utilisation des microorganismes atmosphériques comme traceurs environnementaux.
La souche *P. syringae* 32b74 est ainsi incubée en absence et en présence de mercure biodisponible Hg2+ (5 μg/mL). Un profilage métabolique est réalisé après 2h et 24h d'exposition. Le métabolisme des cellules est stoppé par immersion dans l'azote liquide, et les métabolites intracellulaires sont extraits par un mélange de solvant avant d'être analysés par LC-MS. Par la suite, une analyse en composante principale (ACP) est réalisée afin de discriminer les potentiels signaux biomarqueurs.
L'impact du mercure sur l'écosystème atmosphérique est une question environnementale importante. L'utilisation de la métabolomique nous permettra de l'observer plus finement sur cette souche isolée de l'atmosphère.

Mots-Clés: Mercure, Atmosphere, Pseudomonas syringae 32b74, Métabolomique

Oral Flash 8 – FP8

Comparaison des profils métaboliques et des propriétés antimicrobiennes de produits formulés à base de piment (*Capsicum*)

Inès Remy-Larsonnier[a], Marjolaine Rey[a], Carine Commun[b], Anne Doléans-Jordheim[b], Gilles Comte[a], Marie-Geneviève Dijoux-Franca[a], Camille Rozier[c]

[a] Université Lyon 1, UMR CNRS 5557/INRA 1418, Ecologie Microbienne, CESN , Villeurbanne, France

[b] Université Lyon 1, UMR CNRS 5557/INRA 1418, Ecologie Microbienne, Lyon , France

[c] ID4FEED SAS square du Rhône, 12 avenue du Rhône, BP 10051, Annecy, France

Réduire l'usage des antibiotiques en santé humaine et animale est devenue une absolue nécessité afin de limiter l'émergence de souches bactériennes antibio-résistantes. Les entreprises développant des additifs destinés aux animaux d'élevage tel qu'ID4FEED utilisent les propriétés biologiques des plantes comme base principale de leurs produits. Parmi les sources d'actifs ciblées par ID4FEED, le piment (*Capsicum chinense*) s'est révélé être un candidat majeur[1]. Les capsaïcinoïdes, responsables de la sensation de chaleur provoquée par le piment, sont la famille de métabolites très caractérisés jusqu'à présent chez le piment car ils possèdent une activité biologique d'importance en santé animale (antimicrobienne, antiinflammatoire, analgésique, radical-scavenging)[2]. Il a néanmoins été mis en évidence que d'autres classes de métabolites apportaient leurs activités dans le *totum* que représente la poudre de piment tels que des antioxydants et des antimicrobiens (vitamines A et E, caroténoïdes, polyphénols, phénols, terpènes).

L'objectif de ce travail a été de réaliser un suivi quantitatif et qualitatif des capsaïcinoides et des composés minoritaires de la poudre de piment et de comparer les profils métaboliques de la matière première et des produits commerciaux issus de cette matière première.

Une stratégie d'analyse globale par GC-QQQ et UHPLC-DAD-Q-TOF nous a permis de mettre en évidence des différences (quantitatives et qualitatives), notamment de certains flavonoïdes et volatiles contenus dans le piment. Le lien potentiel entre ces différences de profils métaboliques et les activités biologiques (antimicrobiennes et radical-scavenging notamment) de la matière première et des produits commerciaux est de même évalué en parallèle et nous permettra de renforcer notre compréhension des propriétés biologiques des préparations industrielles.

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Mots clés : *Capsicum* ; additifs ; capsaïcinoides ; UHPLC-DAD-Q-TOF ; GC-QQQ

Oral Flash 9 – FP9

Syringol metabolites as biomarkers of smoked meat intake

Roland Wedekind[1], Pekka Keski-Rahkonen [1], Vivien Viallon [1], Pietro Ferrari [1], Erwan Engel [2], Marc Gunter [1], Inge Huybrechts [1], Augustin Scalbert[1]

[1] Centre International de Recherche contre le Cancer - International Agency for Research on Cancer – Organisation Mondiale de la Santé / World Health Organization Office – France

[2] UR 370 Qualité des Produits Animaux, Equipe Micro-contaminants Arôme et Sciences Séparatives (MASS) (QuaPA, MASS) – Institut National de la Recherche Agronomique - INRA (FRANCE) – INRA Centre de recherches Auvergne-Rhône-Alpes Theix 63122 Saint-Genès-Champanelle, France

Background: Intake of processed meat has been associated with an increased risk of colorectal cancer but the estimation of intake of this heterogeneous food group in epidemiological studies is still challenging because of the lack of sufficient details in dietary questionnaires. Objective: The objective of this work is to identify in a dietary intervention study novel biomarkers for processed meat intake.

Design: A metabolomic approach based on high-resolution mass spectrometry was applied to 21 processed meat products previously digested *in vitro and* a randomized cross-over dietary intervention in which 12 volunteers consumed successively 3 processed meat products (bacon, salami, hot dog) and two other foods used as controls during 3 days. Identified biomarkers were tested for replication in 474 subjects from the European Prospective Investigation into Cancer and Nutrition (EPIC) cross-sectional study for which a detailed 24h dietary recall and food frequency questionnaires were available.

Results: Syringol and four derivatives of syringol were found to be characteristic of digests of smoked meat products. The same compounds present as sulfate esters in urine showed increased levels at 2 and 12 hours following consumption of smoked meat products (hot dog, bacon) in the intervention study. The same syringol sulfates also showed increased urinary excretion in participants of the EPIC cross-sectional study reporting recent or habitual consumption of smoked meat products. These markers showed good ability to predict smoked meat intake with receiver operator characteristic areas under the curve ranging from 0.78 to 0.86 and 0.74 to 0.79 for acute and habitual intake respectively.

Conclusions: The biomarkers of smoked meat intake identified in the present study may improve assessment of smoked meat intake in epidemiological studies.

Mots-Clés: Smoked meat, biomarker, metabolomics

Oral Sponsor 6 – OS6

Platinium

Analyses du plasma par RMN chez les adultes phenylcetonuriquessous prise en charge nutritionnelle montrent des désiquilibres métabolomiques

Claire Cannet, Bruker

Oral 9 – O9

The Exposome-Explorer database in 2019: recent development on dietary biomarkers and their association with cancer risk

Vanessa Neveu [1], Geneviève Nicolas [1], Augustin Scalbert[1]

[1] Centre International de Recherche contre le Cancer - International Agency for Research on Cancer – Organisation Mondiale de la Santé / World Health Organization Office – France

Exposome-Explorer (http://exposome-explorer.iarc.fr/), the first database on biomarkers of exposure to dietary and environmental factors, aims at providing comprehensive data on all known biomarkers of exposures to the diet, pollutants and contaminants measured in population studies.
This data is systematically and manually collected from the scientific literature, evaluated and stored in the database. All data is accessible on-line through a user-friendly web interface. This data includes nature of biomarkers (currently 491 biomarkers), biospecimens and population where measured, analytical methods, concentrations, reproducibility over time.

Since its first official release in February 2017, Exposome-Explorer has been expanded with a list of 180 new putative biomarkers of dietary exposure identified from recent metabolomics studies that can be subjected to further tests for validation purpose. In addition, new information on the application of dietary biomarkers in epidemiological studies on cancer risk has been collected. More than 300 scientific papers describing associations of dietary biomarkers with cancer risk have been compiled. This information is now accessible online.
This recent new development of Explorer-Explorer will be presented. Illustrations of the use of the database by researchers to compare the performance and field of application of various biomarkers of exposure and to identify the specific biomarkers or panels of biomarkers most useful for biomonitoring or disease aetiology studies will be presented.

Mots-Clés: Biomarkers, Databases, Cancer, Environmental exposure, Biocuration

Oral 10 – O10

Dynamic retention time databases for steroidomics

Santiago Codesido[1], Giuseppe Marco Randazzo [1], Fabio Lehmann [2], Victor Gonzalez Ruiz [1], Arnaud Garcia [1], Ioannis Xenarios [2], Robin Liechti [2], Alan Bridge [2], Julien Boccard [1], Serge Rudaz [1]

[1] Analytical Sciences, School of Pharmaceutical Sciences, University of Geneva, University of Lausanne – Suisse

[2] Swiss Institute of Bioinformatics – Suisse

On top of the usual analytical challenges found in metabolomics, steroidomics faces the additional difficulty of working with a large family of compounds with very similar structures. Liquid chromatography (LC) is often the method of choice for their separation, but such structural similarity can be compromising for this separation due to the consequently similar physico-chemical properties. The problem is usually overcome by careful tuning of the mobile phase gradient, to focus the separation on compounds of interest. However, in a standard workflow this is highly problematic for annotation. It requires characterizing a library of known compounds for every fine-tuned configuration. We present a software solution, DynaStI, capable of annotating LCMS (liquid chromatography-mass spectrometry) features by dynamically generating the retention times from a database containing intrinsic properties for a library of metabolites. In this way, the chemical characterization of the library only needs to be performed once, and the generated retention times are adapted to the parameters of each gradient on the fly. We study the influence of experimental vs. in-silico compound properties on the quality of the prediction and the annotation, and we introduce a calibration mechanism to increase accuracy and compensate for deviations in the input parameters. We run tests on both standards and real samples, and observe that the algorithm produces reliable predictions, suitable for steroidomics compound annotation.

Mots-Clés: steroidomics, liquid chromatography, prediction, dynamic, database, annotation

Oral Membre d'honneur

Cloud microorganisms: what does metabolomics tell us?

Anne Marie Delort[1]

[1] Institut de Chimie de Clermont-Ferrand - Clermont Auvergne – SIGMA Clermont, Université Clermont Auvergne : UMR6296, Centre National de la Recherche Scientifique : UMR6296 – 24 Avenue des Landais / 63177 Aubière Cedex, France

A few years ago our team discovered the presence of metabolically active microorganisms in clouds (1). These microorganisms are aerosolized from the earth surface, travel in the air, condense water as any aerosol to form a droplet and become thus part of the cloud. They can finally go back to the earth *via* the precipitations. During this atmospheric cycle they face different stresses (cold temperature, UV light, oxidants, pollutants...), they bio-transform the organic matter in clouds, and finally they also interact with the terrestrial or aquatic ecosystems when they return to the soil.
We have used metabolomics combining LC-MS and NMR to study cloud microorganisms all along this atmospheric cycle.

When exposed to cold stress(2) or to H2O2(3), cloud bacteria completely reorganize their metabolism, as a result this change could impact cloud chemistry.

Work is in progress to study the modulation of the cloud bacteria metabolome facing atmospheric mercury. We also study photosynthetic organisms, and more particularly the interactions between atmospheric algae present in clouds (and rain) with cyanobacteria present in lakes.

Up to now metabolomics' experiments have been performed with isolated strains isolated from clouds sampled at the puy de Dôme station. In future work we plan to perform meta-metabolomics based on the whole cloud microbiota.

(1) A.-M. Delort and P. Amato (eds), Microbiology of aerosols. *Willey*, 2017.https://onlinelibrary.wiley.com/doi/

(2) C. Jousse, C. Dalle, I. Canet, M. Lagrée, M. Traïkia, B. Lyan, C. Mendes, M. Sancelme, P. Amato, A.-M. Delort*. Metabolomic study of the response to cold shock in a strain of *Pseudomonas syringae* isolated from cloud water. *Metabolomics*, 2018, 14, 11.

(3) N. Wirgot , M. Lagrée, M. Traïkia, L. Besaury, P. Amato, I. Canet, M. Sancelme, C. Jousse, B. Diémé, B. Lyan and A.-M. Delort*. Metabolic modulations and resilience of *Pseudomonas graminis* facing H2O2 in cloud water. *Scientific reports*, 2019 *in revision.*

Mots-Clés: Cloud, microorganisms, stresses, ecosystem, NMR, LC, MS

Oral 11 – O11

Fusion de données de transcriptomique et de métabolomique pour une meilleure compréhension des effets d'un contaminant alimentaire

Marie Tremblay-Franco [1,2], **Yannick Lippi** [1], **Cécile Canlet** [1,2], **Roselyne Gautier** [1,2], **Claire Naylies** [1], **Manon Neves** [1], **Philippe Pinton** [1], **Imourana Alassane-Kpembi** [1], **Laurent Debrauwer** [1,2], **Isabelle Oswald** [1]

[1] UMR1331 Toxalim, Université de Toulouse, INRA, ENVT, INP-Purpan, UPS INRA – France
[2] Axiom platform, MetaToul-MetaboHUB, national infrastructure for metabolomics and fluxomics INRA

L'analyse d'un seul bloc de données " omiques " peut s'avérer insuffisante pour fournir une image globale des modulations engendrées par un stimulus biologique. Les métabolites sont les produits finaux de réactions enzymatiques et pourraient donc refléter des modifications d'expression de gènes.
Dans ce contexte, ce travail a pour objet de comparer des méthodes d'intégration de données de transcriptomique et de métabolomique, pour évaluer les corrélations entre ces deux niveaux fonctionnels et identifier d'éventuels gènes candidats à la modification du métabolome suite à une exposition à un contaminant alimentaire.

Nous avons tout d'abord évalué deux familles de méthodes pour sélectionner des variables corrélées intra et inter-blocs : l'analyse des corrélations canoniques (CCA) " sparse ", méthode à variables latentes, et les cartes auto organisées de Kohonen (SOM, 1982), méthode de projection utilisée pour classer les objets dans les unités d'une carte bi-dimensionnelle. La pénalisation " sparse " (Wilms et al. 2016) permet de résoudre les problèmes de sur-ajustement et sélectionner les transcrits et métabolites les plus importants. La méthode O2PLS (Bouhaddani et al. 2016) a ensuite été utilisée pour étudier la relation entre les variables sélectionnées à la 1ère étape et l'exposition au contaminant alimentaire.

Les simulations effectuées ont montré que la méthode SOM, à l'opposé de la méthode sCCA, était très sensible mais très peu spécifique, et permettait une meilleure discrimination des observations en fonction de l'exposition.
Les méthodes sCCA/SOM+O2PLS ont ensuite été appliquées à des données générées à partir de fragments d'intestin exposés à un contaminant alimentaire, qui peut se retrouver dans les denrées alimentaires destinées à la consommation humaine et animale. L'identification de biomarqueurs d'exposition est donc importante pour le suivi de la santé humaine et animale. Le modèle O2PLS ajusté en utilisant les variables sélectionnées par la méthode SOM a permis une meilleure séparation en fonction de l'exposition. Les liens entre les métabolites et les gènes discriminants sont en cours de détermination (réseaux de corrélations et métaboliques).

Mots-Clés: Intégration de données, Transcriptomique, Metabolomique, Contaminant alimentaire

Oral 12 – O12

A new tool for multi-block PLS discriminant analysis of metabolomic data: application to systems epidemiology

Marion Brandolini-Bunlon[1], Mélanie Petera[1], Pierrette Gaudereau [2,3], Blandine Comte [4], Stéphanie Bougeard[5], Estelle Pujos-Guillot [1]

[1] Université Clermont Auvergne, INRA, UNH, Plateforme d'Exploration du Métabolisme, MetaboHUB Clermont – Institut national de la recherche agronomique (INRA) : UMR1019 – F-63000 Clermont-Ferrand, France

[2] Département de médecine, Université de Montréal – Canada

[3] Centre de recherche du centre hospitalier de l'université de Montréal – Canada [4] Université Clermont Auvergne, INRA, UNH – Institut national de la recherche agronomique (INRA) : UMR1019 – F-63000 Clermont-Ferrand, France

[4] Département d'épidémiologie – Anses – France

Metabolomics is a powerful phenotyping tool in nutrition and health research, generating massive and complex data that need dedicated treatments to enrich our knowledge of biological systems. In particular, to deeper investigate relations between environmental factors, phenotypes and metabolism, discriminant statistical analyses performed separately on metabolomic datasets, are often complemented by associations with metadata (anthropometric, clinical, nutritional and physical activity data...). Another relevant strategy is to perform a multi-block partial least squares discriminant analysis (MBPLSDA) that simultaneously analyses data available from different sources, allowing determining the importance of variables and variable blocks in discriminating groups of subjects, taking into account data structure in thematic blocks. In order to propose a full open-source standalone tool, the present objective was to develop an R package allowing all steps of MBPLSDA analysis for the joint analysis of metabolomic and additional data.

The tool was based on the *mbpls* function of the *ade4* R package, enriched with different functionalities, including some dedicated to discriminant analysis. Provided indicators help to determine the optimal number of components, to check the MBPLSDA model validity, and to evaluate the variability of its parameters and predictions. To illustrate the potential of the proposed tool and the associated procedure, MBPLSDA was applied to a real case study involving metabolomics, nutritional and clinical data from a human cohort.

The availability of the different functionalities in a single R package allowed optimizing parameters for an efficient joint analysis of metabolomics and epidemiological data to obtain new insights into multidimensional phenotypes. In particular, we highlighted the impact of filtering the metabolomic variables beforehand, and the relevance of a MBPLSDA approach in comparison to a standard PLS discriminant analysis method.

Mots-Clés: multi block PLS discriminant analysis, metabolomics, epidemiology

Oral Junior 5 – OJ5

Metabolomics and proteomics data integration and feature selection for high-throughput phenotyping

Alyssa Imbert [1], Magali Rompais, Julia Novion Ducassou, Emmanuelle Mouton-Barbosa, Mohammed Selloum, Florence Castelli, Charlotte Joly, Emeline Chu-Van, Aurélie Hirschler, Marie Courçon, Yohann Coute, Christophe Lechaplais, Natacha Lenuzza, Arthur Tenenhaus, Laurent Vasseur, Marion Brandolini-Bunlon, Ludovic Cottret, Yves Vandenbrouck , Olivier Sand, Bruley Christophe, Franck Giacomoni, Fabien Jourdan, Odile Schiltz, Pierre Le Ber, Claudine Medigue, Jérôme Garin, Yann Herault, Dominique Rolin, Christophe Junot, Marcel Salanoubat, Virginie Brun, Alain Perret, Myriam Ferro, Tania Sorg, Estelle Pujos-Guillot, Anne Gonzalez-De-Peredo, François Fenaille, Christine Carapito, David Vallenet, Etienne Thevenot [1]

[1] CEA, LIST, Laboratoire Analyse de Données et Intelligence des Systèmes, MetaboHUB – CEA – France

How complementary are metabolomics and proteomics approaches? What benefit does their combination provide in terms of prediction performance and data interpretation? Which methods and pipelines would enable robust and high-throughput data integration?
To address these pivotal and yet open questions, the ProMetIS consortium, gathering 10 laboratories from the 5 national infrastructures for mouse phenogenomics (PHENOMIN), genomics (France Génomique), proteomics (ProFI), metabolomics and fluxomics (MetaboHUB), and bioinformatics (IFB), has designed a case study focusing on the high-throughput phenotyping of mouse models. Liver and plasma from homozygous mutant mice for the "Linker For Activation Of T Cells" gene (LAT), together with their wild-type littermates (WT), were analyzed by clinical phenotyping, proteomics, and metabolomics complementary LC-MS technologies, resulting in a total of 11 datasets.

To combine such data, we focused on the regularized generalized canonical correlation analysis method (rGCCA). This multi-block approach enables a broad exploration of the relationships between the individual datasets, in an unsupervised (e.g. canonical correlation) or supervised context (e.g. partial least squares), as well as feature selection.

Here, we optimized the design of the relationships between the blocks and the tuning of the penalization parameter to achieve robust and efficient proteomics and metabolomics integrated phenotyping. By comparing the results to the separate analysis of each dataset, we demonstrate the impact of data integration in terms of prediction performance and biological interpretation.

Mots-Clés: Data integration, feature selection, metabolomics, proteomics, high, throughput phenotyping, rGCCA

Oral Sponsor 7

Platinium

Industrializing Flux capabilities

Yoann Fillâtre AB Sciex

Oral Keynote 4 – OK4

Discovery and validation of food intake biomarkers using untargeted metabolomics in human intervention and cross-sectional studies

Natalia Vazquez-Manjarrez [1], Christoph Weinert [2], Marynka Ulaszewska [3], Carina I Mack [2], Pierre Micheau [1], Mélanie Pétéra [4], Stéphanie Durand [4], Estelle Pujos-Guillot [4], Bjorn Egert [2], Fulvio Mattivi [3], Achim Bub [2], Sabine E. Kulling [2], Lars Ove Dragsted [5], Claudine Manach [1]

1 Unité de Nutrition Humaine – INRA): UMR1019, Université Clemont Auvergne – France
2 Max Rubner-Institut – Allemagne
3 Fondazione Edmund Mach - Edmund Mach Foundation [Italie] – Italie
4 PFEM – INR) : UMR1019 – France
5 [5] University of Copenhagen – Danemark
6

Background: Banana is one of the most widely consumed fruits in the world. However, scarce information is available regarding its health effects. Biomarkers of banana intake would allow a more accurate assessment of its consumption in nutrition studies.
Objective: Using an untargeted metabolomics approach, we aimed to identify the bananaderived metabolites present in urine after consumption, and among them new candidate biomarkers of banana intake.
Methods: A randomized controlled study with a crossover design was performed on 12 healthy subjects, with two dietary interventions: 1) 250ml control drink (Fresubin) and 2) 240g banana+150ml control drink. Urine samples collected over 24h were analyzed with UPLC-QTOFMS and GC×GC-MS. The discovered biomarkers were confirmed in a cross-sectional study (KarMeN) where 72 subjects were selected reflecting high, low, and non-consumers of banana. The confirmed biomarkers were examined singly or in combinations, for established criteria of validation for biomarkers of food intake.
Results: We identified 33 potentially bioactive banana metabolites, some of them being newly described. A panel of five metabolites, methoxyeugenol-glucuronide, dopamine-sulfate, salsolinolsulfate, xanthurenic-acid and 6-hydroxy-1-methyl-1,2,3,4-tetrahydro-b-carboline-sulfate, were confirmed as candidate intake biomarkers. We demonstrated that the combination of methoxyeugenolglucuronide and dopamine-sulfate performs best in predicting banana intake in high (AUCtest=0.92) and low (AUCtest=0.87) consumers. The new biomarkers met key criteria establishing their current applicability in nutrition and health research for assessing occurrence of banana intake.
Conclusions: Our metabolomics study revealed new putative bioactive metabolites of banana and a combined biomarker of intake. Those findings will help to better decipher the health effects of banana in future focused studies.
Funding: JPI HDHL FoodBAll project, #ANR-14-HDHL-0002-02

Mots-Clés: Biomarkers of food intake, banana, untargeted metabolomics, dopamine, salsolinol

Oral 13 – O13

Pratiques culturales, qualité des graines de colza et santé du consommateur : une étude preuve de principe " multi-omique " chez la souris au métabolisme dérégulé

Djawed Bennouna [1], Franck Tourniaire [2], Jean-François Landrier [1,3], Thierry Durand [4], Jean-Marie Galano [4], Frédéric Fine [6], Sherazade Benatia [1], Clément Rosique [1], Karl Fraser 4, Claire Vigor [8], Jean-Charles Martin [1]

[1] Centre recherche en CardioVasculaire et Nutrition – INRA UMRs1263
[2] CriBioM – Aix-Marseille Université - AMU – 27 Bd Jean Moulin Faculté de médecine, La Timone, France
[3] UMR INRA 1260
[4] Institut des Biomolécules Max Mousseron [Pôle Chimie Balard] – Centre National de la Recherche Scientifique : UMR5247, Université de Montpellier, Ecole Nationale Supérieure de Chimie de Montpellier – France
[5] terre innovia – TERRES INOVIA – France
[6] AgResearch – Nouvelle-Zélande

Les graines de colza (Brassica napus) sont riches en composés phytochimiques bénéfiques pour la santé humaine ou animale, tels que polyphénols et glucosinolates. Le contenu de ces composés peut fluctuer en fonction des conditions de culture. Nous avons estimé si de telle différence pouvait moduler la valeur nutritionnelle des graines. Parmi les 8 cultivars x 8 régions, 2 cultivars provenant d'Ile et Vilaine (cultivar Bonanza) ou de la Somme (ES-Mambo) présentaient des contenus phytochimiques très contrastés (84 métabolites secondaires mesurés par LCHRMS et MS2). Les extraits ont été administrés pendant 6 semaines à des souris ob. Un extrait commercial de Brassica oleracea (Brocoli) a été ajouté comme référence dans l'essai nutritionnel. La réponse biologique ou " responsome " a été déterminée dans le métabolome intestinal et plasmatique, le transcriptome et le métabolome hépatiques. 768 métabolites, 436 lipides et 24974 transcrits par souris ont été identifiés, avec 64 variables cliniques et 25 types d'isoprostanes (analyses LCMS). Les données ont été regroupées en 72 ensembles fonctionnels ou biochimiques pour le métabolome, 14 pour les transcrits, et examinés par analyse multibloc.
Seul l'extrait de " mambo " réduisait le stress oxydatif, indiquée par la diminution d'isoprostanoïdes plasmatiques, associé aux fonctions de stress oxydatif " omiques ". Ces actions sur le stress oxydant paraissent favorables dans le cadre de la protection vasculaire. En dehors de ces régulations,
le "responsome " montrait une proximité constante entre l'extrait de colza " mambo " et l'extrait de brocoli, liée à des composés phytochimiques bioactifs communs, et ciblaient des régulations métaboliques bénéfiques comme le métabolisme du tryptophane. L'analyse des régulations conjointes multi-échelles montrait que 70% des fonctions biologiques avaient une régulation coordonnée et soulignait l'impact régulateur primordial des extraits sur le microbiote et sa propagation à l'hôte.
Conclusion: les modifications du contenu phytochimique induites par différentes conditions de culture de B. napus peuvent modifier leur valeur santé.
Mots-Clés: colza, environnement, nutrition, santé, multiomique

Oral Junior 6 – OJ6

Découverte de marqueurs précoces de l'altération microbiologique des ovoproduits par des approches de métabolomique croisée (RMN-spectrométrie de masse)

Rémy Coat[1], Jack Legrand [1], Abdellah Arhaliass [1], Olivier Goncalves [1]

[1] Laboratoire de génie des procédés - environnement - agroalimentaire – Université Bretagne Loire, IMT Atlantique Bretagne-Pays de la Loire, Oniris, Centre National de la Recherche Scientifique : UMR6144, Université de Nantes – France

L'ovoproduit est une matrice biologique complexe qui une fois cassé et homogénéisé perd une bonne part de ses propriétés de conservation, devenant ainsi pour certaines bactéries un excellent milieu de croissance. Ce développement bactérien non maitrisé provoque des altérations biochimiques modifiants fortement les qualités organoleptiques de l'ovoproduit. L'objectif du projet ALTOVOP est d'étudier les mécanismes biochimiques impliqués par des approches de métabolomique combinées (ciblée et non ciblée) afin de décrire et de comprendre ces mécanismes jusqu'alors peu ou pas caractérisés et d'identifier *in fine* des marqueurs précoces de ces altérations microbiologiques.
L'approche analytique ciblée concerne des métabolites identifiés comme impliqués dans la dégradation des ovoproduits. Dans le cadre de l'approche analytique non ciblée, des techniques de profilage des macromolécules (FTIR, NIR, Raman) et des métabolites (NMR, GC-MS) ont été employées afin d'établir des profils d'ovoproduits contrastés (frais et dégradés) et d'identifier des marqueurs potentiels d'altération.

Deux études cinétiques de la dégradation réalisées sur des Ovoproduits stériles préparés en laboratoire avec différentes espèces bactériennes ont permis d'établir et de suivre plus en détail l'évolution de la concentration des différents métabolites identifiés au cours de la dégradation et d'identifier différentes voies métaboliques impliquées. Différents liens entre ces voies d'altération et l'évolution des propriétés fonctionnelles des produits ont également pu être proposés. L'altération des propriétés émulsifiantes et gélifiantes, par exemple, serait liée au catabolisme des lipides et aux activités protéolytiques.
Les molécules ainsi identifiées (Glucose et produits de son catabolisme, glycérol et acides organiques, acides aminés...) pourraient être utilisées pour détecter de manière précoce l'altération microbiologique des ovoproduits, permettant un meilleur suivi qualité des produits, affinant leurs DLC et ce faisant évitant de régulières pertes de produit liées à leur altération en milieu industriel.

Mots-Clés: GCMS, RMN, Profilage Métabolique, Empreinte Métabolique, Ovoproduits

Oral 14 – O14

Analyse non ciblée de contaminants dans les aliments : validation d'une approche de type métabolomique sur deux technologies UHPLC-HRMS et deux scénarios de contamination " en aveugle "

Grégoire Delaporte [1], Mathieu Cladière [1], Valérie Camel [1]

[1] UMR Ingénierie Procédés Aliments (GENIAL) – Institut National de la Recherche Agronomique : UMR1145, AgroParisTech, Université Paris-Saclay, Université Paris-Saclay – 1 avenue des Olympiades, F-91744 Massy, France

Le récent intérêt pour les approches métabolomiques dans l'analyse non-ciblée de contaminants dans les aliments a permis le développement de premières approches aux performances contrastées : niveaux de concentrations élevés (mg/kg) et gamme de composés analysés relativement importante [1]. De nouveaux travaux à des concentrations plus faibles (_~10 μg/kg) ont été publiés, mais sans aucune étude de transférabilité sur différents instruments ou scénarios de contamination [2].
Nous avons mis au point une méthode d'analyse non-ciblée de contaminants dans le thé basée sur une extraction générique par solvant, une analyse par UHPLC-ToF-MS, l'extraction des signaux par XCMS (opéré sur W4M) et un prétraitement des données avant une analyse multivariée par analyse en composantes indépendantes (ICA) sur Matlab. A l'issue de ce protocole, les ions suspects sont annotés grâce à une combinaison de recherches dans des bases de données et d'analyse des profils isotopiques [3]. La validation de cette méthode nécessite de la confronter à différents scénarios de contamination, et de vérifier son applicabilité à d'autres technologies HRMS. Ainsi, deux scénarios de contamination ont été étudiés " en aveugle " sur deux instruments (ToF et Orbitrap) : 1) thé vert / 3 groupes / 1 mélange de dopage / teneurs < 30 μg/kg (MTBLS771) ; 2) thé noir / 3 groupes / 2 mélanges de dopage / teneurs < 10 μg/kg (MTBLS772) [4].

L'application de notre méthode aux données Orbitrap a nécessité une adaptation de la stratégie d'imputation des valeurs manquantes en raison d'un bruit trop faible pour le module xcms.fillPeaks. En termes de performances, l'étude " en aveugle " du scénario 1) a montré des réponses sensiblement équivalentes pour les deux instruments avec environ 60% de détection, ainsi qu'un faux positif trouvé sur le ToF. Concernant le scénario 2), plus complexe, les résultats montrent une complémentarité des deux appareils avec 2 (ou 3) molécules détectées uniquement sur le ToF (ou l'Orbitrap), soit un taux global de détection de 100%. Ces résultats sont novateurs car pour la première fois une même méthode d'analyse non-ciblée de contaminants peut être mise en œuvre sur deux plateformes HRMS de technologies différentes, avec la capacité à détecter plusieurs scénarios de contamination.

Mots-Clés: sécurité sanitaire, thé, contaminants, non ciblé, HRMS, ToF, orbitrap

Prix de Thèse RFMF 2019

Nouvelle stratégie de priorisation pour l'étude des produits naturels par l'approche des réseaux moléculaires multi-informatifs,

Florent Olivon

Communications Posters

Session Santé

Poster 1 – P1

Pathogenic E. coli induces a metabolic reprogramming in colonic epithelial cells through the production of colibactin

Romain Villéger [1], Binta Diémé [2,3], Marie Lagrée [2,3], Julien Delmas [1], Gwenaëlle Roche [1], Nicolas Barnich [1], Cyril Jousse [2,3], Mathilde Bonnet [1]

[1] Microbes, Intestin, Inflammation et Susceptibilité de lHôte - Clermont Auvergne – INSERM UMR1071, Université Clermont Auvergne, Centre de Recherche en Nutrition Humaine dÁuvergne, INRA [Auvergne/Rhône-Alpes] – France

[2] Institut de Chimie de Clermont-Ferrand - Clermont Auvergne – SIGMA Clermont, Université Clermont Auvergne : UMR6296, Centre National de la Recherche Scientifique : UMR6296 – France

[3] Plateforme d'Exploration du Métabolisme MetaboHUB Clermont, Clermont-Ferrand, France – INRA (FRANCE), Université Clemont Auvergne – France

Introduction: Recent studies suggest that colonization of the colonic mucosa by pathogenic *E. coli* could be involved in the development of colorectal cancer (CRC), especially through the production of genotoxins such as colibactin. However, the mechanisms underlying the colibactindependent carcinogenesis are still to explore. We propose to use an untargeted metabolomic approach to investigate the mechanisms sustaining the carcinogenic potential of colibactin-positive *E. coli*.

Methods: Human colon carcinoma T84 cells were infected with a colibactin-positive *E. coli* strain 11G5, or its colibactin-negative mutant 11G5-ΔclbQ. Expression of genes coding for enzymes responsible for colibactin synthesis was evaluated by RT-qPCR after 3h, 24h and 48h post-infection. Metabolites were extracted from infected cells by a methanol/water/acetonitrile mixture (2:1:2) 24h post-infection. Extracts were investigated using 1H-NMR / LC-MS and data were processed with XCMS package (Galaxy W4M). PCA and PLS-DA were applied to analyze profiling data.

Results: Colibactin-positive *E. coli* were found intracellularly 3h post-infection, then persisted and multiplied in cells during the following 24h and 48h. A higher expression of *clbC*, *clbN*, *clbP* and *clbQ* genes was observed after 24h when compared to 3h and 48h. At the 24h post-infection time point, 1H-NMR metabolomic analysis of cell lysate retained 881 buckets. For LC-MS analysis, 776 and 413 features were respectively kept in positive and negative ionization mode for multivariate analysis. PCA analysis revealed strong discrimination between non-infected cells and cells infected with 11G5 *E. coli*, but also between cells infected with 11G5-ΔclbQ *E. coli* versus 11G5 *E. coli*. Discriminant metabolites between the 11G5 and the 11G5-ΔclbQ groups suggest the specific effect of colibactin on the disturbance of colonic cell metabolome. Alterations in the amino acids and nucleosides seems to characterize the colibactin-dependent metabolic reprogramming in T84 cells.

Conclusion: These results reveal a specific metabolic landscape in colonic epithelial cells following colibactin-positive *E. coli* infection, and highlight a colibactin-dependent metabolic reprogramming that could reveal attractive targets to inhibit CRC development.

Mots-Clés: colorectal cancer, E. coli, colibactin, metabolomics

Poster 2 – P2

Endometriosis: diagnosis, phenotyping and physiopathology from NMR-based metabolomics

Gildas Bertho [1], **Fatiha Kateb** [1], **Pietro Santulli** [2,3], **Charles Chapron** [2]

[1] Laboratoire de Chimie et de Biochimie Pharmacologiques et Toxicologiques – Université Paris Descartes - Paris 5 : UMR8601, Centre National de la Recherche Scientifique : UMR8601 – France
[2] Service de Gynécologie et Obstétrique II [Cochin] – CHU Cochin [AP-HP] – France [3] Service de Médecine de la Reproduction – Hôpital Cochin – France

Endometriosis is a gynecological disease, characterized by the growth and proliferation of endometrium-like tissues outside the uterus. The symptoms include painful periods, painful ovulation, pain during or after sexual intercourse, heavy bleeding, chronic pelvic pain, fatigue, as well as infertility, and can impact on general physical, mental, and social well-being. Endometriosis affects around 10% of women during their reproductive years, which corresponds to approximately 176 million women in the world. Endometriosis can start as early as a girl's first period, and menopause may not resolve the symptoms. A general lack of awareness by both women and health care providers, due to a "normalization" of symptoms, results in a significant delay from the apparition of symptoms to the diagnosis and treatment. There is no known cure and today laparoscopy is the most common procedure used to diagnose and remove endometriotic tissue.
We used 1H-NMR-based metabolomics to decipher the mechanism inherent to the disease. This work in collaboration with physician at hospital Cochin in Paris consists in a prospective cohort study, including 75 patients: 50 with histologically proved endometriosis and 25 controls with no macroscopic endometriotic lesions during laparoscopic surgery. Endometriosis was precisely classified into 2 groups of 25 patients: endometrioma (OMA) and deep infiltrating endometriosis (DIE).

The serum metabolomic profile of endometriosis patients was characterized by decreased concentrations of amino acids compared with healthy women. Conversely, the concentrations of free fatty acids and ketone bodies biomarkers were significantly increased in the endometriosis group. Therefore, in endometriosis patients the lipolysis is activated followed by an increased ketogenesis where the inflammatory role of 3-hydroxybutyrate could have of major importance. Interestingly, OMA and DIE phenotypes also displayed specific metabolic profiles.
1H-NMR–based metabolomics demonstrated its potential to identify metabolic changes associated to endometriosis and endometriosis phenotypes in serum samples. This information is useful to get a better understanding of the pathogenesis of endometriosis, thus providing support to the rapid and noninvasive diagnosis of this pathology.

Mots-Clés: Endometriosis, Biomarkers, NMR, Serum, Phenotyping, Metabolic Pathways

Poster 3 – P3

Colorectal Cancer : Biomarkers and Effect Size

Nicolas Di Giovanni[1], Marie-Alice Meuwis [2], Edouard Louis [2], Jean-François Focant [1]

[1] Organic and Biological Analytical Chemistry Group, MS Lab, Université de Liège – Belgique
[2] Translationnal Gastroenterology Unit, GIGA-R, CHU (University Hospital), Liège – Belgique

Colorectal cancer affects more than one million new persons each year worldwide, and kills more than 700.000. Nevertheless, its diagnosis is still largely based on invasive tissue sampling and gaps remain in the understanding of its pathogenesis, with complex combinations between lifestyle, genetics, epigenetics, chronic inflammation (IBD) and microbiota. Untargeted metabolomics is one of the approaches that can be used to solve these issues.
In the present study, we analyzed serum samples from patients affected by colorectal cancer (CRC, n = 18) and by colorectal cancer in remission (R-CRC, n = 17), and samples from healthy patients matched for biases (HC, n = 19 and R-HC, n = 17). The aim was to find candidate biomarkers able to diagnose the active state of the disease as well as to compare the concentration levels of the molecules of interest with the remission state to better understand the biolocal processes beneath the observed clinical and metabolic symptoms.

To do so, an optimized and validated (NIST SRM 1950) comprehensive GC×GC-(HR)TOFMS method we developed was used, that also included an in-house QC system and data processing based on multiple statistical techniques.

Because the experimental design prevented a direct comparison between the active and remission samples, which were not directly matched for biases, we used a measure called effect size that has the advantage over significance testing to focus on effect (here signal/concentration variation) magnitude.
This presentation will therefore discuss effect size interest and application in metabolomics. Along with the results obtained in terms of the highlighted candidate biomarkers? Candidates that were identified using full mass spectrum, linear retention indices and accurate mass provided by state-of-the-art high-resolution (HR) time-of-flight mass spectrometry, and which discrimination potential was assessed using supervised and unsupervised models, discriminant analysis and ROC curves.

Mots-Clés: Colorectal cancer, gas chromatography, effect size

Poster 4 – P4

Increased content of Lysophospholipids, diacylglicerides and sphingomyelins in isolated triglycerides-rich lipoproteins and HDL from patients with coronary artery disease

Ljubica Svilar[1], Magali Barchuk, Patricia Ancel , Veronica Miksztowicz , Graciela Lopez , Miguel Rubio , Laura Schreier , Anne Dutour , Benedicte Gaborit , Jean-Charles Martin , Gabriela Berg

[1] CriBioM – Aix-Marseille University, INSERM, INRA, C2VN – France

Introduction: android obesity and the atherogenic dyslipidemia, with increased TG-rich lipoproteins remnants (TRL) and decreased HDL levels, are associated with higher risk of coronary artery disease (CAD). Plasma lipidomics studies have revealed that particular molecular lipid species are involved in the development of atherosclerosis and CAD. However, the lipidomic analysis of isolated lipoproteins (TRL and HDL) has been scarcely studied so far. This qualitative analysis is of interest as it would allow differentiating specific lipid species contribution to the development of CAD.
Materials and Methods: lipidomic analysis was performed in 15 patients undergoing coronary artery bypass graft (CAD) and 12 patients with valve replacement (No CAD). Serum samples were obtained before surgery and TRL and HDL were isolated by ultracentrifugation. For lipidomics, lipids were extracted from each fraction with MTBE. Lipidomics was carried out on a LC C18 column hyphenated to a Q-Exactive plus mass spectrometer, using both positive and negative ionization mode, and acquired in full MS and data dependent MSMS mode. Lipid annotation was performed using a combination of the open source XCMS and ThermoFischer LipidSearch software.

Results: a total of 242 lipid species were identified in both lipoproteins fractions. In CAD patients, higher content of total LPC ($p< 0.002$) and LPE ($p< 0.02$) in TRL were found. In this lipoprotein higher levels of LPC(20:4), LPC(38:5) and LPC(16:0) as well as LPE(18:1) and LPE(16:0) were observed in CAD, while lower content of MG(18:2p) was detected compared to no CAD patients ($p< 0.04$). No differences in the total content of each lipid in HDL were observed between groups, although CAD patients presented higher levels of DG(18:1/20:4) and SM(22:0/20:2) ($p< 0.04$).
Conclusion: The distribution of lipid species, especially bioactive lipids, in TRL and HDL could differentially contribute to atherosclerosis progression and CAD development. Assessment of molecular lipids from lipoproteins may allow a better understanding of the role of these species in the development of atherosclerosis and the risk of CAD.

Mots-Clés: lipidomics, lipoproteins, HDL, coronary artery disease

Poster 5 – P5

New isoprostanoid biomarkers for prostate cancer

Claire Vigor [1], Amandine Rocher [1], Guillaume Reversat [1], Amandyne Linares-Maurizi [1], Camille Oger [2], Jean Marie Galano [1], Thierry Durand [1], Pierre-Jean Lamy [3], Clément Peigné [3], Bruno Segui [3], Vincent Abd El Fattah [3], Grégoire Poinas [3], Xavier Rébillard [3]

[1] Institut des Biomolécules Max Mousseron [Pôle Chimie Balard] – CNRS : UMR5247 – France
[2] Institut des Biomolécules Max Mousseron [Pôle Chimie Balard] – Centre National de la Recherche Scientifique : UMR5247, Université de Montpellier, Ecole Nationale Supérieure de Chimie de Montpellier – France
[3] Clinique Beau Soleil [Montpellier] – Université Montpellier I – France

Context : At present, early detection of prostate cancer relies primarily on digital rectal examination and PSA assays after identifying life expectancy and personal risk factors.[1] The requirement of new biomarkers is real for an improvement of the diagnosis, for a better classification of potentially aggressive cancer allowing the orientation of the patient care. In recent years, numerous epidemiological, experimental or clinical studies have shown a close link between oxidative stress (OS) and prostate cancer.[2] Isoprostanes (IsoPs) derived from the radical oxidation of polyunsaturated fatty acids[3], constitute excellent biomarkers of lipid peroxidation in vivo and thus of OS.[4] The goal of our study is to discover new diagnostic biomarkers through the establishment of an LC-MS/MS profile of isoprostanoids and to assess their relevance and performance in the context of early detection or of identification of population subgroups. *Experimental study :* This study consists of a qualitative and quantitative profile of about 50 IsoP derivatives in urine of patients cohort made of: 30 ”healthy” patients (no suspicion of prostate cancer), 30 risk patients (suspicion of cancer, negative biopsy), 30 patients with localized prostate cancer and 30 patients with locally advanced or metastatic prostate cancer.

Preliminary results : Preliminary data allow observation of trends. Of the 50 isoprostanoids in the method, 5 appear to be higher in patients with highly advanced disease (locally advanced or metastatic) compared to healthy patients. In addition, patients in the risk group according to conventional markers (TR + PSA), but having a negative biopsy, comparable to healthy patients, also have isoprostanoid levels comparable to healthy patients. The analysis of isoprostanoids could help to classify individuals at risk in the category of healthy individuals and avoid the implementation of a biopsy. This preliminary study should be pursued in particular by the comparison between MRI results and pathology for patients with negative biopsies.

Mots-Clés: Biomarker, diagnostic, prostate cancer, isoprostanoids

Poster 6 – P6

Combattre le glioblastome en combinant le témozolomide aux nanoparticules : analyse de l'exométabolome par Résonance Magnétique Nucléaire

Manon Courcelle [1], Ferdaous Sahli [1], Célia Arib [1], Maroua Ben Haddada [1], Nadia Bouchemal [1], Mohamed Triba [1], Philippe Savarin [1], Jolanda Spadavecchia [1], Tony Palama [1]

[1] Laboratoire Chimie, Structures, Propriétés de Biomatériaux et d'Agents Thérapeutiques (CSPBAT) – Université Paris 13, Sorbonne Paris Cité,, CNRS : UMR7244 – France

Les glioblastomes sont des tumeurs cérébrales malignes très fréquentes qui surviennent à tout âge mais dans 70% des cas entre 45 et 70 ans. Ce type de cancer présente un taux de mortalité élevé ainsi qu'un taux important de récidive. Le traitement actuel consiste en une chirurgie d'ablation la plus large possible suivi d'une radio-chimiothérapie. La molécule utilisée en chimiothérapie est le témozolomide (TMZ), appartenant à la famille des agents alkylants. Cependant, les traitements oncologiques conventionnels sont insuffisants et ont une efficacité limitée ainsi qu'une toxicité non négligeable. L'utilisation de nanoparticules permettrait de pallier à ces inconvénients. En effet, elles permettent de cibler le transport du médicament jusqu'à la tumeur et réduisent la dégradation de ce dernier.

La lignée cellulaire U87MG a été étudiée et leur exométabolome a été analysé par résonance magnétique nucléaire (RMN). Les cellules ont été mises en culture dans du milieu DMEM supplémenté en sérum à 10%. Dans un premier temps, la concentration inhibitrice médiane (IC50) a été mesurée afin de déterminer l'efficacité du TMZ seul. Ensuite, des nanoparticules d'or ont été synthétisées contenant à l'intérieur, le témozolomide et en surface un polymère, le polyéthylène glycol (PEG) qui est un agent stabilisant. Ces nanoparticules sont mises en contact avec les cellules U87MG afin de suivre la viabilité cellulaire au cours du temps. A chaque expérience, les milieux de culture des cellules ont été récupérés pour mesurer les métabolites consommés et produits par les cellules, ie l'exométabolome.

Ces résultats contribueront à une meilleure compréhension du métabolisme des cellules U87MG lors de l'utilisation de nanoparticules pour les combattre.

Mots-Clés: nanoparticules, exométabolome, rmn, glioblastome, cancer, témozolomide

Poster 7 – P7

Mechanisms of gastric cancer suppression by taxanes and statins using a pluri-omics approach

George Alzeeb [1], Laurent Corcos [1], Catherine Le Jossic-Corcos [1]

[1] Génétique, génomique fonctionnelle et biotechnologies (UMR 1078) – EFS, Université de Brest, Institut National de la Santé et de la Recherche Médicale : U1078 – France

Gastric cancer (GC) is globally the fourth most common cancer and a leading cause of death amongst cancer patients, who are mostly diagnosed at advanced stages of the disease where treatment options are limited and survival rate is poor. Therapeutic options might include combinations of available drugs. Previous studies by our lab have shown that the association of statins (Lovastatin) and taxanes (Docetaxel) was a powerful trigger of HGT-1 human gastric cancer cells apoptosis. Statins are major anti-hypercholesterolemia drugs, that also trigger apoptosis of many types of cancer cells. Docetaxel is a potent microtubule-stabilising agent. To identify biomarkers of response to these treatments, a project was conducted in collaboration with mass spectrometry (GC-MS and LC-MS) and NMR platforms of the CORSAIRE metabolomics and lipidomics network. The levels of more than 100 metabolites were significantly altered by the treatments (especially lovastatin), including amino acids, organic acids, sugars, and several families of lipids. While most metabolites were increased, fumarate was decreased at all time points and L-Glutamine was the metabolite altered the most, pointing to an important metabolic adaptation of cancer cells. Transposed onto the KEGG pathway (Kyoto Encyclopedia of Genes and Genomes) of the ”Central Carbon Metabolism in Cancer”, our metabolomics, lipidomics and transcriptomics data led to the proposition of a model of the effect of the combined treatment. We aim next to extend this analytical approach by studying the molecular mechanisms (genes) and biochemicals (enzymes and transporters) responsible for the changes in the metabolome and lipidome. Furthermore, our team showed that the combination of docetaxel and lovastatin allowed reduction of tumor volume in a xenograft model in immunocompromised mice. These models, with cells in culture and tumors in mice offer us the opportunity to analyze the metabolic changes resulting from treatments both ex vivo and in vivo.

Mots-Clés: Gastric cancer, statin, taxane, metabolomics, lipidomics, transcriptomics, LGlutamine.

Poster 8 – P8

Identification de biomarqueurs dans le Trouble de Déficit d'Attention avec ou sans Hyperactivité (TDAH)

Camille Dupuy [1], **Laurent Galineau** [1], **Pierre Thomas-Castelnau** [1], **Patrick Emond** [3]

[1] Imagerie et cerveau – Université de Tours : U930, INSERM : U930 – France

[2] Université François-Rabelais, INSERM U930 "Imagerie et Cerveau", CHRU de Tours, 10 Bv Tonnellé, 37044 Tours, France – Inserm – 10 Bv Tonnellé, 37044 Tours, France

Le Trouble Déficit d'Attention avec ou sans Hyperactivité (TDAH) est un trouble du neurodéveloppement hétérogène touchant 3 à 5% des enfants scolarisés et caractérisé par un déficit d'attention, une hyperactivité et une impulsivité. Actuellement le diagnostic se fait principalement à l'aide de tests cognitifs avec un risque non négligeable d'erreurs diagnostiques.
A l'heure actuelle aucune étude ne propose un/des biomarqueurs fiable(s) du TDAH. L'identification de biomarqueurs reste donc un défi important pour le diagnostic précoce et le suivi thérapeutique adapté des patients. Associé à la recherche de biomarqueurs circulants, l'étude du métabolisme central est déterminante pour mieux comprendre la physiopathologie du TDAH. A ce titre, l'utilisation de modèles animaux est pertinente pour étudier le métabolome cérébral. Le modèle de rat le mieux caractérisé et le plus utilisé est la souche SHR/NCrl comparée à la souche témoin WKY/NHsd.

L'objectif de cette étude est d'identifier des biomarqueurs métaboliques, au niveau central et périphérique, chez des rats SHR/NCrl. Pour cela, dix régions cérébrales ont été prélevées, ainsi que des prélèvements périphériques (sang, urines et fèces), puis analysés en LC-HRMS. Les données obtenues ont été traitées par des analyses multivariées, univariées et les voies métaboliques discriminantes ont été recherchées.

Cette étude permet de montrer une discrimination entre les deux souches basée sur leur métabolisme. L'analyse de voies permet en effet de différencier deux réseaux fonctionnels : le réseau ventral limbique et le réseau dorsal cognitif. De plus, l'altération statistiquement significative de voies métaboliques communes est retrouvée dans les régions cérébrales et les compartiments périphériques.
A l'avenir, des études de métabolomiques sur des échantillons périphériques cliniques (urines et sang) vont être réalisées afin de mieux caractériser ce trouble sévère et fréquent.

Mots-Clés: TDAH, biomarqueurs, LC, MS, cerveau

Poster 9 – P9

METABOLOMIC NMR STUDIES AT PRESYMPTOMATIC AND SYMPTOMATIC STAGES OF HUNTINGTON'S DISEASE, ON DROSOPHILA MODEL

Marylène Bertrand [1], Martine Decoville , Hervé Meudal , Serge Birman , Céline Landon [1]

[1] Centre de biophysique moléculaire – Université d'Orléans : UPR4301, Institut National de la Santé et de la Recherche Médicale : UPR4301, Centre National de la Recherche Scientifique : UPR4301 – France

Huntington's disease (HD) is an inherited neurodegenerative disorder, for which diagnosis development and discovery of new therapeutic targets are urgently required. For this study, a model of Huntington's disease has been generated in *Drosophila melanogaster,* in order to identify metabolic biomarkers, at presymptomatic (10 days-old flies) and at symptomatic stages of the disease (16 days-old flies). The pan-neuronal expression of a pathogenic polyglutaminated huntingtin protein (htt-93Q) in transgenic flies induces neuropathology, with most of the characteristics of the human disease. The discriminant metabolites between the disease carrier flies and their controls were identified by 1H-NMR and OPLS-DA multivariate analyses. This study identifies, for the first time, metabolites at a pre-symptomatic stage and at a symptomatic stage of Huntington's disease modeled in *Drosophila melanogaster*. Ten metabolites (NAD+, AMP, fumarate, asparagine, β-alanine, dimethylamine, glutamine, succinate, glutamate, and ethanol) were identified as biomarkers at a presymptomatic stage of the disease and six metabolites (succinate, phosphocholine, acetate, pyruvate and 2-oxoglutarate) as biomarkers at an advanced stage of the disease. These metabolites reveal dysfunctions in energy production, leading to mitochondrial dysfunction and to a disruption of the glycolysis, TCA and glutamate-glutamine cycles. Our data allow a better understanding of the metabolism impairment of HD at presymptomatic and symptomatic stages of the disease, and demonstrate that the metabolites perturbations evolve during the development of the disease.

Mots-Clés: Huntington's disease, neurodegenerative disease, Drosophila, metabolomics, NMR, presymptomatic

Poster 10 – P10

High throughput metabolomics using DI-HRMS on a FT-ICR equipped with a dynamically harmonized cell: a methodology for improving the assessment of metadata quality in epidemiological studies

Baninia Habchi [1,2,3], Sandra Alves [2], Michèle Guillaume [4], Sylvie Streel [4], Anne-Françoise Donneau [4], Brice Appenzeller [5], Douglas N. Rutledge [1], Alain Paris [6], Estelle Rathahao-Paris [2,7]

[1] UMR Ingénierie Procédés Aliments – AgroParisTech, Inra, Université Paris-Saclay, 91300 Massy, France – France
[2] Sorbonne Université – Faculté des Sciences et de l'Ingénierie, IPCM, 75005 Paris, France – France
[3] Aix Marseille Université (AMU) – NORT, UMR INSERM 1062, INRA 1260, F13005 Marseille, France – France
[4] Department of Public Health, University of Liège, 4000 Liège, Belgium – Belgique
[5] Human Biomonitoring Research Unit, Luxembourg Institute of Health (LIH), Rue Henri Koch 29, Luxembourg
[6] Sorbonne Universités – Muséum national d'Histoire naturelle, CNRS, UMR7245 MCAM, 75005 Paris, France – France
[7] UMR Service de Pharmacologie et d'Immunoanalyse – CEA-INRA, Université Paris-Saclay, 91191 Gif-sur-Yvette – France

Some chronic diseases in human could be explained by exposure to toxicants (*Sears et al., J Environ Public Health. 2012;2012*). So, an analytical monitoring of population exposome based on high throughput methods applied to urine, serum or hair matrices is required. The recent development in metabolomics by mass spectrometry provides strong insights in such context. In the present study, a high-throughput approach based on direct introduction high resolution mass spectrometry (DI-HRMS) using a Fourier transform ion cyclotron resonance (FT-ICR) on a 7 T-instrument equipped with a dynamically harmonized cell is proposed to get large cohort phenotyping. Urine samples of approximately 500 subjects enrolled in the "*Nutrition, Environment and Cardiovascular Health – NESCaV*" study were obtained from the Service de Chimie médicale, University Hospital Centre of Liège, and analyzed using flow injection analysis (FIA). A total of 626 injections was performed in positive mode in less than 74 hours.
The so generated data was pre-processed using in-house Matlab scripts. A Principal Component Analysis (PCA) was applied without any known metadata. Analytical data quality was evaluated in a previous work based on more than 50 injections of a quality control sample (*Habchi et al., Anal Bioanal Chem*, *2018*).
PCA showed a separation of some individuals according to drugs administered. As an example, distinction of 16 subjects is related to specific ions at *m/z* 130.109 annotated putatively as [M+H]+ ions of dimethylbiguanide (2 ppm error). This drug is prescribed for non-insulindependent diabetes, a cardiovascular risk factor (CRF) that was studied in the NESCaV study. A second PC separated three individuals from all others based on two variables detected at *m/z* 273.127 and 274.13. These ions correspond probably to [M+H]+ ions of sotalol (1 ppm error) and the corresponding 13C isotope peak, respectively. The sotalol is a drug used for life-threatening arrhythmias, another studied CRF in this cross-sectional study. In both cases, our structural hypotheses resulting from HMDB queries were hopefully confirmed by checking metadata. These results demonstrate the efficiency of the DI-HRMS approach as a very promising tool for high throughput epidemiological studies, specially, to correlate analytical results with metadata.

Mots-Clés: Metabolomics, Epidemiology, Metadata, DIMS, HRMS, PCA

Poster 11 – P11

Study of the toxic effect of diphenyl phosphate on the liver metabolism of mice

Jesús Marín-Sáez [1,2], Aurélie Fildier [2], Audrey Buleté [2], Léa Payen [3], Samia Ruby [3], Myriam Abdallah [3], Jessica Garcia [3], Arnaud Vigneron [3], Emmanuelle Vulliet [2]

[1] Department of Chemistry and Physics, Analytical Chemistry Area, University of Almería, Research Centre for Agricultural and Food Biotechnology (BITAL), Agrifood Campus of International Excellence ceiA3, Carretera de Sacramento s/n, E-04120 Almería, Spain – Espagne

[2] Institut des Sciences Analytiques, UMR 5280 CNRS - Université de Lyon 1, 5 rue de la Doua, 69100 Villeurbanne, France – CNRS : UMR5280 – France

[3] Universite de Lyon, Universite Claude Bernard Lyon 1, INSERM 1052, CNRS 5286, Centre Leon Berard, Cancer Research Center of Lyon, Equipe Labellisée Ligue contre le Cancer, Lyon, France – Centre Hospitalier Lyon Sud [CHU - HCL] – France

Diphenyl phosphate (DPP) is one of the main metabolites of triphenyl phosphate (TPP), organophosphorus additives widely used as flame retardant in substitution of polybrominated diphenylethers and polybrominated biphenyls. In term of exposure, the migration of organophosphate flame retardants constitutes a serious concern for the health of the population. As few studies, mainly carried during in vitro experiments, deal with the impact of DPP on human health, a multi-omics in vivo study on rodent model was performed.
Mice of 3 weeks were fed with water at three concentrations of DPP (0.1, 1, 10 mg/mL) during seven weeks, dose chosen through their relevance in term of human exposure. After a solid-liquid extraction, the extract was split in two before to be analyzed by HPLC-HRMS on a C18 and HILIC columns. The extraction was performed by two operators and from eight independent samples for each concentration to obtain a suitable statistical analysis. The analysis were carry out with an UHPLC system (U3000 Thermo ®) coupled to a QToF mass spectrometer (MaxisPlus, Bruker ®). The data were processed using MetaboScape 4.0 and were statistically treated using univariate and supervised statistical tools.

The principal discriminant compounds were fatty acyls (oleic acid, linoleic acid) and their carnitinederivatives involved in their mitochondrial oxidation. Confirming these results, RNAseq (NextSeq™ 500 and HiSeq ® X Systems) performed on separate treated livers unmask the existence of a disturbed genetic network associated with lipids and fatty acid catabolism. Finally, multiple immunohistochemistry analysis demonstrated aberrant expression of main regulators of lipids metabolism and lipid droplets contents in liver exposed to DPP.
The results indicated clearly a disruption of the control pathways of lipid catabolism and bioenergetics, explaining weight alteration of exposed animals. To the best of our knowledge, this is the first time that the effect of DPP is studied in vivo experiments. This work confirms the hypothesis of other studies where other OPEs have disturbed lipid metabolism and transportation. The successful combination of analytical and biological methodologies shows the huge potential of these techniques to evaluate the effect of these and other kind of compounds in the body functioning.

Mots-Clés: diphenyl phosphate, metabolism, HPLC, HRMS, RNAseq

Poster 12 – P12

Glioma biomarker discovery in a Drosophila melanogaster model using NMR spectroscopy

Marion Maravat [1], Martine Decoville [2,3], Marylène Bertrand [3], Celine Landon [3], Séverine Morisset-Lopez [3], Franck Fayon [1], Vincent Sarou-Kanian [1]

[1] Conditions Extrêmes et Matériaux : Haute Température et Irradiation – Université d'Orléans, Centre National de la Recherche Scientifique : UPR3079 – France

[2] Université d'Orléans – Ministère de l'Enseignement Supérieur et de la Recherche Scientifique – 6 Avenue du Parc Floral, 45067 Orléans Cédex 2, France

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

Human malignant glioma is the most common type of primary brain tumor. Composed of glial cells and their precursors, it is aggressive, highly invasive and resistant to standard therapies which leads to a poor prognosis. Despite the mutated pathways responsible for tumorigenesis being identified, targeted therapies have proven mostly ineffective so far. Novel therapeutic approaches are needed to improve patients' life expectancy and comfort. Studying glioma cells metabolism could lead to the identification of new therapeutic targets.

Drosophila melanogaster is a compelling genetic model to study human neurological diseases because of its high conservation in signaling pathways and cellular content of the brain. In this regard, tumor-like proliferation was successfully induced in *Drosophila* by constitutively co-activating the Epidermal Growth Factor Receptor (EGFR) and the Phosphatidyl-Inositol-3 Kinase (PI3K) signaling pathways in glial cells.

Different Nuclear Magnetic Resonance (NMR) spectroscopy techniques were used to obtain metabolic profiles of dissected third instar larval brains. Fresh organs were directly analyzed by High Resolution – Magic Angle Spinning (HR-MAS) and brain extracts were analyzed by solution-state 1H-NMR. Biomarkers were then identified through statistical analyses of the NMR data.

The results of this study not only validate further the *Drosophila melanogaster* model for human glioma but also open the possibility of a better understanding of the biochemical mechanisms of these tumors. The identified metabolites provide the possibility of metabolic therapy by targeting specific pathways but also a mean to follow tumor evolution during pharmacologic treatments.

Mots-Clés: glioma, nmr, biomarker, Drosophila melanogaster

Poster 13 – P13

Etude longitudinale d'une exposition aiguë à uranium par approche métabolomique

Baninia Habchi[1], Stéphane Grison[2], Dimitri Kereselidze [2], Celine Gloaguen [2], Christelle Elie [2], Victor Magneron [2], Jean-Charles Martin [1], Mâamar Souidi [2]

[1] Aix Marseille Université – Aix Marseille Université (AMU), NORT, UMR INSERM 1062, INRA 1260, F13005 Marseille, France – France

[2] Institut de Radioprotection et de Sûreté Nucléaire – Institut de Radioprotection et de Sûreté Nucléaire, PSE-SANTE, SESANE, LRTOX, Fontenay-aux-Roses, F-92260 France – France

Ce travail cherche à mettre en évidence des signatures métabolomiques associées à une contamination aiguë à l'uranium chez le rat dans le cadre d'une étude basée sur les effets des expositions environnementales ou accidentelles aux faibles doses de radionucléides.

Des prélèvements de plasma et urines ont été réalisés périodiquement pendant 9 mois tout au long du protocole expérimental à partir d'animaux issus de quatre groupes de doses d'expositions croissantes (2 non toxiques et 2 toxiques) et d'un groupe témoin. 900 échantillons d'urine et 600 échantillons de plasma ont été analysés par chromatographie liquide couplée à un spectromètre de masse (Q-Exactive Plus) en mode switch positif-négatif sur deux colonnes C18 et HILIC. Un échantillon de contrôle qualité a permis de contrôler la qualité des analyses et corriger les dérives analytiques entre les différents batchs d'analyse. Plusieurs étapes de prétraitements et différents outils d'analyses multivariées ont été appliqués et utilisés.
Dans l'urine, une ACP (Analyse en composante principale) a permis de montrer l'existence d'un effet temporel. L'analyse discriminante PLSDA (Partial Least Square - Discriminant Analysis) a révélé l'existence d'un effet dose entre les témoins et les doses toxiques mais aussi entre les témoins et les doses non toxiques. Des sous modèles PLSDA, ont aussi été réalisés entre les témoins et les doses non toxiques pour rechercher des marqueurs discriminants selon les périodes temporelles. A partir de ces marqueurs, des scores composites ont pu être calculés et validés par courbe ROC (Receiver Operating Characteristic). Ces scores pourraient être utilisés pour élaborer des tests de diagnostic d'exposition. Des étapes de validations des biomarqueurs discriminants sont encore nécessaires, tout comme l'exploitation des données plasmatiques qui pourraient être complémentaires aux résultats obtenus dans l'urine.

Mots-Clés: Métabolomique, Biomarqueurs, uranium, exposition aiguë, HRMS, LC/MS, PLS, DA.

Poster 14 – P14

Leukemic cells develop metabolic reprogramming to adapt their proliferation to nutrient deprivation.

Caroline Lo Presti [1,2], Florence Fauvelle [3,4], Pascal Mossuz [1,2]

[1] Department of biological hematology, CHU Grenoble Alpes – Centre Hospitalier Universitaire [Grenoble], Centre Hospitalier Universitaire [Grenoble] – France

[2] Institute for Advanced Biosciences / Institut pour lÁvancée des Biosciences (Grenoble) – Institut National de la Santé et de la Recherche Médicale : U1209, Centre National de la Recherche Scientifique : UMR5309, Université Grenoble Alpes – France

[3] Grenoble Institut des Neurosciences (GIN) – Université Grenoble Alples, U1216 Inserm – Chemin Fortuné Ferrini Batiment Edmond J Safra F-38700 La Tronche, France

[4] IRMaGe (IRMaGe) – UGA université Grenoble Alpes, Inserm US17, CNRS UMS 3552, Gesellschaft für Schwerionenforschung – Chemin Fortuné Ferrini Batiment Edmond J Safra F-38700 La Tronche, France

Background: Acute Myeloid Leukemia are a set of malignant proliferations leading to an accumulation of blasts in the bone marrow and blood. The prognosis is pejorative due to their molecular complexity and pathways implicated in leukemogenesis. Studying the metabolism of leukemic cells would help to better characterize AML. For that aim, we have used HRMAS-NMR based metabolomics on different leukemic cell lines. This method has the advantage of allowing the analysis of intact cells, with few preparation, which could facilitate the transfer of our results to clinic.

Objectives: The objective was to compare the metabolic profiles of leukemic cells in basal conditions of culture and in deprivation conditions, in order to investigate their behavior under metabolic stress.

Methods: Five human leukemic cell lines, KG1, K562, HEL, HL60 and OCIAML3 were studied. For stress experiments, the cells were cultured without serum and samples for HRMAS NMR were prepared at three time points: 2h, 24h and 48h. The samples were analyzed on a 500MHz Bruker Avance III spectrometer (IRMaGE, CEA Grenoble). MAS spin rate was set at 4000 Hz and sample temperature at 277K. A CPMG pulse sequence was used to minimize lipid contribution. All the spectra were corrected for phase and baseline distortions and the spectral region between 8.5 and 0.5 ppm was divided into buckets with equal width of 0.001 ppm using the NMRprocflow software. Buckets were transferred to the SIMCA R V14.1 software for multivariate statistics (PCA and OPLS-DA), in order to find metabolites that are over or under expressed in the different cell lines and depending on the experimental conditions.

Results: We showed that each cell line has a specific metabolic profile at basal state, characterized by overexpression of a specific metabolite: glutamine for HL60, phosphocholine for KG1, myoinositol for HEL, creatine/phosphocreatine for K562 and aspartate for OCIAML3.

When cultured in serum-free medium, they developed a rapid metabolic adaptation which allows them to continue to proliferate despite the lack of nutrients.

Conclusion: Our results showed that human leukemic cell lines display different metabolic fingerprint in their basal state and develop a common metabolic reprogramming that allowed them to survive under stress conditions.

Mots-Clés: acute myeloid leukemia, metabolomics, HRMAS NMR

Poster 15 – Associé FP6

New insight in Metabolomics based study of Age Related Macular Degeneration (AMD): Lipoprotein profile and subclass analysis.

Matthieu Schoumacher[2] , Vincent Lambert , Justine Leenders [1], Bernadette Govaerts [2], Sylvain Hansen , Jean-Marie Rakic , Agnès Noël , Pascal De Tullio [1]

[1] Centre Indisciplinaire de Recherche sur le Médicament (CIRM) – Institut de Pharmacie Quartier HOPITAL - Av. Hippocrate, 15 (Bât. B36) Sart-Tilman B-4000 Liège, Belgique
[2] Institut de Statistique, Biostatistique et Sciences Actuarielles (ISBA) – Université Catholique de Louvain, Voie du Roman Pays 20, 1348 Louvain-La-Neuve, Belgique

Age-related macular degeneration (AMD) is the leading cause of blindness among the elderly population in developed countries. 90% of all vision loss due to AMD result from the exudative form of this pathology, which is characterized by a choroidal neovascularization (CNV). Currently, diagnosis of AMD relies on ophthalmologic exams and treatments of the exudative form are based on the use of anti-angiogenic drug targeting vascular endothelial growth factors. Despite these advance, several clinical challenges have to be overcome. Among those, the identification of biomarkers that could allow to refine patient stratification, to follow disease progression and evaluate responses to treatment are mandatory.For this purpose, we decide to apply NMRbased metabolomics approach on both AMD patients and on a laser-induced murine choroidal neovascularization experimental model.
In the clinical study, the metabolomics approach does not allow a complete differentiation between control and AMD patients. However, focusing only on AMD group, a clear-cut separation between active and non-active phases could be highlighted.
In the mice model, discrimination between laser-induced and control mice occurs only when CNV is install ed.In both human and animal studies, lactate and lipoprotein profile were identified as the main biomarkers. Mechanistically, we demonstrated that lactate, plays a critical role in the onset of the inflammatory and angiogenic phases and could be correlated with the CNV development. Then, controlling lactate level appears as a new therapeutic approach of AMD.
On the other hand, lipoprotein profile is of particular interest for patient follow-up. Indeed, evaluation of lipoprotein profile change trough a simple methods allowed us to establish clear modification of profiles according to the active or non-active status of the patient and to the induced or non-induced status of the mice.
This work focuses on the lipoprotein profile and on the development of a methodology that could be used to characterize and compared the different profiles in the human and the mice studies.

Mots-Clés: NMR, metabolomics, AMD, Lipoprotein

Poster 16 – Associé FP1

A combined metabolomics and lipidomics approach enables the stratification of acute-on-chronic liver failure patients according to their severity

Noemie Butin [1], **François Fenaille**[1], **Benoit Colsch** [1], **Christophe Junot** [1], **Joan Clària** [2,3], **Richard Moreau** [4,5], **Vicente Arroyo** [5]

[6] CEA Saclay, Laboratoire d'Etude du Métabolisme des Médicaments, MetaboHUB-Paris, DRF/IBITEC-S/SPI, 91191 Gif-sur-Yvette Cedex, France. – CEA, CNRS, Université Paris-Saclay, CEA Saclay 91191 Gif sur Yvette France – France

[7] European Foundation for the Study of Chronic Liver Failure (EF-CLIF), Barcelona, Spain – Travessera de Gràcia, 11, Planta 7ª, 08021 Barcelona, Spain, Espagne

[8] Department of Biochemistry and Molecular Genetics, Hospital Clinic, Institut d'Investigacions Biomèdiques August-Pi-Sunyer (IDIBAPS), University of Barcelona, Barcelona, Spain – Carrer del Rosselló, 149, 08036 Barcelona, Spain, Espagne

[9] INSERM and Université Paris Diderot-Paris 7, Centre de Recherche sur l'Inflammation (CRI), Paris and Clichy, France – Institut National de la Santé et de la Recherche Médicale - INSERM – France

[10] European Foundation for the Study of Chronic Liver Failure (EF-CLIF), Barcelona, Spain – Espagne

Acute-on-Chronic Liver Failure (ACLF) is a recently recognized syndrome characterized by acute decompensation (AD) of cirrhosis, an organ/system failure(s) and extremely poor survival. ACLF can be triggered by a precipitating event (e.g. bacterial infection) and is invariably associated with exacerbated systemic inflammation. According to the European Foundation for the study of Chronic Liver Failure (EF-CLIF), patients with ACLF can be classified into three groups, essentially according to the number of impaired organs. In the present project, we investigated whether metabolomics and lipidomics can identify potential new diagnostic biomarkers of ACLF.

In our study, a cohort of more of 800 serum samples from decompensated cirrhotic patients with and without ACLF were analyzed and compared to healthy subjects by LC-HRMS. Data mining procedures using multivariate and univariate analyses were then performed to highlight discriminant metabolites with a preliminary work on data processing to normalize data and to overcome analytical biases occurring in the analysis of a large biological cohort. Our data confirmed the metabolic and lipidomic cirrhosis signatures obtained in previous studies, especially regarding to the levels of glycerophosphatidylcholines, amino acids and energy metabolites. Furthermore, our approach enabled to discriminate between decompensated cirrhotic patients with ACLF and those without ACLF, and a specific metabolite signature associated with the ACLF grade was obtained. Moreover, we found that Kynurenine pathway is activated in patients with acute decompensation, culminate in patients with ACLF and may be involved in the pathogenesis of ACLF, clinical course and mortality.

Mots-Clés: Metabolomics, ACLF, LC/MS

Poster 17 – Associé FP2

Metabolic characterization of different phases of Parkinson's disease

David Mallet [1], Thibault Duffourd [1], Carole Carcenac [1], Sébastien Carnicella [1], Emmanuel Barbier [1], Sabrina Boulet [1], Florence Fauvelle [1]

[1] Grenoble Institut des Neurosciences – CEA, CHU Grenoble, Inserm : U1216, Université Grenoble Alpes – France

Context: Parkinson's disease (PD) is the second most common neurodegenerative disorder, affecting almost 1% of the population beyond the age of 60. Currently, its diagnosis relies on the expression of the well-known motor symptoms (akinesia, rigidity, and tremor) which appear in the late stage of the disease. Detecting the disease earlier represents a key step to develop curative treatments which are so far only symptomatic. Long considered as a purely motor disease, PD is nevertheless also characterized by neuropsychiatric disorders (apathy, depression, anxiety...) that can develop during the early stages of the disease as well as later on.

Objectives: Our aim is to find specific molecular markers of early phases of PD, when only the neuropsychiatric

symptoms are expressed, using 1HNMR-based metabolomics on serum samples and on brain tissues of a rodent model allowing investigation of different phases of PD.

Methods: The animal model is based on a specific, partial, bilateral 6-OHDA-induced lesion in dopaminergic neurons. For each rat, motor functions and apathetic-like behaviors were assessed using a stepping test and operant sucrose self-administration, respectively.

Both serum and intact tissue samples were analyzed using a CPMG pulse sequence by respectively liquid NMR at 950 MHz (IBS Grenoble) and HRMAS-NMR at 500 MHz (IRMaGE, CEA Grenoble). Spectra were submitted to multivariate statistics (SIMCA-v14) in order to investigate if metabolic profile is correlated to behavioral and histological data.

Results/conclusion: In our animal cohort we observed a gradation in the symptoms, well in line with PD progression, from only neuropsychiatric, to the expression of neuropsychiatric associated with motor symptoms. For more precise evaluation, a score based on behavioral performances and on the striatal dopaminergic denervation was built. Serum and tissue spectra showed a good correlation of this score with the gradation of symptoms at different phases of PD.

In both samples, the energetic pathways seems to be modified with the progression of the disease, while some amino acids, like alanine and serine, are also dysregulated prior to the appearance of motors symptoms. We will further use the same methodology in serum with *De Novo* patients.

Mots-Clés: Parkinson, Biomarkers, liquid 1HNMR, Tissu, HRMAS NMR (High resolution magic angle spinning NMR)

Communication Posters

Session Pharmacologie - Toxicologie

Poster 18 – P18

Metabolomic profiling of polarized human lung macrophages with liquid- and gas-chromatography coupled to mass spectrometry

Fanta Fall [1], Natacha Lenuzza [2], Elodie Lamy [1], Marion Brollo [3], Emmanuel Naline [3,4], Philippe Devillier [3,4], Etienne Thevenot [2], Stanislas Grassin Delyle[1,3]

[1] Plateforme de spectrométrie de masse MasSpecLab, INSERM U1173, UFR Sciences de la Santé Simone Veil – Université Versailles - Saint Quentin en Yvelines – France
[2] CEA, LIST, Laboratory for Data Sciences and Decision, MetaboHUB – CEA – France
[3] Département des maladies des voies respiratoires, Hôpital Foch, Suresnes, France – Hôpital Foch – France
[4] UPRES EA220, UFR Simone Veil - Santé, Université Versailles – Saint Quentin en Yvelines, Université Paris Saclay, Montigny le Bretonneux, France – UPRES EA220 – France

Rationale: Lung macrophages (LM) are in the first line of defense against inhaled pathogens. These cells can undergo phenotypic polarization to the proinflammatory M1 or to the immunomodulatory M2 phenotype. The objective of the present work was to characterize the metabolic alterations occurring during the experimental M1 or M2 LM polarization.

Methods: Human LM were obtained from resected lungs and cultured for 24h in medium alone or with 10 ng/mL LPS (M1 polarization) or 10 ng/mL IL-4 (M2 polarization). Cells and culture supernatants were subjected to extraction for metabolomic analysis with high-resolution LC-MS (HILIC and reverse phase -RP- chromatography in both negative and positive ionization modes) and GC-MS. The data were analyzed with the R software and the Worklow4Metabolomics online infrastructure.

Results: For the intracellular content, 7,527 features were detected in HILIC-MS, 1021 in RPMS and 193 in GC-MS. For supernatants, 1,894 features were detected in HILIC-MS, 2,187 in RP-MS and 274 in GC-MS. 52 features had expression levels significantly altered between experimental conditions, mainly with LPS. Annotation with HMDB suggested a regulation in the expression of metabolites from the eicosanoid (prostaglandin, leukotriene, lipoxin), retinoid, kynunerin and Krebs cycle pathways. Identification with reference standards was successful for several compounds and quantitative methods were developed for culture supernatants. In the presence of LPS, malic acid increased from 568±58 ng/mL to 902±111 ng/mL, quinolinic acid from 40±10 ng/mL to 108±20 ng/mL and kynurenin from 92±13 ng/mL to 280±45 ng/mL whereas tryptophan decreased from 173±22 mg/L to 51±10 mg/L.

Conclusions: Macrophages polarization is accompanied by changes in the cell metabolome. Metabolites differentially expressed are mainly involved in the promotion and regulation of inflammation. In depth analysis will allow a better understanding of the molecular mechanisms underlying LM polarization.

Mots-Clés: Lung macrophages, Proinflammatory, immunomodulators, Metabolomic, HILIC, Reverse phase

Poster 19 – P19

Identification des modulations du lipidome des cellules hépatiques HepaRG exposées à du tétrachloro-bisphénol A (TCBPA) par RMN du proton et du phosphore-31

Cécile Canlet [1,2], Marie Tremblay-Franco [2,1], Justine Bertrand Michel [3], Roselyne Gautier [1,2], Aurélie Batut [3], Laurent Debrauwer [2,1], Nicolas Cabaton [1]

[1] Toxalim, Université de Toulouse, INRA, Toulouse

[2] Plateforme MetaToul-AXIOM, Toulouse – Institut National de la Recherche Agronomique - INRA – France

[3] Inserm U1048, Plateforme MetaToul-LIPIDOMIQUE, Toulouse – Institut National de la Santé et de la Recherche Médicale - INSERM – France

Les bisphénols halogénés suscitent aujourd'hui un questionnement sociétal en toxicologie environnementale et alimentaire. Ces composés présentent une forte affinité pour le récepteur nucléaire PPARg et le tétrabromo-bisphenol A (TBBPA) a un effet avéré sur les phospholipides et la fluidité membranaire. Des études ont montré des altérations des concentrations en lipides plasmatiques et une inflammation au niveau hépatique

L'objectif de ce projet est d'approfondir l'exploration des voies lipidiques modulées par le TCBPA par deux approches complémentaires : la RMN et la spectrométrie de masse (MS), à partir des mêmes extraits de cellules HepaRG exposées au TCBPA, cellules métaboliquement compétentes et très proches d'hépatocytes humains. La RMN du proton permet de quantifier certaines familles de lipides et la RMN du phosphore-31 permet de quantifier de manière absolue les différentes classes de phospholipides (PL). Ces analyses ont été complétées par des approches classiques de lipidomique ciblée afin de comparer les résultats obtenus par les deux techniques et de montrer les avantages et les limites de chaque technique.

Des tests préliminaires ont été effectués afin d'optimiser la quantité de cellules nécessaire, le protocole d'extraction, le solvant de reprise des extraits lipidiques pour les analyses RMN. Les cellules HepaRG ont été ensemencées à un taux de 3 millions de cellules/puits et ont été exposées pendant 24h à différentes concentrations de TCBPA. Les extraits lipidiques ont été analysés par RMN du proton et du phosphore-31, et par MS. Pour les PL, les résultats obtenus en RMN sont similaires à ceux obtenus en MS. Bien que moins sensible que la MS, la RMN permet de quantifier de manière absolue les phospholipides. En revanche, seulement trois familles ont pu être quantifiées en RMN. Les analyses statistiques multivariées de toutes les espèces lipidiques quantifiées en RMN ont montré une perturbation du métabolisme lipidique dans les cellules exposées au TCBPA. Les analyses ciblées par MS des lipides neutres et acides gras totaux sont en cours pour préciser la nature des lipides modulés par l'exposition au TCBPA. Ces résultats préliminaires montrent d'ores et déjà la complémentarité des 2 approches.

Mots-Clés: lipidomique, RMN du proton, RMN du phosphore, bisphénols halogénés

Poster 20 – P20

Untargeted profiling of toxicologically relevant metabolites: case study of reactive aldehydes

Loic Mervant [1,2], Robin Costantino [1,2], Jean-François Martin [1,2], Laurent Debrauwer [1,2], Françoise Guéraud [2], Emilien Jamin [1,2]

[1] Axiom platform, MetaToul-Metabo-HUB, National Infrastructure for Metabolomics and Fluxomics, Toulouse – Institut national de la recherche agronomique (INRA) – France

[2] Toxalim, UMR1331, Université de Toulouse, INRA, ENVT, INP-Purpan, UPS, Toulouse – Institut national de la recherche agronomique (INRA) – France

Human exposure to toxic substances, particularly through diet, is a major societal concern, and the most complete characterization of exposure is required to link food consumption and toxic effect. However, current methods to assess exposure are mainly focused on some families of compounds. The concept of exposomics aims to assess this exposure in a most comprehensive way by the use of untargeted methods.

Here we propose an untargeted UPLC-HRMS method to specifically profile electrophilic metabolites, which are known to be toxic due to their reactivity towards biomolecules such as DNA, proteins... The main route to detoxify these compounds is by conjugation to glutathione, and excretion in urine as mercapturic acids conjugates (MACs) after enzymatic cleavage.

As proof of concept we applied this method in the toxicological context of colorectal cancer promotion linked to the consumption of red meat/heme iron. Heme iron contained in red meat is known to catalyse lipid peroxidation in the intestinal tract, leading to the formation of toxic alkenals. Urines of rats fed various oils with heme iron or free iron supplementation were analysed by UPLC-MS, and untargeted MACs profiling was performed on QC samples by using "all ion MS/MS mode" on a Waters Synapt G2-Si instrument since MACs display a characteristic neutral loss in MS/MS. Then the corresponding MACs signals were measured in all urine samples.

Thus, dozens of MACs could be detected without a priori with this approach, including expected ones (e.g. DHN-MA) as well as unexpected MACs derived from aldehydes or other chemical classes. Interestingly, multivariate statistical analyses carried out only on the MACs yielded a much better segregation of groups compared to results obtained from a classic untargeted metabolomic approach. Therefore, our approach not only allowed to highlight metabolites of lipid peroxidation, but also opens the way to the untargeted detection of toxicologically relevant compounds.

Mots-Clés: toxicology, reactive metabolites, mass spectrometry

Poster 21 – P21

Variation du phénotype métabolique des mousses forestières liée à l'exposition de dépôts métalliques

Alain Paris[1], Truong Anh Nguyen , Arul Marie [2], Caroline Meyer , Sébastien Leblond [3]

[1] UMR 7245 - Molécules de Communication et Adaptation des Micro-organismes (MCAM) – Museum National d'Histoire Naturelle, Centre National de la Recherche Scientifique – France

[2] Molécules de Communication et Adaptation des Micro-Organismes (MCAM) – CNRS : UMR7245 – 57 rue Cuvier 75005 Paris, France

[3] MNHN – Museum National d'Histoire Naturelle – Rue Buffon, 75005 Paris, France

La pollution atmosphérique par des éléments métalliques représente une menace pour la santé publique dans les zones urbaines et une cause majeure de contamination des écosystèmes terrestres. Ces dernières décennies, les émissions métalliques d'origine anthropique (industries, transports routiers, etc.) ont dominé les émissions naturelles. Les métaux émis dans l'atmosphère et transportés sur de longues distances se déposent au niveau des sols et des écosystèmes. Cependant, les effets chroniques toxiques sur la biosphère restent peu documentés.

L'évaluation métabolomique de l'impact des dépôts en métaux d'origine atmosphérique sur le métabolisme des mousses forestières a consisté en une analyse des corrélations canoniques pouvant exister entre les concentrations en métaux et métalloïdes mesurées par ICP-MS, d'une part, et les variations d'analytes détectés dans le métabolome d'extraits hydrosolubles de pièces végétales, brutes ou réduites en poudre au préalable, qui est établi par FIA-ESI-Q-ToF-MS en mode positif ou négatif, d'autre part. Trois espèces de mousse (*Pseusdoscleropodium purum, Hypnum cupressiforme, Thuidium tamariscinum*) ont été prélevées au printemps 2016 sur 550 forestiers en France dans le cadre du dispositif de Biosurveillance des Retombées Atmosphériques des Métaux par les Mousses (BRAMM), inclus dans le volet européen de l'International Cooperative Programme – Vegetation. Dans cette pré-étude, 32 sites ont été choisis de façon à représenter les variations les plus contrastées en dépôts en métaux lourds (Cd, Pb, Hg) mesurées sur ces espèces sur tout le territoire.

L'approche métabolomique utilisée a permis de montrer qu'à partir des variables métaboliques détectées (entre 50 et 200 selon le mode d'ionisation et la matrice utilisés), une corrélation globale entre la variation des teneurs en Cd, Zn et Hg et celle en certains métabolites était établie significativement. Les trois espèces de mousse adoptent un comportement homéostatique similaire en réponse à la contamination chronique par ces métaux. L'identification de quelques marqueurs métaboliques a été tentée grâce à la base de données métaboliques Metlin.

Mots-Clés: pollution atmosphérique, métaux, bryophyte, FIA, ESI, Q, ToF, MS, analyse canonique

Poster 22 – P22

Central and peripheral metabolomics in a model of depression: biosignatures of drug responder and non-responder mice

Sylvie Mavel[1], Antoine Lefèvre[2], Marc Legrand[1], Laurent Galineau[1], Lydie Nadal-Desbarats[1,2], Catherine Belzung[1], Patrick Emond[1,2]

[1] UMR 1253, iBrain, Université de Tours, Inserm, Tours, France
[2] Université François-Rabelais, PPF Analyse des Systèmes Biologiques (ASB), 10 Bv Tonnellé, 37044 Tours, France

Major depressive disorder is a heterogeneous disorder with a wide spectrum of symptoms leading to disability, suicide and physical disorders. Selective serotonin reuptake inhibitors (SSRIs) are ones of the most commonly used drugs of treatment of depression. For unknown reasons, a great number of patients do not show any improvement during drug treatment (30-40%). The underlying molecular mechanisms of depression remain unknown. To improve knowledge of pathophysiology and to search biomarkers to help prognostic of antidepressant treatment response in patient, we employed a LC-HRMS metabolomics approach in a BALB/c induced mouse model of depression to investigate metabolic changes in 6 specific brain regions, after 5 weeks with a fluoxetine treatment. From 770 cumulated targeted metabolites, 68 discriminant Variable Importance in Projection (VIP) were shown to discriminate vehicle-treated group from drug responder and non-responder mice, after OPLS-DA. This central metabolomics approach is completed by a peripheral analysis through plasma analysis. A less robust OPS-DA model was obtained, but 27 discriminant metabolites (over 197 robustly analyzed) were altered in plasma. Comparison of central VIP with peripheral VIP leads to 7 common metabolites. This study shows the complementary of these two compartments, i.e. cerebral and plasma metabolome.

From this, specific brain regions shown particular metabolism which could explain some aspects of the depressive pathophysiology and some biomarkers could be proposed to anticipate antidepressant treatment response.

Communication Posters

Session Alimentation

Poster 23 – P23

De bonnes habitudes alimentaires: impact sur le profil métabolique d'échantillons de sérum analysés par RMN du proton

Tony Palama[1], Marinos Fysekidis [2,3], Nadia Bouchemal [1], Mohamed Triba [1], Philippe Savarin [1]

[1] Laboratoire Chimie, Structures, Propriétés de Biomatériaux et d'Agents Thérapeutiques (CSPBAT) – Université Paris 13, Sorbonne Paris Cité - CNRS (France) : UMR7244 – France

[2] Service d'endocrinologie diabétologie, nutrition (Hôpital Jean Verdier) – Assistance publique Hôpitaux de Paris (AP-HP), université Paris 13, Hôpital Jean Verdier – France

[3] Equipe de Recherche en Epidémiologie Nutritionnelle (EREN) – Université Paris 13, Sorbonne Paris Cité,, Inserm (U1153), Inra (U1125), Centre d'Epidémiologie et Statistiques Paris Cité, CNAM, COMMUE Sorbonne-Paris-Cité, F-93017 Bobigny – France

Les mécanismes métaboliques en lien avec la nutrition-santé restent peu explorés jusqu'ici. Leur élucidation est cruciale pour mieux comprendre la survenue des maladies et améliorer les stratégies de prévention. Le but de l'étude Nutrivasc réalisée par l'équipe de recherche en épidémiologie nutritionnelle (EREN) était d'étudier les relations entre la qualité nutritionnelle de l'alimentation évaluée par les recommandations du Programme National Nutrition Santé (PNNS) et des facteurs de risques cardiovasculaires. Nous avons utilisé des échantillons de sérum collectés lors de cette étude pour en faire l'analyse du métabolome. L'utilisation de la métabolomique dans le domaine de l'épidémiologie est une approche récente et alternative permettant d'explorer ces relations afin de découvrir de nouveaux biomarqueurs.Objectifs: 1/Comparer les profils métaboliques RMN des sérums des individus ayant un mauvais comportement alimentaire (score bas PNNS) de ceux qui respectent les recommandations nutritionnelles (score PNNS élevé). 2/Identifier les biomarqueurs durant la période postprandiale dans les deux groupes. Matériels et méthodes : 49 individus appartenant au 1er et 4ème quartile des scores PNNS ont été sélectionnés. Lors de l'étude, des prélèvements sanguins ont été réalisés à jeun (T0), puis 1, 2, et 3 heures (T+1h, T+2h et T+3h) après la prise d'un petit déjeuner standardisé. Les échantillons ont été analysés par résonance magnétique nucléaire (RMN) du proton 1H. Les spectres acquis sur un spectromètre RMN Bruker à 500 MHz en utilisant la séquence CPMG ont été soumis à des analyses statistiques multivariées (ACP, OPLS). Résultats : Les métabolites responsables de la principale source de variabilité ont été identifiés. En prenant en compte l'ensemble de la population aucune discrimination n'a été possible entre le 1er et le 4ème quartile par ACP et OPLS. Cependant une discrimination des échantillons, suivant le temps de prélèvement, a été observée. Les métabolites discriminants ont été identifiés par comparaison avec la base de donnée HMDB (www.hmdb.ca) et l'analyse des spectres RMN 2D (J-resolved, TOSCY, HSQC).

Mots clefs : métabolomique clinique, RMN, analyse multivariée, serum

Poster 24 – P24

Modelling hepatic metabolic changes during the onset of obesity using NMR metabolomics arterio-venous blood exploration in minipigs

Nathalie Poupin [1], **Marie Tremblay-Franco** [1,2], **Aurélien Amiel** [1,2], **Cécile Canlet** [1,2], **Didier Rémond** [3], **Laurent Debrauwer** [1,2], **Dominique Dardevet** [3], **Fabien Jourdan** [1], **Isabelle Savary-Auzeloux** [3], **Sergio Polakof** [3]

[1] UMR 1331 Toxalim - INRA-ENVT-INP-EIP-UPS – Institut national de la recherche agronomique [Toulouse] – France
[2] Metatoul Axiom platform - Metabohub national Infrastructure INRA [Toulouse] – France
[3] INRA UMR1019 Unité de Nutrition Humaine – Université Clermont Auvergne, INRA, UNH – France

Phenotypic alterations associated with obesity are well known, but metabolic adaptations occurring during its onset are poorly characterized. This study aims at understanding the hepatic metabolic processes modulated during the onset of obesity by combining paired arterial and venous metabolome analyses with computational modeling. It relies on the principle that differences in the metabolite composition of blood flowing in and out of the liver reflect its metabolic functioning. Blood samples were collected in a mini-pig model of obesity [1], consisting in over nutrition with a high-fat-high-sucrose (HFHS diet) for 60 days. Blood was sampled at the fasting state in 5 catheterized mini-pigs from incoming (abdominal artery and portal vein) and outgoing hepatic vessels at days 0 and 60 of HFHS feeding. From 1H-NMR analyses, we identified metabolites with significantly changed levels between arterious and venous blood. Calculated 1H-NMR integration ratios between arterious and venous blood allowed us to identify the metabolites with significant positive (resp. negative) balance between inflow and outflow, reflecting a release (resp. uptake) by the liver. These data were analyzed within the frame of an hepatic genome-scale metabolic network model using constraint-based modeling approaches to predict the changes in intra-tissular metabolic fluxes [2]. Constraints were set on exchange reactions in the metabolic model to enforce uptake and release of metabolites in accordance to experimental data and *in silico* flux balance analysis methods were used to predict possible flux ranges for hepatic metabolic reactions. We predicted that HFHS was associated with changes in the glucose metabolism and the sources of gluconeogenesis, but also in the catabolism of tryptophan and lysine, for which further support was found through complementary biochemical and molecular analyses.

Polakof S, *et al*. Metabolic adaptations to HFHS overfeeding: how whole body and tissues postprandial metabolic flexibility adapt in Yucatan mini-pigs. *Eur J Nutr.* 2018;57(1):119-35.

Mots-Clés: arterio venous differences, metabolic networks, constraint, based modelling, obesity, mini, pig

Poster 25 – P25

Metabolomic and lipidomic analysis of serum and liver samples following Curcuma longa extract supplementation in high-fructose and saturated fat fed rats

Fabrice Tranchida [1], Laetitia Shintu [1], Zo Rakotoniaina [1], Léopold Tchiakpe [2], Mehdi Yemloul [1], Valérie Deyris [1], Pierre Stocker [3], Nicolas Vidal [3], Abel Hiol [4], Stefano Caldarelli [1]

[1] Institut Sciences Moléculaires de Marseille UMR 7313 – Aix-Marseille Université - AMU – France
[2] Laboratoire de Nutrition-Dietétique, Faculté de Pharmacie, Marseille – Aix-Marseille Université AMU – France
[3] Institut de Chimie Radicalaire UMR 7273 – Aix-Marseille Université - AMU – France [4] CIRAD UMR QualiSud – Université de la Réunion – France

Fructose consumption from corn syrup, a common sweetener used in the food industry, has increased dramatically over the past few decades in industrialized countries, and its impact on health has been recently reviewed. Similarly, the intake of saturated fats has risen during, the same time period. It has been reported that the current high dietary intake of fructose and saturated fats (HFS) contributes to the epidemic of insulin resistance (IR). Native to Southeast Asia, Curcuma longa (CL) also known as turmeric is one of the most studied natural products. A metabolomics approach based on NMR spectroscopy and GC-MS was performed to highlight metabolic variations of serum and liver tissues of rats fed either a standard diet, a HFS diet, or a HFS diet with the addition of a Curcuma longa extract at a dose nutritionally relevant with common human use (1% in curcuminoids) for ten weeks. Orthogonal projections to latent structures discriminant analysis (OPLS-DA) on the NMR profiles and fatty acid composition (determined by GC/MS) showed a clear discrimination between HFS groups and controls involving metabolites such as glucose, glycogen, amino acids, acetate, choline, pyruvate, creatine, phosphocholine/glycerophosphocholine, lysophosphatidylcholine, phosphatidylethanolamine, and βhydroxybutyrate, VLDL as well as an increase of MUFAs and a decrease of n-6 and n-3 PUFAs. Although the administration of CL did not counteract the IR induced by the HFS diet, discriminating metabolites were observed between groups fed HFS alone or with addition of a Curcuma longa extract, namely some n-6 PUFA, n-3 PUFA, betaine and glycoproteins, glutamine, methanol respectively in liver and serum samples. CL extract addition in rats appears to increase some of the natural defences preventing the development of fatty liver by acting on the choline metabolism (betaine was found to rise by 35%) to increase fat export from the liver. Whereas in serum, a depression of the alcohol oxidation, detected through an enhanced concentration of methanol, and a possible activation of the hexosamine biosynthesis pathway, consistent with a reduction of glutamine and glycoprotein levels were observed. All of these points to possible specific and local, rather than systemic, protective effects of Curcuma longa extract.

Mots-Clés: Fructose, Saturated fatty acid, Curcuma longa, Metabolomics, NMR, Serum, Liver

Poster 26 – P26

Evaluation de l'approche non-ciblée pour l'analyse des biotoxines marines dans les coquillages via la plateforme Galaxy w4m

Sophie Mompelat [1], Inès Dom , Vincent Hort , Gwenaëlle Lavison-Bompard , Marina Nicolas , Thierry Guerin , Dominique Hurtaud-Pessel , Ronel Bire

[1] Laboratoire de Fougères, Laboratoire National et Européen de Référence pour les Résidus de Médicaments Vétérinaires dans les Denrées Alimentaires d'Origine Animale (ANSES) – Anses – 10B rue Claude Bourgelat, 35306 FOUGERES, FRANCE, France

Les biotoxines marines sont des composés toxiques produits par des microalgues. En raison du risque avéré qu'elles représentent pour le consommateur, leur présence dans les coquillages est réglementée et surveillée. La tendance actuelle est à l'abandon des tests biologiques au profit de méthodes chimiques pour des raisons éthiques notamment. Depuis 2010, la chromatographie liquide couplée à la spectrométrie de masse en tandem (LC-MS/MS) est la méthode officielle d'analyse des biotoxines lipophiles. Si cette méthode s'applique parfaitement aux toxines ciblées par la règlementation, elle ne permet pas de mettre en évidence la présence de toxines inconnues ou émergentes.

Cette étude vise donc à évaluer la pertinence et l'apport pour le contrôle sanitaire de nouvelles approches récentes d'analyses dites non-ciblées afin de mettre en évidence la présence de toxines émergentes. La stratégie analytique développée repose sur la comparaison d'empreintes biologiques obtenues via le criblage non ciblé (mode full-scan) par spectrométrie de masse à haut pouvoir résolutif (HRMS, QTOF) afin de discriminer des groupes de coquillages témoins de groupes de coquillages contaminés par des biotoxines. Cet essai de preuve de concept a été réalisé sur quatre lots différents de trois types de coquillages (moules, huîtres et coquilles StJacques) supplémentés avec un mélange de quatre toxines connues (13-déméthyl spirolide C, azaspiracide 1, gymnodimine A et pinnatoxine A) à trois niveaux de concentrations. Le workflow appliqué à partir des outils de la plateforme Galaxy Workflow4metabolomics et recouvrant les étapes principales d'analyse de données allant du prétraitement des signaux HRMS jusqu'à l'annotation seront décrits. Les résultats démontrent que cette approche semble constituer une alternative envisageable pour discriminer des coquillages contaminés de coquillages propres à la consommation au regard des biotoxines. Les limites ainsi que l'évaluation de la robustesse de cette approche à considérer seront abordées.

Mots-Clés: biotoxines marines, contrôle sanitaire, analyse non ciblée, LC, MS/MS, HRMS

Poster 27 – P27

Hepatic metabolomics and transcriptomics provide valuable insights on inflammatory and metabolic features in rats fed on a high fat diet but showing similar clinical phenotyping alterations

Sergio Polakof [1], Marie Tremblay-Franco [2], Cécile Canlet [2], Jérémie David [1], Fabien Jourdan [3], Nicolas Cabaton [3], Nathalie Poupin [3], Blandine Comte [1], Daniel Zalko [3]

[1] INRA UMR1019 Unité de Nutrition Humaine – Université Clermont Auvergne, INRA, UNH – France
[2] Plateforme MetaToul-AXIOM, Toulouse – Institut National de la Recherche Agronomique - INRA – France
[3] INRA Toxalim - Université de Toulouse – Institut national de la recherche agronomique [Toulouse] : UMR1331 – France

The complex processes of obesity development involve genetic and environmental factors. Genetic factors are considered to account for only a small fraction of the overall susceptibility to high fat (HF) induced obesity. Under certain conditions, foetal environment can trigger an adaptive response. This involves the further onset of insulin resistance to optimize the development of key organs (brain, heart...), to the detriment of others organs such as the liver. We studied the impact of a perinatal exposure to a HF diet on rats liver metabolome and transcriptome. Pregnant rats were fed with a normal fat (NF) or a HF diet during gestation and lactation. After weaning, males from the F1 generation were fed either the NF or the HF diet, until PND142, resulting in 4 groups depending on perinatal and post-weaning diet, respectively (NFxNF, NFxHF, HFxNF and HFxHF). H-NMR spectroscopy and mRNA (TLDA) analyses were performed on male livers extracts.

Clinical phenotyping were different according to post-weaning diet but not according to maternal diet. The multivariate OPLSDA models discriminated F1-animals based on F1 diet (NFxNF and HFxNF *vs* NFxHF and HFxHF). When dams were fed on a standard diet (NFxNF *vs* NFxHF)

HF-fed F1 animals showed an increased expression of genes participating to hepatic inflammation and a reduction in oxidative stress protectors (lower glutathione in the metabolome). In contrast, feeding dams and pups with a HF diet (HFxNF *vs* HFxHF) resulted in major changes at the metabolic level, including a reduced expression of lipogenic genes and several actors involved in branched-chain amino acid metabolism. The latter result was further supported by the increased hepatic levels of leucine (metabolome). The most extreme scenario compared to the control one (NFxNF *vs.* HFxHF) resulted in a more deleterious phenotype, which is the consequence of the combination of the inflammatory and metabolic features described above.
In conclusion, despite the fact that HF feeding at the F1 level induced similar changes in clinical phenotyping, metabolomics and transcriptomics analyses at the liver level were able to discriminate between animals, providing valuable information on the underlying inflammatory and metabolic processes specifically signing the consequence of different maternal feeding.

Mots-Clés: liver, metabolomics, transcriptomics, high fat, metabolism, maternal feeding, obesity

Poster 28 – Associé FP9

Syringol metabolites as biomarkers of smoked meat intake

Roland Wedekind[1], Pekka Keski-Rahkonen [1], Vivien Viallon [1], Pietro Ferrari [1], Erwan Engel [2], Marc Gunter [1], Inge Huybrechts [1], Augustin Scalbert[1]

[3] Centre International de Recherche contre le Cancer - International Agency for Research on Cancer – Organisation Mondiale de la Santé / World Health Organization Office – France

[4] UR 370 Qualité des Produits Animaux, Equipe Micro-contaminants Arôme et Sciences Séparatives (MASS) (QuaPA, MASS) – Institut National de la Recherche Agronomique - INRA (FRANCE) – INRA Centre de recherches Auvergne-Rhône-Alpes Theix 63122 Saint-Genès-Champanelle, France

Background: Intake of processed meat has been associated with an increased risk of colorectal cancer but the estimation of intake of this heterogeneous food group in epidemiological studies is still challenging because of the lack of sufficient details in dietary questionnaires. Objective: The objective of this work is to identify in a dietary intervention study novel biomarkers for processed meat intake.

Design: A metabolomic approach based on high-resolution mass spectrometry was applied to 21 processed meat products previously digested *in vitro and* a randomized cross-over dietary intervention in which 12 volunteers consumed successively 3 processed meat products (bacon, salami, hot dog) and two other foods used as controls during 3 days. Identified biomarkers were tested for replication in 474 subjects from the European Prospective Investigation into Cancer and Nutrition (EPIC) cross-sectional study for which a detailed 24h dietary recall and food frequency questionnaires were available.

Results: Syringol and four derivatives of syringol were found to be characteristic of digests of smoked meat products. The same compounds present as sulfate esters in urine showed increased levels at 2 and 12 hours following consumption of smoked meat products (hot dog, bacon) in the intervention study. The same syringol sulfates also showed increased urinary excretion in participants of the EPIC cross-sectional study reporting recent or habitual consumption of smoked meat products. These markers showed good ability to predict smoked meat intake with receiver operator characteristic areas under the curve ranging from 0.78 to 0.86 and 0.74 to 0.79 for acute and habitual intake respectively.

Conclusions: The biomarkers of smoked meat intake identified in the present study may improve assessment of smoked meat intake in epidemiological studies.

Mots-Clés: Smoked meat, biomarker, metabolomics

Poster 29 – Associé FP3

Impaired postprandial skeletal muscle metabolism in a minipig model of insulin resistance: insights from arteriovenous and biopsy-based metabolomics analyses

Marie Tremblay-Franco [1,2], Nathalie Poupin [1], Florence Castelli , Jérémie David , Marianne Jarzaguet , Didier Rémond , François Fenaille [3], Dominique Dardevet [4], Sergio Polakof [4]

[5] UMR 1331 Toxalim - INRA-ENVT-INP-EIP-UPS – INRA [Toulouse] – France
[6] Metatoul Axiom platform - Metabohub national Infrastructure – Toulouse – France
[7] CEA-INRA UMR 0496/DRF/Institut Joliot/SPI/LEMM, Université Paris Saclay, MetaboHUB – CEA – Centre d'Etudes Nucléaires de Saclay, 91191 Gif-sur-Yvette, France, France
[8] INRA UMR1019 Unité de Nutrition Humaine – Université Clermont Auvergne, INRA, UNH – France

The postprandial phase (PP) represents one of the most challenging phenomena in wholebody metabolism, as the metabolism must adapt to major changes in blood composition following meal intake. This capacity of adaptation is strongly dependent on the metabolic flexibility of the individual, which is compromised in insulin resistant (IR) conditions, particularly at the skeletal muscle level (1). We applied an untargeted LC/MS-based metabolomics approach to skeletal muscle biopsies obtained before (0) and 1.5, 3 and 6 h after a regular meal in a minipig (dexamethasone treated) model of IR (2). Biopsies analyses were complemented by the assessment of the incoming (arterial) and outgoing (venous) blood metabolomes. As the consequence of the anabolic effect of the meal, in healthy animals we observed increased PP muscle levels of metabolites related to glucose (lactate, glucose-1,6biP, lactoylglutathione), energy (citrate, malate), amino acids (AA; methionine, glutamate, aspartate) and nucleotide (GTP, inosine, guanosine) metabolisms. In contrast, these metabolites showed a blunted PP profile in IR minipigs, which illustrates the loss of response to the meal induced by such condition. These include the inability to uptake and use glucose through glycolysis, stimulate protein synthesis due to the lack of AA entry into the muscle and net loss of other nitrogen resources (nucleotides). For some of these metabolites (lactate, AA) the observed changes in the biopsies were in line with the arteriovenous differences across the muscle, which confirms that they were actively uptaken, while for other (nucleotides) the altered profiles in the muscle were the consequence of intramuscular metabolic changes. As a whole, our high-throughput arteriovenous metabolomics approach across the muscle allowed us to further explore the PP metabolic changes observed in the muscle biopsies from IR minipigs. This strategy could be therefore useful to determine in the systemic circulation metabolites (potential biomarkers) able to sign the IR condition without having access to the skeletal muscle biopsies.

(3) Galgani et al. Am J Physiol. 2008;295(5):E1009-17.
(4) Revel et al. PLoS ONE. 2017;12(10):e0186204.

Mots-Clés: insulin resistance, human nutrition, minipig, arteriovenous metabolome, biopsy, postprandial

Poster 30 – P30

Characterization of GMO or glyphosate effects on the composition of maize grain and maize-based diet for rat feeding

Stéphane Bernillon [1,2], Mickaël Maucourt [1,2], Sylvain Chéreau [3], Catherine Deborde [1,2], Daniel Jacob [1,2], Nathalie Priymenko [4], Bérengère Laporte [4], Xavier Coumoul [5], Bernard Salles [4], Rogowsky Peter [6], Florence Forget [3], Annick Moing [1,2]

[1] UMR 1332 Biologie du Fruit et Pathologie INRA : UMR1332, Universtité de Bordeaux – France
[2] Plateforme Métabolome Bordeaux (PMB-MetaboHUB) – INRA – France
[3] UR1264 MycSA INRA Bordeaux Nouvelle Aquitaine –INRA (FRANCE) : UR1264 MycSA – France
[4] Toxalim (Research Centre in Food Toxicology), Université de Toulouse, INRA, ENVT, INP-Purpan, UPS, Toulouse –INRA
[5] Université Paris Descartes - Paris 5 (UPD5) – Institut National de la Santé et de la Recherche Médicale - INSERM – 12, rue de l´cole de Médecine - 75270 Paris cedex 06, France
[6] Laboratoire Reproduction et Développement des Plantes – Université Claude Bernard et ENS Lyon – France

The yearly production of maize (*Zea mays* L.) grains is largely used for animal feed and human nutrition. In addition to classical breeding methods to improve yield, stress tolerance or quality traits, additional traits of agronomic interest were created by the insertion of foreign genes leading to genetically modified organisms (GMOs). Controversial studies about the potential impact of maize and other crop GMOs on human health and the environment have been published [1-3]. Studies about the effect of a given transgene on maize grain or leaf biochemical composition are not always confirmed and frequently result in contradictory conclusions [4-6] such as those related with herbicide tolerant maize.

To overcome these conflicting results, our objective was to combine NMR- and MS-based untargeted metabolomics analyses to assess possible compositional changes in maize grains induced by two bacterial gene-insertion events: a *Bacillus* toxin gene providing insect resistance, and a glyphosate-insensitive enzyme providing herbicide tolerance. Composition of GMO grains was compared with that of their closest non-GMO counterpart, each genotype pair cultivated in a given environmental condition. Untargeted metabolomic approaches were used to identify metabolite signatures and/or metabolites associated with the GMO event or with the glyphosate application in the case of the herbicide-tolerance event. We also checked if the changes observed in the grains were still visible in the feed containing 11 or 33% grain ingredient of GMO maize. This work on grain and pellet composition contributes to characterize the material used further in a rat feeding experiment [7] aiming at the detection of exposure or health effect biomarkers able to differentiate GMO and non-GMO or glyphosate-treated and control diets [8].

Mots-Clés: Maize composition, GMO, rat diet, Glyphosate

Poster 31 – P31

LABERCA ANALYTICAL CHEMISTRY PLATFORM: METABOLOMIC AND STRUCTURAL ANALYSIS

Anne-Lise Royer [1], Yann Guitton [1], Sadia Ouzia [1], Lauriane Rambaud [1], Karinne Pouponneau [1], Fabrice Monteau [1], Bruno Le Bizec [1]

[1] LABERCA, Oniris, INRA, Université Bretagne-Loire, 44307, Nantes, France – Institut national de la recherche agronomique (INRA) : UMR1329, Ecole Nationale Vétérinaire de l'Alimentation et de l'Agroalimentaire, Nantes Atlantique - ONIRIS, route de Gachet, CS 40706, 44307 Nantes Cedex 03, France – France

Metabolomics is an area of increased interest in many scientific fields such as chemical food safety and toxicology in order to investigate and identify (bio)markers of exposure, effect and/or susceptibility. However, the different parts of a metabolomic workflow are sources of many issues. Biological samples contain hundreds or even thousands of metabolites with different physicochemical properties limiting the analysis of the entire metabolome using a unique analytical technique. Therefore, the use of a multi-analytical platform in combination with appropriated sample preparation is required to enlarge the number of metabolites detected. Since 2007, our analytical chemistry platform is engaged in the development of tools and methods for untargeted metabolomics 1. Through the years we've faced the different challenges of untargeted metabolomics studies (from experimental design to statistical analysis). Along the years we've worked with several matrices and developed dedicated sample preparation procedure 2,3. Our expertise also covers bioinformatics tools development through the workflow4metabolomics.org infrastructure 4 and more targeted tools for halogen (Cl, Br) compound retrieval 5. In 2017, our platform work was pushed a step further with the ISO17025 accreditation of an LC-HRMS methods derived from a metabolomics model dedicated to the detection of β-agonist administration in bovines that has been developed and fully validated. allowing for the very first official implementation of a metabolomics based strategy within French National Monitoring Plans 6.

Thanks to its environment, our platform has access to new technologies (IMS, APGC, ASAP, REIMS...) and has been able to investigate them in untargeted metabolomics studies 7–9.

Mots-Clés: Metabolomique, Accreditation, plate, forme

Poster 32 – P32

Linking cocoa polyphenol composition to chocolate quality with average mass spectra fingerprints

Véronique Cheynier [1], Noémie Fayeulle , Emmanuelle Meudec [1], Jean-Claude Boulet [2], Clotilde Hue , Renaud Boulanger , Nicolas Sommerer [1]

[1] INRA - UMR1083 SPO - Plateforme d'analyse des polyphénols (INRA - PFP) – Institut National de la Recherche Agronomique - INRA – UMR1083 SPO 2 place Viala 34060 Montpellier, France

[2] INRA, UMR Sciences pour l'Oenologie (INRA, UMR SPO) – INRA : UMR1083 – 2 Place Viala 34060 Montpellier cedex 2, France

Chocolate quality is largely due to the presence of polyphenols, and especially flavan-3-ols that are present in large amounts in cocoa beans and contribute bitterness and astringency to the final product.
Prediction of chocolate quality from cocoa composition would be of great help for chocolate makers who currently base their sourcing on a time consuming and costly procedure involving sensory analysis of chocolates made using a standardized process.

The aim of the present work was to investigate the potential of polyphenol-fingerprints for discrimination of cocoa samples and their attribution to chocolate sensory groups.

Sixty cocoa bean samples have been processed into chocolates and attributed to four different sensory groups by sensory analysis. Cocoa polyphenol extracts have been prepared from the ground cocoa beans and analyzed by UHPLC-ESI-IT-MS. Mass spectra recorded on the whole UHPLC profile provided cocoa polyphenol fingerprints which were processed with chemometrics (PCA, PLS-DA) to select the most meaningful molecules for discrimination of the chocolate sensory groups.

Sixteen samples representative of the four sensory groups were used for selection of the most discriminant variables. Non supervised PCA analysis of the average mass spectra showed that IT-MS is sensitive and quantitative enough to discriminate the sensory groups without prior selection of target compounds, *i.e* polyphenolic markers. A supervised chemometrics treatment, PLS-DA, was applied to this data to select the most relevant molecules for the discrimination. A larger set of 44 cocoa samples was used to validate the results. The fingerprinting method proved to be quick and efficient and the chemometrics analysis highlighted 32 mass signals of known and unknown molecules that were finally targeted [1], enabling sensory-group discrimination. Most of these signals were attributed to flavan-3-ols, including 2 newly described ethyl-bridged flavan-3-ols arising from condensation of flavan-3-ols with acetaldehyde [2].

Mots-Clés: cocoa, chocolate, polyphenols, fingerprinting, mass spectrometry, chemometrics

Poster 33 – P33

Etude par spectroscopie 1H-NMR du profil métabolique du liquide allantoïque au cours du développement embryonnaire de deux lignées de poulets divergentes pHu- et pHu+.

Lydie Nadal-Desbarats[1,2], Angélique Petit [3], Sophie Réhault-Godbert [3], Cécile Martias [1], Frédéric Montigny [2], Elisabeth Le Bihan-Duval [3], Sonia Metayer-Coustard [3]

[1] UMR 1253 iBrain, Université de Tours, Inserm, F-37000 Tours, France – Université de Tours – France
[2] PST-ASB, Université de Tours, F-37000 Tours, France – Université de Tours – France
[3] UMR BOA, INRA, Université de Tours, F-37380 Nouzilly, France –INRA – France

La sélection des souches de poulet de chair a porté sur l'augmentation de la croissance et du rendement en viande. Ceci conduit à un amenuisement des réserves énergétiques musculaires. Ce caractère, héritable, détermine génétiquement le niveau du pH ultime (pHu) de la viande, pour lequel deux lignées divergentes (pHu+ et pHu-) ont été sélectionnées. L'objectif de cette étude est d'identifier des biomarqueurs précoces du statut énergétique des animaux *via* la caractérisation du liquide allantoïque (LC) des lignées divergentes pHu- et pHu+ par 1H-NMR à 10, 14 et 17 jours de développement embryonnaire.

Les LC ont été prélevés,centrifugés, ultrafiltrés puis conservés à -80◦ C. Les échantillons ont été ajoutés à un tampon phosphate deutéré contenant du TSP. Les spectres 1H ont été réalisés sur un spectromètre Bruker 600MHz. Les intensités spectrales sont normalisées à l'aire de la référence pour une analyse statistique multivariée (SIMCA-P+, Umetrics, Umea, Sweden).

L'analyse conjointe des 3 stades de développement montre des profils spectraux très différents (PLS-DA, R^2Y=0,9, Q^2=0,84, X=26) à E10, E14, ou E17. Si le facteur temps de développement apparaît plus discriminant que le facteur " lignée ", les 2 souches se distinguent en fonction de leur génotype au stade E14 (PLS-DA, R^2Y=0,65, Q^2=0,6, X=19, CV-Anova p=0,003) mais pas aux autres stades. A E14, les principaux métabolites discriminants sont l'hypoxanthine (p=0,001), la xanthosine (p=7,12E-5), la 3-HOKynurenine (p=0,005), ainsi que certains acides aminés (tels que glutamate, valine, lysine, glycine, histidine).
Cette étude illustre le changement de composition du liquide allantoïque au cours du développement embryonnaire pendant lequel les sources de nutriment évoluent. C'est à E14, âge auquel les embryons changent de source de nutriments, qu'une signature métabolique différente est mise en évidence entre les 2 lignées. Ceci suggère des mécanismes d'utilisation métabolique différents en fonction de la génétique de l'embryon et ouvre des perspectives d'une détection précoce, *in ovo*, du statut énergétique de l'animal.

Mots-Clés: metabolomics, oeuf, SRM

Poster 34 – Associé FP8

Comparaison des profils métaboliques et des propriétés antimicrobiennes de produits formulés à base de piment (*Capsicum*)

Inès Remy-Larsonnier[a], Marjolaine Rey[a], Carine Commun[b], Anne Doléans-Jordheim[b], Gilles Comte[a], Marie-Geneviève Dijoux-Franca[a], Camille Rozier[c]

[a] Université Lyon 1, UMR CNRS 5557/INRA 1418, Ecologie Microbienne, CESN , Villeurbanne, France
[b] Université Lyon 1, UMR CNRS 5557/INRA 1418, Ecologie Microbienne, Lyon , France
[c] ID4FEED SAS square du Rhône, 12 avenue du Rhône, BP 10051, Annecy, France

Réduire l'usage des antibiotiques en santé humaine et animale est devenue une absolue nécessité afin de limiter l'émergence de souches bactériennes antibio-résistantes. Les entreprises développant des additifs destinés aux animaux d'élevage tel qu'ID4FEED utilisent les propriétés biologiques des plantes comme base principale de leurs produits. Parmi les sources d'actifs ciblées par ID4FEED, le piment (*Capsicum chinense*) s'est révélé être un candidat majeur[1]. Les capsaïcinoïdes, responsables de la sensation de chaleur provoquée par le piment, sont la famille de métabolites très caractérisés jusqu'à présent chez le piment car ils possèdent une activité biologique d'importance en santé animale (antimicrobienne, antiinflammatoire, analgésique, radical-scavenging)[2]. Il a néanmoins été mis en évidence que d'autres classes de métabolites apportaient leurs activités dans le *totum* que représente la poudre de piment tels que des antioxydants et des antimicrobiens (vitamines A et E, caroténoïdes, polyphénols, phénols, terpènes).

L'objectif de ce travail a été de réaliser un suivi quantitatif et qualitatif des capsaïcinoides et des composés minoritaires de la poudre de piment et de comparer les profils métaboliques de la matière première et des produits commerciaux issus de cette matière première.

Une stratégie d'analyse globale par GC-QQQ et UHPLC-DAD-Q-TOF nous a permis de mettre en évidence des différences (quantitatives et qualitatives), notamment de certains flavonoïdes et volatiles contenus dans le piment. Le lien potentiel entre ces différences de profils métaboliques et les activités biologiques (antimicrobiennes et radical-scavenging notamment) de la matière première et des produits commerciaux est de même évalué en parallèle et nous permettra de renforcer notre compréhension des propriétés biologiques des préparations industrielles.

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Mots clés : *Capsicum* ; additifs ; capsaïcinoides ; UHPLC-DAD-Q-TOF ; GC-QQQ

Communication Posters

Session Environnement - Plante

Poster 35 – P35

Processus allélopathique et bio-herbicide : étude métabolomique de la diversité microalgale et caractérisation de substances allélochimiques assistée par les réseaux de similarités

Slimane Chaïb [1], Delphine Raviglione [1], Carole Vialleix [2], Annabel Levert [3], Jean-Paul Cadoret[2], Cédric Bertrand [1], Isabelle Bonnard[1]

[1] CRIOBE USR3278, Université de Perpignan Via Domitia – Univ de Perpignan Via Domitia – France
[2] Greensea – Greensea – France
[3] Akinao – S.A.S. AkiNaO – France

Le glyphosate est la molécule active d'un des herbicides les plus connus au monde : le Roundup de Monsanto homologué en 1974. En mars 2015, l'évaluation du Centre international de recherche sur le cancer (CIRC), une agence de l'Organisation mondiale de la santé (OMS), classait le glyphosate dans la catégorie des produits à fort impact sur la biodiversité.
Dans ce contexte, la biomasse issue de la production de micro-organismes photosynthétiques est une alternative à mettre en avant. En effet, les micro-algues constituent, au sein du milieu marin, une source variée de molécules bioactives. Il est mentionné dans la littérature la présence de substances allélopathiques connues comme ayant divers effets inhibiteurs et/ou toxiques tel que la fischerelline A qui est fortement algicide et antifongique.

Le projet " ALL DREAM " a pour but de développer un outil innovant adapté à l'analyse de la diversité chimique des micro-algues et à l'évaluation de leur potentiel comme biocide. Dans ce cadre, 150 extraits de micro-algues issues de diverses familles ont été soumises à un test d'inhibition de la photosynthèse ainsi qu'à un test antioxydant. Les résultats sont couplés à une approche métabolomique et aux réseaux de similarités.

Les données sont tout d'abors traitées via la plateforme W4M et le logiciel R. Elles font ensuite l'objet d'une analyse comparative entre la plateforme GNPS et MzMine/MetGem avant d'être traitées sous Cytoscape pour l'annotation. Puis, les extraits actifs sont soumis à un fractionnement bioguidé via un couplage LC-PDA-ELSD avec collecteur de fractions et LC-HRMS pour obtenir une caractérisation du/des métabolites actifs. Les extraits actifs sont ensuite testés sur des jeunes plantules afin d'analyser leur effet herbicide.

Ce poster présente l'ensemble des résultats mettant en avant le potentiel de cette ressource " bleue " que sont les micro-algues ainsi que l'alternative " verte " qu'elles représentent pour l'agriculture du futur.

Source de financement : Bourse Région/FEDER, AKINAO, GREENSEA

Mots-Clés: Allélopathie, Micro, algues, Bio, herbicide, Métabolomique, Réseaux de similarités

Poster 36 – P36

Evidence of wheat plant ability to control Pseudomonas secondary metabolism

Laura Rieusset [1], Marjolaine Rey [1], Florence Gerin [2], Daniel Muller [2], Claire Prigent-Combaret [2], Gilles Comte [1]

[1] Laboratoire d'écologie microbienne - CESN – CNRS : UMR5557, Institut national de la recherche agronomique (INRA) : UMR1418, Université Claude Bernard-Lyon I - UCBL (FRANCE) – France
[2] Laboratoire dÉcologie Microbienne – CNRS : UMR5557, Institut national de la recherche agronomique (INRA) : UMR1418, Université Claude Bernard-Lyon I - UCBL (FRANCE) – France

The plant root adhering soil houses an important microbial community. Roots exude a wide variety of secondary metabolites able to attract and/or control a large diversity of microbial species. In return, among the root microbiota, some bacteria are able to promote plant development, using plant growth-promotion and plant protection properties. These bacteria are named PGPR for Plant Growth-Promoting Rhizobacteria. Some PGPR belong to the *Pseudomonas* genus. *Pseudomonas* are known to produce a wide diversity of secondary metabolites that could have biological activity on the host plant and other soil microorganisms. But yet, the impact of host plant on *Pseudomonas* secondary metabolism is still poorly understood.

The aim of our project is to better understand the impact of the host plant on secondary metabolite production by fluorescent *Pseudomonas* strains.

A metabolomic approach was developed in order to decipher how plant may modulate secondary metabolites production in *Pseudomonas*. Five different fluorescent *Pseudomonas* strains were thus cultivated in the presence of root extracts of three wheat genotypes, at low concentration. This experimentation allows us to evaluate the impact of root metabolites on *Pseudomona*s secondary metabolism.

Analysis of our metabolomic workflow revealed that the production of several *Pseudomonas* secondary metabolites were significantly up- or down-regulated when bacteria were cultivated with root extracts. This shows that wheat root metabolites may act as signal compounds and modulate *Pseudomonas* secondary metabolism, including metabolites involved in plant stimulation and plant protection properties. Interestingly, root extract modulation differs according to wheat genotypes and *Pseudomonas* strains.

Mots-Clés: Wheat genotypes, Pseudomonas, secondary metabolites, metabolomics, plant/bacterial interactions

Poster 37 – Associé FP7

Étude de la réponse métabolique de Pseudomonas syringae 32b-74 isolée des nuages à une exposition sub – létale au mercure

Christelle Ghaffar , Martine Sancelme[1], Binta Diémé[2], Marie Lagrée[3], Cyril Jousse[4,5], Pierre Amato [3]

[6] Institut de Chimie de Clermont Ferrand (ICCF) – Université Clermont Auvergne, CNRS, SIGMA Clermont – France
[7] Université François-Rabelais, INSERM U930 "Imagerie et Cerveau", CHRU de Tours, 10 Bv Tonnellé, 37044 Tours, France –
[8] Institut de Chimie de Clermont-Ferrand - Clermont Auvergne (ICCF) – SIGMA Clermont, Université Clermont Auvergne : UMR6296, Centre National de la Recherche Scientifique : UMR6296 – 24 Avenue des Landais / 63177 Aubière Cedex, France
[9] Institut de Chimie de Clermont-Ferrand (ICCF) – SIGMA Clermont, Université Clermont Auvergne : UMR6296, Centre National de la Recherche Scientifique : UMR6296 – 24 Avenue des Landais / 63177 Aubière Cedex, France
[10] Plateforme d'Exploration du Métabolisme, MetaboHUB Clermont-Ferrand, Clermont-Ferrand, France (PFEM) – Université Clermont Auvergne, INRA, Université Clermont Auvergne – France

Ces dernières décennies, les fortes activités industrielles ont conduit à une augmentation de la pollution aux métaux lourds de l'atmosphère, et notamment au mercure (1,7 ng d'Hg / m3 d'air) 1. Celui-ci est présent sous différentes formes dans l'environnement : élémentaire (Hg0), ionique (Hg2+) et méthylé (Me – Hg). Ce dernier agit sur les groupes thiols des protéines, siège de sa toxicité. Certaines bactéries présentent un mécanisme de résistance codé par l'opéron *mer* et sous le contrôle du régulateur transcriptionnel MerR 2.

Des études montrent que des microorganismes subsistent dans l'atmosphère et les nuages, avec des conséquences possibles sur les processus physico-chimiques atmosphériques 3 4. Ces derniers sont donc exposés à la pollution atmosphérique du mercure, et notamment à la forme ionique Hg2+.

Nous étudions ici l'impact du mercure à dose sub – létale sur le métabolisme d'une souche bactérienne précédemment isolée d'eau de nuage et dépourvue de l'opéron *mer* : *Pseudomonas syringae* 32b74 5. L'objectif est d'identifier des marqueurs métaboliques spécifiques de l'exposition au mercure, dans l'optique d'une utilisation des microorganismes atmosphériques comme traceurs environnementaux.

La souche *P. syringae* 32b74 est ainsi incubée en absence et en présence de mercure biodisponible Hg2+ (5 μg/mL). Un profilage métabolique est réalisé après 2h et 24h d'exposition. Le métabolisme des cellules est stoppé par immersion dans l'azote liquide, et les métabolites intracellulaires sont extraits par un mélange de solvant avant d'être analysés par LC-MS. Par la suite, une analyse en composante principale (ACP) est réalisée afin de discriminer les potentiels signaux biomarqueurs.

L'impact du mercure sur l'écosystème atmosphérique est une question environnementale importante. L'utilisation de la métabolomique nous permettra de l'observer plus finement sur cette souche isolée de l'atmosphère.

Mots-Clés: Mercure, Atmosphere, Pseudomonas syringae 32b74, Métabolomique

Poster 38 – P38

Characterizing the effect of Zn contamination on variegated scallop, Mimachlamys varia, using metabolomics

Pascaline Ory [1], Vincent Hamani [1], Pierre-Edouard Bodet [1], Emmanuel Dubillot [1], Laurence Murillo [1], Marianne Graber [2,3]

[1] University of La Rochelle, UMR 7266 CNRS-ULR, LIENSs – Université de La Rochelle, CNRS : UMR7266 – France
[2] LIttoral ENvironnement et Sociétés [La Rochelle] (LIENSs) – CNRS : UMR7266, Université de La Rochelle – Bâtiment Marie Curie Avenue Michel Crépeau 17 042 La Rochelle cx1 - Bâtiment ILE 2, rue Olympe de Gouges 17 000 La Rochelle, France
[3] LIENSs, CNRS, Université de la Rochelle – Université de La Rochelle - CNRS – France

The variegated scallop (*Mimachlamys varia*) is a filter feeder bivalve of the *Pectinidae* family encountered in marine regions of the Atlantic coast. In particular, it is present in the La Rochelle marina (France), where it is used for the biomonitoring of marine pollution, due to its ability to strongly bioaccumulate pollutants. In this semi-closed environment, contamination generated by port activities leads to an accumulation of both organic pollutants and metals. Zinc has been identified as one of these pollutants. It arises, amoung others, from sacrificial anodes used to protect ship's hulls and engines from corrosions.

This study investigated the effects of 48 h zinc exposure upon the metabolic profiles of *Mimachlamys varia* using UHPLC/QTOF tandem mass spectrometry metabolomics. The objective was to determine the potential impairment of cell energy synthesis processes (glycolysis and respiratory chain pathways) by zinc in *M. varia* and highlight other metabolic pathways changes due to zinc exposure.

After acclimatization of a pool of individuals in mesocosms recreating *in situ* conditions, both controls and exposed bivalves with zinc chloride (nominal concentrations:150 μg/l) were shucked and deprived of the digestive gland and gills after 48h, and stored at –80 ◦ C before metabolite extraction.

UHPLC/QTOF tandem mass spectrometry was performed to study metabolite composition of samples. The obtained data point out a sensible metabolic response of bivalves to 48 h metal exposure. Our study demonstrated that the metabolomics approach is a useful tool for obtaining comprehensive and novel insights into the molecular toxicity of environmental pollutants.

Mots-Clés: metabolomics, zinc contamination, bivalves, marine environment

Poster 39 – P39

Metabolomics approach to study the environmental impact and residues of biocontrol products (BP)

Mélina Ramos [1,2,3], Marie-Virginie Salvia, Raviglione Delphine [4], Mercè Llugany Ollé [5], Esther Badosa [2], Emilio Montesinos [2], Cédric Bertrand[1]

[1] CRIOBE USR3278, Université de Perpignan Via Domitia – Univ de Perpignan Via Domitia – France
[2] Universitat de Girona [Girona] – Espagne
[3] Universitat Autònoma de Barcelona [Barcelona] – Espagne
[4] PSL Université Paris: EPHE-UPVD-CNRS, USR 3278 CRIOBE, Bat T, Université de Perpignan,
[5] Universitat Autònoma de Barcelona (UAB) – Espagne

Biopesticides or Biocontrol Products (BP) represents an interesting alternative to the conventional pesticides. However, there is a need of technical efficacy studies and ecotoxicological profile references. The PALVIP project (local Mediterranean crops' alternative protection) aims to fill that gap while evaluating new BP developed by small local businesses partners. To reach that goal, the BP selected in the project are studied according to their efficiency through field experimentations, their effect on plants and their environmental impact.
In a first stage the CRIOBE / University of Perpignan Via Domitia will contribute to the part of the project regarding the evaluation of BPs' environmental impact. To date, the half-life, t1/2, was often used to study the fate of pesticides in environmental matrices. However, this value doesn't give any information regarding the formation of by-products and the effect on biodiversity. Consequently, an innovative approach based on metabolomics (LC-MS), the Environmental Metabolic Footprinting (EMF), was recently developed in the lab.

On one hand, the EMF gives rise to a new integrative proxy, the resilience that corresponds to the time needed for the compound dissipation and its effects on the matrix (meta-metabolome). On the other hand, the EMF can be used in order to determine the preharvest interval (PHI) that corresponds to the time needed to have no residue (xenometabolome) difference between the treated sample and the control.

The degradation of the BP on vine leaves was monitored. Vines were treated against powdery mildew with 2 fungicides BPs. The vine leaves were sampled at different time points between 2 treatments in order to study the BP degradation (kinetics). The extraction steps were optimized and the extracts were analyzed using a UHPLC-HRMS instrument. For now, only the xenometabolome was studied and the preliminary results showed a degradation kinetics for the 2 fungicides BP with a PHI between 4 and 10 days. The endometabolome is currently analyzed. The same study will be performed for the 2019 field experiment (the project lasts 3 years (from 01/2018 to 12/2020) and sampling are done each year). Also, other matrices will be studied in the project: peach treated against brown rot, grape treated against grey mold and soil treated against weed development.

Mots-Clés: biocontrol compounds, environmental impact, LC, MS, metabolomics, resilience time

Poster 40 – P40

Analyse du métabolome d'une actinobactérie fixatrice d'azote : Frankia

Andrea Calhau [1], Marjolaine Rey [1], Hasna Boubakri [2], Aude Herrera Belaroussi [3], Anne-Emmanuelle Hay [1]

[1] Laboratoire d'Écologie Microbienne - CESN – CNRS : UMR5557, Institut national de la recherche agronomique (INRA) : UMR1418, Université Claude Bernard-Lyon I - UCBL (FRANCE) – France
[2] Université Lyon 1 – CNRS : UMR5557 – Ecologie microbienne, Bâtiment Mendel, Villeurbanne cedex, France
[3] Laboratoire d'Ecologie Microbienne (LEM) – CNRS : UMR5557 – Villeurbanne, France

Frankia est une actinobactérie saprophyte capable d'établir une symbiose racinaire avec près de 260 espèces de plantes dites actinorhiziennes, parmi lesquelles les Betulaceae, dont le genre *Alnus* (Benson *et al.,* 1993). 15% des entrées d'azote fixé biologiquement sur Terre proviennent de cette symbiose actinorhizienne. Cette interaction assure à ces plantes un avantage sélectif dans des écosystèmes où l'azote est limitant (*e.g.* moraines glaciaires, remblais miniers ...) et où elles constituent les premiers maillons d'une implantation pérenne d'un écosystème plus divers. Afin de mieux comprendre les interactions entre ces deux partenaires, une meilleure connaissance du métabolome de *Frankia* s'avère nécessaire. En effet, des approches *in-silico* ont permis de prédire une grande diversité de métabolites secondaires à partir du génôme de *Frankia* (Udwary *et al.*, 2011). Sous l'hypothèse que les métabolismes primaire et secondaire sont modifiés selon l'environnement de la bactérie, connaître le métabolome de *Frankia* en culture pure pourrait nous permettre d'interpréter les variations observées en conditions symbiotiques, dans le nodule. Cependant, malgré l'intérêt porté aux métabolites secondaires bioactifs produits par les actinobactéries (Solecka *et al.*, 2012), la métabolomique au sens propre est une approche encore peu utilisée chez les bactéries filamenteuses. L'étude présentée ici vise à mettre au point un protocole d'extraction à partir d'une culture pure de *Frankia* ACN14a (infective *d'Alnus (*Normand et Lalonde *1982*), afin d'explorer les profils métaboliques obtenus par HPLC-FLD, UHPLC-QTOF et GC-QQQ. Pour commencer, nous nous sommes intéressés à l'effet temporel sur le métabolisme de la bactérie en optimisant le protocole sur deux cultures d'âge différent : 10 jours (fin de phase exponentielle) et 2 mois (phase stationnaire). Ce protocole a été testé sur cette même souche au cours d'une cinétique de croissance et permis d'observer des variations dans le métabolome en fonction de l'activité physiologique de la bactérie.

Mots-Clés: Métabolome, Frankia ACN14a, symbiose

Poster 41 – P41

Metabolic foot-printing approach to study long term environmental fate and impacts of biological and synthetic Biocidal products in sediment

Chandrashekhar Patil [1], Amani Ben Jrad [1], Hikmat Ghosson [1], Raviglione Delphine [1], Marie-Virginie Salvia [1], Cédric Bertrand [1]

[1] PSL Université Paris: EPHE-UPVD-CNRS, USR 3278 CRIOBE, Bat T, Université de Perpignan, 58 Avenue Paul Alduy, 66860 Perpignan Cedex, France – Univ de Perpignan Via Domitia – France

Mosquitoes can carry infectious diseases from person to person and from place to place. Presence and establishment of invasive mosquito species such as *Aedes aegypti* and *Aedes albopictus* are rapidly increasing in the European environment. The most efficient means to fight against mosquitos is the use of insecticides. However, most of the insecticides were removed from the market as they showed severe risks for human and animal health as well as for the ecosystem. The European Directive in 1998 led to the increasing use of biological insecticides such as cry proteins produced by the bacterium *Bacillus thuringiensis israelensis* (Bti) that kill mosquito larvae after being ingested. Considering the interest in Bti as more environmentally sustainable bioinsecticide, it is important to examine in detail environmental fate and impact of Bti. The available tool such as half-life, t1/2, does not consider the biodegradation and biotransformation phenomenon of complex formulations. To address this challenge, 'Environmental Metabolic Footprinting' (EMF), giving an idea of the resilience time was recently developed in the laboratory (Patil et al. 2016; Salvia et al, 2017) to evaluate the impact of synthetic, botanical and microbial insecticides on soil and sediment matrix respectively.

The project 'EnvFate' aims to employ an EMF approach, to dynamically characterize environmental markers of Bti pollution found among the sediment matrix meta-metabolome and to determine the resilience time upon exposure. Metabolome characterization will require to develop and optimize extraction and detection protocols using LC-MS platform. Emphasis will be placed on better standardisation, data interpretation and evaluation that will build confidence in the value of "omics technologies – this being essential to increase their (regulatory) use. Based on our preliminary results, we performed a year-long experiment to know the changes in the meta-metabolome and if resilience has reached after Bti and α-cypermethrin treatment in three different sediment matrices. We used climatic chambers to mimic natural conditions. These activities will advance our understanding of environmental risks associated with test insecticides, and pave the way for the development and adaptation of new environmental monitoring tools.

Mots-Clés: Natural products, biopesticides, Bti, metabolomics, environment, fate, LC, MS

Poster 42 – P42

Preliminary metabolomic approach on the microalgae Nitzchia palea exposed to herbicide diuron

Léa Gauthier [1], Nicolas Mazzella [1], Juliette Tison-Rosebery [1]

[1] Irstea-Bordeaux Unité EABX – Institut national de recherche en sciences et technologies pour l'environnement et l'agriculture - IRSTEA (FRANCE) – France

Freshwater ecosystems are presently under a range of increasing pressures, some of which being directly due to anthropogenic activities such as agricultural practices or urban activities. Biofilms are communities of microorganisms (bacteria, algae, protozoa, fungi) and meiofaune integrated in an extracellular polysaccharide matrix fixed on immersed substrates. At the base of the trophic chain, biofilms play a key role in aquatic ecosystems. Due to their capacity to rapidly react to contamination, they are seen as efficient bioindicators and are increasingly used for water quality evaluation. Diatoms in particular dominate the algal biomass of biofilms and are routinely used in ecotoxicology to assess the impact of contaminations on freshwater ecosystems.
Traditional ecotoxicology approaches rely on quantitative concentration-response relationships highlighted on a batch of biological endpoints such as mortality, reproduction, growth and development, but all those approaches are materially and time expensive. We believe that metabolomics can enhance our knowledge of contaminant mode of actions, resulting in the identification of stress induced biomarkers.

The present study aims to highlight metabolome changes in the freshwater diatom *Nitzchia palea* exposed to an herbicide, the diuron (0.3 μg/L and 3 μg/L), during 4 hours. An extraction protocol as well as a non–targeted metabolomic approach using high resolution mass spectrometry (LC-TOF-MS) was developed in order to decipher the impact of contamination on a metabolic level.
Ongoing works will be dedicated to optimize both extraction and analysis methods to determine relevant biomarkers for risk assessment.

Mots-Clés: microalgae, diatom, environmental metabolomics, ecotoxicology

Poster 43 – P43

Etude de l'impact d'agents de contraste iodés sur deux organismes aquatiques modèles par des approches métabolomiques et métallomiques (projet ACTIONS)

Gaelle Daniele [1], Sandra Mounicou [2], Carine Arnaudguilhem [2], Alain Geffard [3], Odile Dedourge-Geffard [3], Anne Bado-Nilles [3], Jean-Marc Porcher [3], Emmanuelle Lebeau [1], Aurélie Fildier [1], Christelle Bonnefoy [1], Emmanuelle Vulliet [1]

[1] Institut des Sciences Analytiques – CNRS : UMR5280, Université Claude Bernard Lyon 1 – France

[2] Institut des sciences analytiques et de physico-chimie pour lénvironnement et les materiaux – Université de Pau et des Pays de l'Adour, Centre National de la Recherche Scientifique : UMR5254 – France

[3] Stress Environnementaux et BIOsurveillance des milieux aquatiques – Institut National de l'Environnement Industriel et des Risques : UMR-I 02, Université de Reims Champagne-Ardenne : UMR-I 02, Université Le Havre Normandie :

La contamination des milieux aquatiques par les produits pharmaceutiques n'est plus à démontrer. Parmi ces composés, les agents de contraste iodés (ACI) sont utilisés depuis les années 50 en imagerie médicale pour augmenter artificiellement le contraste et ainsi mieux visualiser les organes, tissus, tumeurs,... . Ces composés sont présents ubiquitairement dans les eaux à des teneurs pouvant atteindre quelques μg/L voire mg/L. Bien que rapidement métabolisés et excrétés par l'organisme, les produits de contraste sont connus pour interférer avec la thyroïde, notamment par l'intermédiaire de l'iode qu'ils contiennent, et ainsi interagir avec les fonctions endocriniennes. De même, ces molécules peuvent induire des réactions immunitaires et inflammatoires ou encore une néphrotoxicité. Ainsi, une exposition en continu aux ACI pourrait provoquer des effets néfastes sur les organismes aquatiques, non seulement sur les poissons qui présentent un rôle prépondérant dans la structure et le fonctionnement des réseaux trophiques aquatiques, mais également sur les organismes filtreurs, dont le caractère filtreur en fait de bons bioaccumulateurs.
Le projet ACTIONS propose d'évaluer, sur la base d'expositions à des teneurs environnementales, l'impact de 2 ACI d'osmolalité différente régulièrement détectés dans les eaux, l'acide diatrizoïque et l'iohexol, sur deux espèces modèles de nos rivières : un mollusque bivalve, *Dreissena polymorpha*, et un poisson Téléostéen, *Gasterosteous aculeatus*.

Après l'étude de la répartition et de la spéciation de l'iode dans les différentes parties des organismes par imagerie élémentaire, la détermination de la bioaccumulation de l'iode et ses potentielles interactions avec les biomolécules seront menées par une approche métallomique combinant spectrométrie de masse élémentaire (ICP-MS) et moléculaire (ESI-MS/MS). En parallèle, l'étude des effets des ACI sur le métabolisme des organismes sera réalisée par une approche métabolomique en LC-HRMS. Cette approche favorisera une vue d'ensemble des voies de métabolisation des molécules tout en proposant des marqueurs pertinents d'exposition aux ACI.

Ce projet est financé par le PNREST Anses, 2018/1/222.

Mots-Clés: métabolomique, métallomique, espèces sentinelles, LC, HRMS, HPLC, ICP, MS, bioimagerie LA, ICP MS, environnement

Poster 44 – P44

Etude métabolomique de la réponse au stress de lins déficients en lignanes

Kamar Hamade

Les lignanes sont des composés polyphénoliques largement distribués dans le monde végétal. Le lin, cultivé pour son huile ou sa fibre, renferme de grandes quantités d'un lignane de type dibenzylbutane : le sécoisolaricirésinol, qui est stocké dans le tégument de la graine sous forme diglucosylée, dans une macromolécule contenant de nombreux composés phénoliques assemblés par des liaisons esters (Ford et al., 2001). De nombreuses études s'intéressent aux effets bénéfiques sur la santé des lignanes et du sécoisolaricirésinol en particulier (Gutte et al., 2015), du fait de propriétés antioxydantes marquées, néanmoins le rôle de ces molécules in planta n'est pas clairement élucidé ; un rôle potentiel dans les mécanismes de réponse aux stress est toutefois proposé (Davin et al., 2008).

Une enzyme clé de la biosynthèse du sécoisolaricirésinol est la PLR (Pinorésinol Laricirésinol Réductase). Des lins RNAi PLR ont montré l'absence d'accumulation de SDG dans les graines (Renouard et al., 2014). Il est donc envisagé dans cette étude métabolomique de comparer le contenu en métabolites (semi-)polaires de lins accumulant du SDG ou non, en condition de stress osmotique ou en condition témoin. Les premières analyses réalisées en RMN et en LC-MS sur les extraits racinaires montrent une différence du profil métabolique due à la lignée de lin utilisée (avec ou sans le transgène RNAi PLR) ainsi que des variations métaboliques induites par l'application du stress osmotique.

Mots-Clés: Métabolomique, lins, lignanes, PLR, stress osmotique.

Poster 45 – P45

Leaf specialized metabolome of Quercus pubescens exposed to amplified drought

Amélie Saunier [1,2], Stéphane Greff [2], Catherine Fernandez [2], Elena Ormeno Lafuente [2]

[1] Department of Environmental and Biological Sciences – Finlande
[2] IMBE – Aix Marseille Univ, Univ Avignon, CNRS, IRD, IMBE, Marseille, France – France

The intensification of summer drought expected with climate change in the Mediterranean region can induce metabolism modifications in plants to help them cope with such conditions. In this experiment, we studied the specialized metabolome of *Quercus pubescens* leaves under natural (ND) and amplified drought (AD). An *in situ* experimental site, equipped with a rainfall exclusion device, allowed reduction of natural rainfall by 30% over the tree canopy. Leaves of ND and AD plots were collected in spring, summer and fall during 3 years, corresponding to the 2nd, 3rd and 4th years of drought application. We used a targeted approach to focus on phenolic metabolites and an untargeted approach to study a broader metabolome variation according to drought. Metabolic profiles (targeted approach) showed that the concentrations of quercetins, catechins and myricitrin were mostly decreased with drought stress, opposite to the concentrations of kaempferols that increased. Multivariate analysis of *Quercus* fingerprints (untargeted approach) demonstrated that metabolic contents remained almost unchanged under AD. Nevertheless, seven metabolites were highlighted as drought biomarkers using an univariate analysis (Venn diagrams). These biomarkers, still under study, likely belong to the phenylpropanoid pathway with one corresponding to kaempferol pentoside. Targeted and untargeted approaches permitted to demonstrate a time lag in leaf phenology when trees were exposed to drought with a modification of the phenylpropanoid pathway. Quercetins, catechins and myricitrin, specific to summer leaves were down-regulated to favor the biosynthesis of kaempferol, specific to fall leaves. Further studies would be necessary to understand how this time lag phenology may impact ecosystem functioning.

Mots-Clés: Quercus pubescens, amplified drought, Mediterranean forest, flavonoids, time lag phenology

Poster 46 – P46

Molecular networking on wild "Tali" (Erythrophleum, Fabaceae) from Central tropical Africa

Nausicaa Noret [1], Cécile Vanhaverbeke [2], Cédric Delporte [3], Caroline Stévigny , Florence Mondeguer [4], David Touboul [5], Florence Souard [6]

[1] Laboratoire d'écologie végétale et de biogéochimie – Université Libre de Bruxelles Bâtiment BC - 5e niveau - aile C Campus de la Plaine - CP244 Boulevard du Triomphe 1050 Bruxelles, Belgique

[2] Département de Pharmacochimie Moléculaire, CNRS : UMR5063 – Université Grenoble Alpes – France

[3] RD3 - Pharmacognosy, Bioanalysis and Drug Discovery Unit Analytical Platform of the Faculty of Pharmacy, ULB – Belgique

[4] Laboratoire Phycotoxines – Institut Français de Recherche pour l'Exploitation de la Mer IFREMER – Nantes, France

[5] Institut de Chimie des Substances Naturelles (CNRS-ICSN) – CNRS UPR2301, Univ. Paris-Sud, Université Paris-Saclay –, Avenue de la Terrasse, 91198 Gif-sur-Yvette, France, France

[6] Département de Pharmacochimie Moléculaire, CNRS UMR5063 – Université Grenoble – France

Plant secondary (or specialized) metabolites (SM) are essential for plants which rely on specific set of molecules to handle basic life functions like reproduction (e.g. pollinator attraction) or defense (e.g. herbivore deterrence). As metabolites are the result of a complex network of gene expression, protein expression and interactions and other regulatory processes, they are therefore closer to the phenotype than transcriptome or proteome. Untargeted metabolomics allows to investigate the metabolic phenotypes of plants including the diversity of SM. As SM are produced by specific biosynthetic pathways, most metabolites have characteristic structural features and often show a clear phylogenetic signature.

The tropical woody genus *Erythrophleum* (Fabaceae, Caesalpinioideae) contains original cassaine diterpenes, a subfamily of the cassane diterpenes restricted to various Fabaceae genera. The cassane skeleton is structurally characterized as a tricyclic diterpene with a substitution by ethyl group in C-13 position and a methyl group at C-14 position. Cassaine diterpenes are known for their non-steroidal inhibition of Na+/K+-ATPase in a way similar to that of the digitalis glycosides. In addition to this cardiotonic effect, the traditional use of *Erythrophleum* is based on emetic, antimalarial, antibacterial and anti-inflammatory properties.

In this study, we used two closely related species of *Erythrophleum*, *E. suaveolens* and *E. ivorense*, to investigate the metabolic diversity of their cassaine diterpenes. After a metabolomic exploration by LC-HRMS and multi-block correlation of neutral genetic diversity with metabolome, we have developed a molecular networking dereplication pipeline based on MS/MS focusing on the cassane diterpenes family. For molecular network construction the user-friendly Metgem software has been used. Principal results of dereplication will be presented in the poster and interpretations of the metabolome will be proposed for the different genotypes.

Mots-Clés: Erythrophleum, ecometabolomics, plantomics, environmental metabolomics : natural variation

Poster 47 – P47

Combining metabolomics and molecular networking as a tool to target and identify phenolic compounds in flax leaves

Elvis Otogo Nnang[1], Sébastien Rigaud [2], Job Tchoumtchoua [1], David Mathiron [2], Emmanuel Petit [1], François Mesnard [1], Roland Molinié [1], Anthony Quéro [1]

[1] EA3900 BIOPI – Biologie des Plantes et Innovation, UFR de Pharmacie – Université de Picardie Jules Verne – France
[2] Plateforme Analytique – Université de Picardie Jules Verne, Chemin du Thil, 80000 Amiens – France

Flax (*Linum usitatissimum*) is a crop that is widely cultivated as a source of fiber or oil1. It belongs to the genus *Linum* and family Linaceae2. The components from flax have diverse uses. Although flaxseed chemical composition is deeply studied, little is known about the phytochemistry of the flax leaves. This study aims at identifying the secondary metabolites contained in flax leaves using a metabolomics approach and the molecular networking as a dereplication strategy3. This computer-based approach is emerging as a promising method to visualize and organize tandem *MS*/*MS* data sets and to automate database searches for secondary metabolite identification within complex mixtures. This strategy led to the rapid detection of more than twenty flavonoids and lignans. After characterization, the metabolite profiling of 14 flax cultivars was performed by LC-MS. PCA analysis showed a clear discrimination between leaves of spring and winter flax. These results suggest the implication of phenolic compounds in flax cold tolerance.

Mots-Clés: Flax (Linum usitatissimum), metabolomics, molecular networking, PCA analysis

Poster 48 – P48

LC-MS and NMR metabolomics study of a tomato (Solanum lycopersicum L.) mutant affected in the fruit pericarp development

Constance Musseau [1], Stéphane Bernillon [1,2], Catherine Deborde [1,2], Daniel Jacob [1,2], Joana Jorly [1], Stéphanie Gadin [1], Annick Moing [1,2], Frédéric Gévaudant [1], Lucie Fernandez [1]

[1] UMR1332 Biologie du Fruit et Pathologie – Institut national de la recherche agronomique (INRA), Universtité de Bordeaux – France

[2] Plateforme Métabolome de Bordeaux - MetaboHUB – (INRA), Université de Bordeaux (Bordeaux, France)

Metabolomics is widely used as a tool in plant genomic studies to decipher gene functions [1] and has been applied to the model species tomato to explore development and metabolism of fleshy fruit [2]. To improve metabolome coverage, the combination of LC-MS and NMR analysis has been shown to be very successful [3] .The aim of this study was to characterised the metabolome of tomato plant mutants for a gene coding for a large GTPase involved in the regulation of the fruit tissue morphology and whose function has not yet been described in plants. Two mutants for this gene were generated from two independent CRISPR-Cas9 transformation events. Mutant and control tomato plants were grown in greenhouse. Tomato pericarp samples were harvested at 4 fruit growth stages, *i.e*. 15, 20, 25 days post anthesis (DPA) and red ripe. They were analysed with both NMR and LCMS methodologies. For NMR analysis, 1H spectra were acquired with a 500 MHz Avance III NMR spectrometer (Bruker, France) and then processed using the software NMRProcFlow [4]. For LCMS analysis, data were acquired with a UHPLC-LTQ-Orbitrap-MS (Thermo Scientific, Germany) in both positive and negative ESI mode with a C18 separation. Acquisitions were processed using the software MS Dial [5]. 2D NMR and MS^2 data were also acquired to enable annotation or identification of metabolites.

Processed data were explored with multivariate analysis that showed distinct metabolic trajectories for control and mutant pericarp tissues suggesting a deep perturbation of the whole metabolism in the mutants. Major modifications occurred at 25 DPA that correlated with pericarp cellular alterations. The identified metabolites belong to various classes of primary and specialised metabolisms such as sugars, organic and amino acids, phenolic compounds and glycoalkaloids. Besides, both processing softwares enabled visualisation and were interactive, making the data processing effortless while allowing the analyst to control and possibly correct automatic output.

Acknowledgements: MetaboHUB (ANR-11-INBS-0010) References:

Mots-Clés: LC, MS, NMR, tomato, processing

Poster 49 – P49

Etude métabolomique des interactions chimiques dans le dépérissement de la lavande

Bénédicte Héral [1], Florence Nicolé [2], Xavier Fernandez [1], Thomas Michel1

[1] Institut de Chimie de Nice – CNRS UMR 7272, Université de Nice-Sophia Antipolis – France
[2] Laboratoire BVpam – UMR 7272, 23 rue du Dr Michelon 42023 Saint Etienne Cedex 2 : Université de St Etienne – France

Les cultures de lavande (*Lavandula angustifolia*) et de lavandin (Lavandula x intermedia) sont, depuis une dizaine d'années, affectées par le phytoplasme du Stolbur (*Candidatus phytoplasma solani*), agent pathogène transmis par la cicadelle (*Hyalesthes obsoletus*) (FranceAgriMer, 2013). Cet insecte se nourrit de la sève de la lavande transmettant la bactérie de plant infecté en plant sain (Gasparich, 2010). Le déperissement de la lavande a des conséquences économiques importantes sur la filière lavandicole et les moyens de lutte sont actuellement inexistants ou restreints. Par ailleurs, les connaissances autour des interactions chimiques entre la cicadelle, le phytoplasme et la lavande sont encore limitées, c'est pourquoi il est important d'étudier les composés organiques non volatiles qui pourraient avoir une influence dans cette interaction tripartite.

Une approche métabolomique globale a été utilisée afin de déterminer les bio-marqueurs spécifiquent :
- des variétés (Maillette et 7713 pour la lavande, et Grosso et Abrial pour le lavandin)
- de l'état symptomatique des plants (sain ou infecté).

Une campagne d'échantillonnage en champ a été mené sur le plateau de Sault (Vaucluse, France) en 2017, au cours de laquelle des échantillons de feuille et d'inflorescence ont été collectés. Les extraits eau-éthanol (50:50, v/v), obtenus par extraction assistée par ultra-sons, ont été analysés par UPLC-HRMS (XevoG2 QTOF, Waters). Les données ont par la suite été retraité sur la plateforme Workflow4Metabolomics 3.0. Le retraitement a nécessité un prétraitement comprenant la détection des ions (CentWave), l'alignement (PeakDensity), la correction des biais expérimentaux, tels que les dérives analytiques intra et inter séquence (loess). La qualité du jeu de données a ensuite été évalué grâce à des statistiques descriptifs (ACP), puis celui-ci a été réduit pour réaliser les analyses discriminantes (PLS-DA). Les premiers résultats obtenus sur les extraits de feuille seront présentés ici.

Mots-Clés: Lavande, phytoplasme, dépérissement, métabolomique

Poster 50 – P50

Metabolite profiling at the graft interface of grapevine in response to grafting

Duyên Prodhomme [1], Josep Valls Fonayet [2], Cyril Hévin [1], Céline Franc [2], Ghislaine Hilbert [1], Gilles De Revel [2], Tristan Richard [2], Nathalie Ollat [1], Sarah Jane Cookson [1]

[1] Ecophysiologie et Génomique Fonctionnelle de la Vigne – Institut National de la Recherche Agronomique : UMR1287, Université de Bordeaux, Institut des Sciences de la Vigne et du Vin (ISVV), Ecole Nationale Supérieure des Sciences Agronomiques de Bordeaux-Aquitaine – France

[2] Unité de Recherche Œnologie USC 1366, EA 4577 – Institut National de la Recherche Agronomique INRA, Université de Bordeaux, Institut des Sciences de la Vigne et du Vin (ISVV) – France

Grafting with rootstocks is essential for the culture of many perennial fruit crops and is increasing being used in the production of annual fruit and vegetables. In viticulture, grafting is used to facilitate grapevine cultivation in soils infected with the Phylloxera, a soil-dwelling insect pest introduced to Europe from America at the end of the 19th century. Our previous work based on microarrays showed that transcripts encoding enzymes of both primary and secondary metabolism were differentially expressed during graft union formation in both homo-grafts (a genotype grafted with itself) and hetero-grafts (two different genotypes grafted together). The aim of this study was to profile primary and secondary metabolites, and quantify the activity of phenylalanine ammonia lyase (PAL) and neutral invertase (NI) in the scion and rootstock tissues and the graft interface of homo and hetero-grafts of grapevine one month after grafting. Table-top grafting was done on over-wintering stems of grapevine and the graft interface tissues were compared to the surrounding rootstock and scion tissues. The objective was to identify compounds involved in graft union formation and hetero-grafting responses.

A total of 54 compounds from primary and secondary metabolism and the activity of two enzymes were measured using different metabolomic tools. The results revealed that graft interface was associated with an increase in the accumulation of the branched-chain amino acids, basic amino acids, certain stilbene compounds and higher PAL and NI activity in comparison to the surrounding woody stem tissues. Eight amino acids and six stilbenes were identified as being accumulated differently between the graft interfaces of the scion/rootstock combinations in a manner which was unrelated to their concentrations in the surrounding woody stem tissues. This study revealed the modification of primary metabolism to support callus cell formation and the stimulation of stilbene synthesis at the graft interface, and how these processes are modified by hetero-grafting. Knowledge of the metabolites and/or enzymes required for successful graft union formation offer us the potential to identify markers that could be used by nurseries and researchers for selection and breeding purposes.

Mots-Clés: Grafting, grapevine, wood, stilbenes, flavanols, sugars, amino acids, HPLC, LC, MS/MS

Poster 51 – P51

Isoprostanoids quantitative profiling of macroalgae submitted to copper stress

Claire Vigor[1], Guillaume Reversat [1], Amandine Rocher [1], Camille Oger [1], Jean Marie Galano [1], Joseph Vercauteren , Thierry Durand [1], Thierry Tonon [2], Catherine Leblanc [3], Philippe Potin [3]

[1] Institut des Biomolécules Max Mousseron – CNRS : UMR5247 – France
[2] Centre for Novel Agricultural Products, Department of Biologie, University of York – Heslington, York, YO10 5DD, Royaume-Uni
[3] Station biologique de Roscoff [Roscoff] (SBR) – Université Pierre et Marie Curie (UPMC) - Paris VI, CNRS : FR8227 – Place Georges Teissier - BP 74 29682 ROSCOFF CEDEX, France

Macroalgae are living organisms inhabiting a broad range of particularly hostile aquatic environments that can cause them to undergo oxidative stress (OS). Considering that isoprostanoids derivatives (phytoprostanes, phytofuranes, isoprostanes) constitute the most relevant oxidative stress biomarkers, we are interested in this work to their qualitative and quantitative profile in six macroalgae submitted to a heavy metal exposure (copper). Thus, 9 phytoprostanes, 3 phytofuranes (observed for the first time in such matrices), and 3 isoprostanes are quantified through a new micro-LC-MS/MS method. The isoprostanoids profiles vary greatly among all the samples, the *ent*-16(*RS*)-9-*epi*-ST-14-10-PhytoF and the 5(*RS*)-5-F2t-IsoP being the major compounds for most of the macroalgae studied. Concentrations of these metabolites, at basal conditions range from 2.5 to 342.6 ng/g of algal tissue. After 24h exposure of macroalgae to cupric action, as expected, in response to copper-induced stress, an increase of lipid oxidation biomarkers is observed in the majority of cases, corroborating the original hypothesis. Only *L. digitata* seems to derogate from the rule with a reduction in the content of ent-9-L1-PhytoP.

Mots-Clés: PUFAs, oxidative stress, biomarkers, micro, LC, MS/MS

Poster 52 – P52

EXPERIMENTAL STUDY OF THE EFFECTS OF OXIDATIVE STRESS ON THE AQUATIC FLORA AND ENVIRONMENT

Khaoula Menaiaia [1]

[1] 1Department of Biology, Faculty of Nature and Life Sciences, University of Mohamed Cherif Messaadia PO Box 1553. 41000, Souk-Ahras, Algeria – Algérie

In recent decades, the increase and diversity of human activities have been accompanied by dispersion into the environment of sometimes considerable quantities of xenobiotics harmful to human health. In many countries, the accumulation of pollutants in ecosystems has led, over the years, to deteriorating environmental quality, forest decline, and declining agricultural productivity.
The accumulation in the soil of heavy metals, which are inherently non-degradable and potentially toxic, increases the risk of exposure of living beings: microorganisms, plants, animals, and humans, which is at the end of the food chain.

Our research is about the effect of zinc oxide on the behavior of *Phragmites australis*. For this purpose, samples of *Phragmites australis* were run at three concentrations based on ZnO (3, 6, 12 nmol/ml) for 7, 14 and 21 days. In order to evaluate the influence of the various concentrations of this nanometric molecule, metabolic and enzymatic parameters characteristic of the oxidative stress were measured.
Regarding the metabolites (proline, total sugars) and the enzymatic biomarker (CAT), no significant difference was observed, which reflects the tolerance of this species to zinc oxide (ZnO).

Mots-Clés: Phragmites australis, ZnO, Enzymatic activity, Oxidative stress.

Poster 53 – P53

Optimisation of 1D 1H-NMR profiling protocol for plant samples: from extract preparation, standardization, automation to spectra processing.

Catherine Deborde [1,2], Jean-Xavier Fontaine [3], Daniel Jacob [1,4], Adolfo Botana [5], François Mesnard [3], Annick Moing [1,2], Roland Molinié [3]

[1] Plateforme Métabolome Bordeaux, MetaboHUB, CGFB INRA, Université de Bordeaux, France
[2] Biologie du Fruit et Pathologie – Institut National de la Recherche Agronomique - INRA : UMR1332, Université de Bordeaux : UMR1332, Université de Bordeaux : UMR1332 – France
[3] Laboratoire de Biologie des Plantes et Innovation – Université de Picardie Jules Verne : EA3900 – France
[4] JEOL-UK – Royaume-Uni

1H NMR-based metabolomic profiling has a range of applications in plant sciences. In order to minimize uncontrolled variability in plant sample preparation before and during NMR metabolomic profiling, we provide advice and show examples for taking into account sample composition, including its specificity in terms of pH and paramagnetic ion concentrations, and NMR spectrometer performances.
A pH extract adjustment step reduces variations of pH-sensitive chemical shifts and addition of paramagnetic ion chelatant, like EDTA, improves citrate and malate resonance detection.
An automation of spectrometer preparation routine standardization before NMR acquisition campaign was implemented and tested on three plant sample sets (extracts of durum wheat, *Arabidopsis* and flax). We performed 1H-NMR spectroscopy in three different sites on the wheat sample set utilizing instruments from two manufacturers with different probes and magnetic field strengths. The three collections of spectra were processed separately with the NMRProcFlow web tool using intelligent bucketing, and the resulting buckets were subjected to multivariate statistical analysis. The comparability of large- (*Arabidopsis*) and medium-size (flax) datasets measured at 600 MHz and from the wheat sample set recorded at the three sites (400, 500 and 600 MHz) was exceptionally good in terms of spectral quality. The coefficient of variation of the full width at half maximum (FWHM) and the signal-to-noise ratio (S/N) of two selected peaks was comprised between 5 and 10% depending on the size of sample set and the spectrometer field.
A collection of 22 samples of wheat spikelet extracts, used as a proof of concept, showed that the data collected at the three sites on instruments of different field strengths and manufacturers yielded the same discrimination pattern of the biological groups.
We showed that standardization or automation of several steps from extract preparation to data reduction improved data quality for small to large collections of plant samples of different origins.

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Mots-Clés: Metabolomics, NMR acquisition, NMR spectra processing, Sample preparation, Plant

Poster 54 – P54

Utilisation des approches de métabolomique pour l'étude des interactions plantes-microorganismes

Virginie Puech Pages [1], Sylvie Fournier[1], Bernard Dumas[1]

[1] UMR5546, LRSV – CNRS : UMR5546, Université Paul Sabatier-Toulouse III - UPS – France

L'environnement naturel des plantes est caractérisé par un microbiote extrêmement diversifié comportant des microorganismes bénéfiques pour leur développement, comme les bactéries fixatrices d'azote (bactéries en symbiose avec les légumineuses) et les champignons mycorhiziens (symbiose plante-champignon très ancienne et très répandue), ou pouvant provoquer des maladies (bactéries, champignons, oomycètes....).Dans ce contexte, les métabolites végétaux ou microbiens sont des acteurs essentiels, favorisant la mise en place puis la régulation des interactions symbiotiques et contribuant à la reconnaissance, et au rejet, des microorganismes pathogènes. Au Laboratoire de Recherche en Sciences végétales (LRSV, Castanet Tolosan) plusieurs équipes étudient le métabolome dans les interactions plantes microorganismes en tirant profit des avancées dans les approches de spectrométrie de masse (métabolomique ciblée et globale) qui sont développées sur la plateforme MetaToul " Métabolites végétaux " du LRSV (https://www.lrsv.upstlse.fr/metatoul/).
Une illustration de nos travaux sera faite avec les métabolites impliqués dans le dialogue moléculaire intervenant i) dans la mise en place de la symbiose mycorhizienne, avec les molécules d'origine végétale identifiées (strigolactones (Gomez-Roldan *et al.* 2008), blumenols (en cours), les signaux d'origine fongiques (lipochitooligosaccharides (Maillet *et al.* 2011), chitooligosaccharides (Genre *et al.* 2013), karrikines et hormones (en cours)) ii) dans la régulation de la symbiose mycorhizienne, avec des approches en métabolomique globales (Laparre *et al.,* 2014 et travaux en cours).

Mots-Clés: champignons, symbiose, pathogènes, interactions plantes microorganismes

Poster 55 – P55

Comprehensive computational approaches to prioritize high-value natural products isolation from a chemodiverse collection.

Arnaud Gaudry[1Δ], Luis Quirós[1Δ], Adriano Rutz[1], Miwa Dounoue[1], Marcel Kaiser [2], Laurence Marcourt[1], Bruno Davide[3], Emerson Ferreira Queiroz[1], Pierre-Marie Allard[1]* and Jean-Luc Wolfender[1]*

1 Phytochimie et Produits Naturels Bioactifs, Section des Sciences Pharmaceutiques, Université de Genève, CH-1211 Genève 4, Switzerland.
2 Swiss Tropical and Public Health Institute, Socinstrasse 57, CH-4002 Basel, Switzerland; University of Basel, Petersplatz 1, 4003 Basel, Switzerland.
3 Green Mission Pierre Fabre, Institut de Recherche Pierre Fabre, 3, Avenue Hubert Curien, BP 13562, 31562 Toulouse, France.

Nature is an inexhaustible source of chemical diversity and bioactive molecules. Given the complexity of natural extracts matrices, identification of novel natural products potentially bioactive continues as the hardest bottleneck in phytochemistry and pharmacognosy nowadays, and recurrent isolation of known compounds discourage further studies [1].
In this work, we report the dereplication of a large chemodiverse library composed by 1600 plants extracts (156 families, 533 genera, 783 species) by means of high resolution mass spectrometry profiling and multi-informative MS^2 spectral organization (Molecular Networks, MN) [2]. Integration of MS^1 identification, MS^2 *in silico* annotations [3] and taxonomical consistency of the annotations biosources [4] and bioactivity results mapping (3 different trypanosomatids and cytotoxicity assays), brings out potentially new bioactive scaffolds within the extracts.
We centered the prioritization from two perspectives: ***Bioactive NP discovery***, approach based on the bioactivity mapping information from which we were able to highlight in the MN features and molecular families specific to active extracts. ***Structure-focused discovery***, approach based on structure novelty considering combinations several criteria: MS^1 and MS^2 *in silico* annotations, annotation's taxonomical consistency, cluster's specificity at the taxa level and literature regarding the highlight species.
Using the bioactivity visualization, we were able to spot *Melochia sp*. quinoline alkaloids derivatives such as Waltherione G, a known trypanocidal compound, as a proof of concept. Selected species were subjected to a straightforward one-step isolation procedure using a gradient transfer combined with a dry-load injection of the sample at a semi-preparative HPLC scale [5]. This approach allowed the targeted isolation of a series of 14 original *β*-agarofuran alkaloid derivatives (structural approach), which were fully characterized by 2D NMR and HRMS.
Our workflow is capable to spot novel natural products from a large and highly chemodiverse collection of plants, which are then efficiently isolated in the required amount for further bioactive analysis without extensive and time consuming purification steps.

Communication Posters

Session Développement Analytique

Poster 56 – P56

Usefulness of retention time prediction for plant food bioactives

Dorrain Low [1], Pierre Micheau [1], Jan Stanstrup [3], Claudine Manach [1]

[1] Unité de Nutrition Humaine – Institut national de la recherche agronomique (INRA) : UMR1019, Université Clemont Auvergne – France

[2] University of Copenhagen – Danemark

Plant food bioactives and their metabolites are chemically diverse, and receive widespread interest for their protective health effects. However, the large chemical space of plant food bioactives and their host/microbial metabolites makes their identification in untargeted metabolomics profiles a challenging feat. Spectral libraries are incomplete for these compounds and standards are often costly or not commercially available. Retention time (RT) is valuable for assisting the certainty of identification of unknown features to MSI levels I and II. It also helps to narrow the number of hypotheses within an observed RT window to a manageable number of compounds to purchase or synthesize for confirmation. In the framework of the COST Action POSITIVe (https://www6.inra.fr/cost-positive, we evaluated the usefulness of PredRet (http://predret.org), an open access RT database, to predict retention times of plant food bioactives and their metabolites in a multi-laboratory test of 18 laboratories and 24 reversed-phase LC-MS or LC-PDA Chromatographic Systems (CS). Participants shared datasets of RT in their own CS for 29 to 104 compounds, covering a total of 471 chemically diverse chemicals, including highly polar to lipophilic aglycones, glycosides, as well as glucuronidated and sulfated metabolites and gut microbial metabolites of flavonoids, alkaloids, coumarins, phenolic acids, and others. Depending on its compatibility with other CSs (conditions and number of common compounds), every platform obtained predicted RTs for 67 to 667 compounds that were not yet analysed in their conditions. The predictions were very accurate, with a median prediction error ranging from 0.03 to 0.76 min, depending on the systems. Such level of prediction allowed distinguishing isomeric compounds. It also provided information in all CSs for rare standards. In conclusion, prediction of retention time with PredRet has proven very efficient and useful to facilitate annotation of plant food bioactives in metabolomics studies.

Mots-Clés: Plant food bioactives, Retention time, Prediction, PredRet, Multiplatform test

Poster 57 – P57

Fluxomic analysis of biomass production through a combination of analytical tools.

Arnaud Vigneron[1], Patrick Jame[2], Erik Bonjour[2], Xavier Saupin[2], Himaïji Chevrotee[1]

[1] Centre de Recherche en Cancérologie de Lyon – Centre Léon Bérard [Lyon], Université Claude Bernard Lyon 1, Institut National de la Santé et de la Recherche Médicale : U1052, Centre National de la Recherche Scientifique : UMR5286 – France
[2] Institut des Sciences Analytiques – Centre National de la Recherche Scientifique : UMR5280, Université Claude Bernard Lyon 1, École Normale Supérieure - Lyon : UMR5280 – France

Biomass production set the flux of the main metabolic paths in proliferating cells. Precise knowledge of biomass production and nutritive distribution could thus highly constrain metabolic reconstruction. For these reasons, we developed new methods to determine how the atomic species of a nutrient distribute into biomass of cancer cells by using a combination of several analytical tools such as Total Organic Carbon analysis, Elementary analysis, Ionic Exchange chromatography coupled to Isotopic Ratio Mass Spectrometry (IRMS), and IRMS of macromolecular entities. Complete flux map can be generated from these analyses, tracing the fate of a particular nutrient into the cell biomass.

Mots-Clés: IRMS Cancer cell Metabolic Reconstruction

Poster 58 – P58

Comparaison inter-laboratoires de profils d'oxylipines par lipidomique ciblée dans le cadre du projet OXYGENATE

Céline Dalle [1], Malwina Mainka [2], Jessica Dalloux-Chioccioli [3], Annika Ostermann [4], Michael Rothe [5], John W. Newman [6], Justine Bertrand-Michel [3], Cécile Gladine [1], Nils H. Schebb [2,4]

1 Unité de Nutrition Humaine - Clermont Auvergne – Université Clermont Auvergne : UMR1019, INRA
2 University of Wuppertal – Allemagne
3 Plateforme MetaToul Lipidomique, I2MC – Centre de Recherche Inserm : UMR1048 – France
4 Institute for Food Toxicology, University of Veterinary Medicine Hannover – Allemagne
5 Lipidomix – Allemagne
6 USDA-ARS Western Human Nutrition Research Unit – États-Unis

Actuellement 20 à 25% de la population adulte mondiale présente un syndrome cardiométabolique. Ce syndrome est un exemple de maladie multifactorielle en lien avec l'alimentation et se caractérise par des troubles cardiovasculaires, métaboliques et inflammatoires. Ces troubles amènent au développement du diabète de type II et des maladies cardiovasculaires. Cependant le diagnostic de ce syndrome n'est pas satisfaisant car pas assez intégratif et précoce pour permettre une prise en charge nutritionnelle.
Les oxylipines, métabolites issus de l'oxygénation des acides gras polyinsaturés via différentes voies de biosynthèse, sont connues pour être des médiateurs lipidiques impliqués dans la régulation de processus biologiques liés au syndrome cardiométabolique et dont la biosynthèse est modulée par le statut cardiométabolique et aussi par l'alimentation. D'où l'hypothèse qu'un profilage complet des oxylipines pourrait révéler des perturbations précoces du statut cardiométabolique et pourrait être un nouvel outil d'évaluation de l'efficacité des préventions et interventions nutritionnelles.

Le projet OXYGENATE a pour but d'identifier et valider des oxylipines discriminant le statut cardiométabolique et la qualité de l'alimentation. Pour cela, il est nécessaire d'avoir une méthode fiable de profilage quantitatif des oxylipines permettant d'obtenir des profils comparables entre différents laboratoires. Une comparaison inter-laboratoires de notre méthode a été réalisée afin d'estimer les variabilités analytiques et d'identifier les oxylipines critiques. Cinq laboratoires ont préparé (en triplicat et sur 2 jours différents) et analysé 7 plasmas présentant des profils d'oxylipines très contrastés. Les variabilités intra- et inter-jour ainsi que la part de variabilité attribuable à la préparation et à l'appareillage sont évalués.
Au-delà de l'intérêt pour le projet, cette comparaison inter-laboratoires unique permettra d'harmoniser les profils d'oxylipines réalisés par l'ensemble de la communauté scientifique.

Mots-Clés: lipidomique ciblée, comparaison inter, laboratoires, oxylipines, syndrome cardiométabolique

Poster 59 – P59

Have a break, Have a Robot!

Julia Soullier [1], Anthony Tournadre [1], Pauline Le Faouder [1], Amandine Hueber [1], Jessica Dalloux-Chiccioli [1], Anaelle Durbec [1], Justine Bertrand-Michel [1], Aurélie Batut [1]

[1] MetaToul-Lipidomic Core Facility – Institut des Maladies Métaboliques et Cardiovasculaires (I2MC), Centre de Recherche Inserm – France

Lipids are ubiquitous biomolecules essential to all life, found in every cellular type, ranging from the human body and vegetal organisms, down to bacteria. They have many different functions in cell structuration, energy storage and signalling so they are natural biomarkers for different diseases like cancer, cardiovascular disease, neurodegenerative disease, lung disease, their study and quantification are then crucial. Mass spectrometry (MS) coupled with liquid chromatography or gas chromatography is mainly used for global and specific analysis of lipids. But before their analysis, there is an important and time consuming step of sample preparation. Due to their amphiphilic properties, there are usually two types of extractions: liquid-liquid extraction (LLE) and solid phase extraction (SPE). LLE and SPE are two very long protocols which are tedious when there are massive numbers of samples. They can also be the source of many errors, considering the experimenter-dependant possible repeated artefacts. To circumvent this point and increase the analytical service delivery, a TECAN robot has been acquired by MetaToul-Lipidomic facility to automate the sample preparation. Due to the specificity of lipid extraction, the robot needs a lot of optimization. This presentation will show part of adaptation we had to perform to validate fatty acid, neutral lipid and phospholipid profiling. That was related to the precision of solvent sampling which implies new adjustment accuracy for each of them, adding air gap, but also to modify the speed... First lipidomic's results obtained with a complete automated sample preparation will be presented for plasma sample.

Mots-Clés: sample preparation, lipidomics, automation, robot

Poster 60 – P60

Development of lipidomic profiling by SFC-HRMS

Pauline Le Faouder [1], Jean-François Martin [2], Marion Regnier [3], Hervé Guillou [4], Justine Bertrand-Michel [5]

[1] Institut des Maladies Métaboliques et Cardiovasculaires (I2MC) – Université Paul Sabatier - Toulouse 3, Hôpital de Rangueil, Institut National de la Santé et de la Recherche Médicale : U1048 – 1 avenue du Prof Jean Poulhes - BP 84225 - 31432 Toulouse Cedex 4, France

[2] ToxAlim (ToxAlim) – Institut national de la recherche agronomique (INRA) : UMR1331 – 180 chemin de tournefeuille 31300 Toulouse, France

[3] ToxAlim (ToxAlim) – Institut National Polytechnique [Toulouse], Institut National de la Recherche Agronomique : UMR1331, Université Paul Sabatier - Toulouse 3, Ecole Nationale Vétérinaire de Toulouse – 23 ch. Capelles 31076 TOULOUSE, France

[4] INRA, Centre de Recherche en Toxicologie Alimentaire (INRA Toxalim) – Université de Toulouse, INRA, ENVT, INP-Purpan, UPS – 180 chemin de Tournefeuille, 31027 Toulouse, France [5] METATOUL – Inserm – France

Lipids are essential cellular constituents that have many critical roles in physiological functions. They are involved in energy storage, cell signaling as second messengers, and are major constituents of cell membranes including lipid rafts1. Their crucial role is highlighted by their involvement in a large number of heterogeneous diseases such as cancer, diabetes, neurological disorders and inherited metabolic diseases2,3.

Due to the high structural diversity of lipid species arising from various combinations of fatty acyls and functional headgroups, the presence of isomeric and isobaric lipid species and their occurrence at a large concentration scale, a complete lipidomic profiling of biological matrices remains a challenge. In this context, the aim of our study presented in this poster is to develop an untargeted lipidomic approach by using supercritical fluid chromatography high resolution mass spectrometry to be able to propose a relative quantification of main lipids class in various biological sample.

To develop the method, the optimization of the separation and the detection of lipid species were performed on pure standards and then on liver lipid extract from 12-weeks-old C57BL6/J mice exposed to a control or an HFD (60% lipids in the diet) during 14 weeks. These analyses allowed the building of a homemade lipid data bank. An automatic process was then developed to produce the relative quantification of lipids species belonging to the 6 main class of lipids. The optimization of this method will be present with the first results obtained on liver disease model.

Mots-Clés: lipidomic profiling, Data processing, SFC, HRMS

Poster 61 – P61

” Less is More " : Analyse de données multivariées issues de plans d’expériences multifactorielles

Víctor González-Ruiz [1,2], Domitille Schvartz [2,3], Jenny Sandström [2,4], Julian Pezzati [1], Fabienne Jeanneret [1,2], David Tonoli [1,2], Julien Boccard [1,2], Florianne Monnet-Tschudi [2,4], Jean-Charles Sanchez [2,3], Serge Rudaz [1,2]

[1] Analytical Sciences, School of Pharmaceutical Sciences, University of Geneva – Suisse
[2] Swiss Centre for Applied Human Toxicology (SCAHT) – Suisse
[3] Translational Biomarker Group, Department of Internal Medicine Specialties, University of Geneva – Suisse
[4] Department of Physiology, University of Lausanne, Switzerland – Suisse

Les plans d’expériences (Design of Experiments, DOE) permettent l’évaluation systématique des différents effets potentiels lorsque plusieurs facteurs expérimentaux sont étudiés simultanément. Toutefois, plusieurs sources de variabilités influencent les signaux mesurés et la décomposition de la variance présente une difficulté supplémentaire dans le cadre de données métabolomiques complexes. Dans cette étude, nous appliquons l’algorithme ANOVA Multiblock OPLS (AMOPLS), à l’étude des processus neuro-inflammatoires dans des cultures de cellules souches neurales 3D de rats.
Les cultures cellulaires ont été exposées au trimethylétain (TMT) et trois facteurs expérimentaux ont été considérés dans le DOE, à savoir (i) la maturité des cellules, (ii) le temps d’exposition et (iii) la concentration en TMT. Les métabolites ont été mesurés et identifiés à l’aide de quatre analyses LC-MS (RP+/- et HILIC+/- avec Q-TOF). L’analyse des données a été effectuée à l’aide d’un modèle consensus-AMOPLS sous MATLAB.

La méthode AMOPLS permet la décomposition et la quantification des sources de variabilité provenant de chaque effet expérimental. Les contributions les plus importantes proviennent de l’état de maturation, du temps d’exposition et de leur interaction (22.3%, 15.3% et 13.5% de la variabilité totale). Bien que faible (6.3%), la contribution de la dose de TMT est très significative (p-value < 0.01%). Une étape originale de déconvolution s’appuyant sur les valeurs des VIP2 permet de dévoiler la variabilité de chaque métabolite en relation avec chaque effet étudié. Les métabolites avec les plus grandes valeurs de VIP2 pour l’effet de la dose ont été utilisés pour l’analyse de voies métaboliques et mettent en évidence des altérations de (i) la différentiation neuronale, (ii) la signalisation nicotinique et (iii) le métabolisme du GABA en conséquence de l’exposition au TMT.
Dans cette étude, l’algorithme AMOPLS a permis lánalyse des données incluant un DOE. À partir d’un modèle global, les métabolites reliés à chaque facteur expérimental ont été mis en évidence. Nos résultats ont montré une forte perturbation de la neurotransmission GABAergique après l’exposition au TMT.

Mots-Clés: analyse multivariée, toxicologie, décomposition d’effets

Poster 62 – P62

LipidQuan : A Targeted Lipid Profiling Solution Using UPLC/MS/MS

Mickael Hybois [1], Nyasha Munjoma , Giorgis Isaac , Gethings Lee , Jimmy Hunk , Robert Plumb

[1] Waters Corporation – Waters Corporation – Saint Quentin en Yvelines, France

Although advances in mass spectrometry (MS) have allowed for more in-depth lipdomics analysis, unambiguous identification and quantification has proven difficult as lipids exhibit a high number of isomeric and isobaric species. Furthermore, MS spectra often contain peaks and fragments from multiple compounds making confident identification and relative quantification of specific molecular species difficult and time consuming. As a result, the transfer of lipidomic data between laboratories is severely hindered, making multi-site study interpretation a challenge.

A hydrophilic interaction chromatography (HILIC) based approach for the separation of lipids by class prior to MS analysis is a proven method of reducing identification ambiguity. An additional benefit of separating lipid species by class is that fewer stable isotope labelled (SIL) standards are required for quantification, conferring a cost saving.

Key Features :

- Streamlined and integrated lipidomics workflow (from sample preparation through to biological interpretations)
- Highly specific MRM transitions based on the fatty acyl chain fragments when applicable instead of the typical head group fragments to improve identification and specificity
- Routine targeted quantification of common lipids in plasma and serum
- Lipid class based separation reduces the number of stable isotope lipid standards (SILS) which results in significant cost saving
- Over 2000 lipid species MRMs
- Selection of screening method application notes available for download

Mots-Clés: Lipid, Lipids, LipidQuan, MetaboQuan, LC/MS/MS, lipidomics, HILIC, Waters

Poster 63 – P63

Étude par RMN du monde caché d'un holobionte : à la recherche de la relation entre éponge et champignon marin

Aurore Michaud [1,2,3], Jonathan Farjon [2], Serge Akoka [2], Yves-François Pouchus [1], Emmanuel Gentil [1], Olivier Grovel† [1], Patrick Giraudeau [2,4]

[1] Mer, molécules et santé EA 2160, Université de Nantes, Nantes – Université de Nantes : EA2160 – France
[2] Chimie Et Interdisciplinarité : Synthèse, Analyse, Modélisation, (CEISAM), Université de Nantes, CNRS, UMR 6230, Nantes
[3] Plateforme CORSAIRE, Biogenouest, Université de Nantes – Université de Nantes, Plateforme Génomique Santé Biogenouest R ,
[4] Institut Universitaire de France – Ministère de l'Education nationale, de l'Enseignement supérieur et de la Recherche, Ministère de lÉducation nationale, de l'Enseignement supérieur et de la Recherche, Ministère de lÉducation nationale, de l'Enseignement supérieur et de la Recherche, Ministère de lÉducation nationale, de l'Enseignement supérieur et de la Recherche, Ministère de lÉducation nationale, de l'Enseignement supérieur et de la Recherche – France

Les holobiontes sont la source de nombreuses questions en écologie chimique. L'une d'entre elles concerne l'intérêt de chacun des partenaires à vivre en cohabitation. Notre projet, s'intéressant à la souche fongique *Eurotium* sp. MMS1599 isolée de l'éponge marine *Tetilla* sp., n'échappe pas à cette interrogation et consiste en l'étude par métabolomique des dialogues moléculaires entre organismes.
Pour plonger au cœur de l'expression du métabolome de MMS1599 lorsqu'elle est en présence de son hôte originel, une double approche analytique associant spectroscopie de résonance magnétique nucléaire et spectrométrie de masse est employée. Ici, nous nous focalisons sur la démarche appliquée, comportant des outils rapides et sensibles en RMN multi-impulsionnelle. En effet, si la RMN du proton est couramment employée en analyse métabolomique pour sa rapidité et sa sensibilité, elle présente néanmoins un inconvénient majeur que sont les recouvrements de signaux lors d'analyses de mélanges complexes. Pour pallier cela, il faut alors envisager des séquences à deux dimensions permettant l'éclatement des signaux tout en gardant des temps d'analyse raisonnables. Nous présenterons ici une boîte à outils constituée d'expériences RMN récemment développées au laboratoire, ayant une excellente répétabilité et un temps d'expérience réduit. Trois types d'expériences ont été explorées : une INEPT 13C adiabatique, une 2D COSY 1H ultrarapide et une 2D HSQC 1H-13C accélérée et hautement résolue. Ces techniques optimisées pour le cas d'extraits de cultures de champignons marins permettent d'obtenir le meilleur compromis entre sensibilité, répétabilité, dispersion des signaux et temps d'expérience. Leur potentiel sera évalué pour le profilage métabolomique par RMN multi-dimensionnelle, une approche dont nous avons récemment démontré l'intérêt par rapport aux approches classiques. La combinaison des données RMN à celles de spectrométrie de masse permettra à terme, en utilisant des approches statistiques, de découvrir les mécanismes d'expression ou répression métaboliques en jeu chez *Eurotium* sp. lorsqu'il se trouve en présence de son organisme hôte *Tetilla* sp. et ainsi de comprendre les voies biosynthétiques mobilisées.

Mots-Clés: Ecologie chimique, Holobionte, éponge, souche fongique marine, RMN, méthodes rapides, INEPT, COSY ultrarapide, QUIPU, HSQC

Poster 64 – P64

Corsaire, plate-forme de métabolomique du Grand Ouest

Léa Cabioch[1]

[1] Biogenouest – Biogenouest – France

Corsaire est une fédération de plateaux analytiques dédiée à l'analyse de petites molécules biologiques. Elle couvre les domaines de la métabolomique, la fluxomique, l'isotopomique et l'analyse structurale. La biologie marine, l'agronomie, la santé et l'écologie chimique sont le cœur de l'activité de Corsaire. Ses approches analytiques vont de l'approche non ciblée pour la recherche de biomarqueurs, à l'approche ciblée pour l'analyse qualitative et quantitative de molécules d'intérêt. Pour ce faire, la plate-forme dispose d'un large parc analytique, composé d'équipements de RMN et de spectrométrie de masse, associés à différentes techniques séparatives adaptées à des matrices biologiques de nature et d'origines variées. Un accompagnement de la procédure analytique est proposé depuis la prise en charge des échantillons biologiques, l'extraction, les phases séparatives, l'acquisition des données brutes et, au besoin, leur traitement chimiométrique.

Mots-Clés: métabolomique, plateforme, Biogenouest, RMN, Spectrométrie de Masse

Poster 65 – P65

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

Isabelle Schmitz-Afonso[1], Justine Ferey [1], Marion Larroque [2], Brice Bouyssière [3], Sandra Mounicou [3], Olivia Sgarbura [4], François Quenet [4], Sébastien Carrere [4], Carlos Afonso [1]

[1] Normandie Univ, FR 3038; IRCOF, (COBRA) - Université de Rouen, CNRS : UMR6014, INSA Rouen – Université de Rouen,
[2] ICM/ Unité de Recherche Translationnelle, Campus Val d'Aurelle – ICM – 208 rue des Apothicaires, 34296 Montpellier, France, France
[3] CNRS/UPPA, UMR 5254, LCABIE – LCABIE – France
[4] ICM/ Service Chirurgie A2, Campus Val d'Aurelle – ICM – France

Peritoneal carcinomatosis is a common extension of colon cancer. Platinum derivatives, such as oxaliplatin, are common metallodrugs applied during the Heated Intraoperative Chemotherapy (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. Previous 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 Spectrometer (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 αcyano-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 isotopic fine structure.

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

Mots-Clés: Mass Spectrometry Imaging, FTICR, metabolite

Poster 66 – P66

The Metaboscope : a metabolomic platform for the comprehensive profiling of metabolites

Florian Vasaï[1], Raphaël Lugan[1]

[1] UMR Qualisud – Université d'Avignon – France

The METABOSCOPE is a new metabolomic platform located at Avignon university, designed to meet the needs of scientists in a wide range of fundamental or applied researches. . It is composed of 5 mass spectrometers hyphenated to liquid and gas chromatographs enabling untargeted or multi-targeted profilings, MS imaging and metabolite purification. Highly sensitive quantitative analysis of multiple targets can be performed by multiple reaction monitoring (MRM), with a triple quadrupole coupled to an ESI source and an UPLC UPLC-ESI-TQ. Non-targeted analyzes are achieved with an UPLC-Q-TOF ; this high resolution (40000) mass spectrometer is also implemented with an ion mobility cell for further metabolites separation, and comes with a MALDI source for *in situ* localization of compounds. A GC-TOF is dedicated to the profiling of primary metabolites (small carbohydrates, fatty acids, amino acids and organic acids), after a fully automated online chemical derivatization insuring high replicability; another GC-MS is fitted with a fully automated dynamic headspace collection system to analyze volatile compounds and a pyrolyzer for polymer structural characterization. Finally, molecules can be purified with a semi-preparative HPLC-MS enabling fraction collection based on mass signals. The Metaboscope provides analytical services but also method development, tips and practical training courses. It is opened to any collaboration with private or public partners.

Mots-Clés: metabolomic platform, analytic, collaboration

Poster 67 – P67

Headspace-Solid Phase Micro Extraction-Gas Chromatography-Quadrupole Mass Spectrometry-based metabolomics for kinetics tracking of natural herbicides' volatile residues: a simple non-destructive method

Hikmat Ghosson[1,2], Delphine Raviglione [1,2], Marie-Virginie Salvia [1,2,3], Cédric Bertrand [1,2,3,4]

[1] UFR Sciences Exactes et Expérimentales, Université de Perpignan Via Domitia, 52 Avenue Paul Alduy, 66860 Perpignan
[2] PSL Université Paris: EPHE-UPVD-CNRS, USR 3278 CRIOBE, Université de Perpignan,
[3] Laboratoire d'Excellence « CORAIL », Université de Perpignan, 52 Avenue Paul Alduy, 66860 Perpignan Cedex, France – EPHE-CNRS-UPVD – France

Despite their known risks, herbicides are still essential for agriculture. Thus, natural herbicides are increasingly recommended to replace synthetic ones. They are supposed less harmful on human health and ecosystem. However, there are still limitations in studying the environmental fate of several of them. The main reason is the lack of methods dedicated for this type of herbicides. In fact, they usually consist of complex mixtures of substances, of which several are unknown.

Hence, in the framework of the Environmental Metabolic Footprinting (EMF) approach, the current work presents a method dedicated to analyze the volatile residues of herbicides applied on soil. The aim is to track the evolution of the volatile compounds issued from the herbicide by an untargeted metabolomics-based kinetics, in order to determine the "resilience time" of the gaseous phase above the soil. The approach aims to explore the environmental fate of these herbicides by combining it to other methods of the EMF. Moreover, it can estimate the exposure of farmers, insects and plants to potential toxic volatile substances.

The method is based on Headspace-Solid Phase Micro Extraction-Gas Chromatography-Quadrupole Mass Spectrometry (HS-SPME-GC-Q MS). The HS-SPME provides a non-destructive extraction. This allows reducing the number of samples. The GC separation technique provides high reproducible analysis for volatile compounds. In addition, it allows the calculation of the Retention Index (RI) as a tool for the molecular characterization. On the other hand, despite the low resolution of the Quadrupole mass analyzer, the Electronic Impact provides reproducible MS fragmentation patterns used for spectral library search and putative identification of unknown compounds.

The method was experimented for a pilot study. It was applied on a natural herbicide: the extract of *Myrica gale*, containing the Myrigalone A active substance. The setup of the analytical tool was performed by optimizing the system parameters. Then, a 38 days kinetics study was applied on control and spiked soil samples. Results proved the reliability of the method and demonstrated the robustness of the system and low matrix effect. Thus, new high scale experiments are planned. The integration of other natural and synthetic herbicides will be also considered.

Mots-Clés: Volatolomics, Headspace, Solid Phase Micro Extraction, GC, MS, Environmental Metabolic Footprinting, Natural herbicides

Poster 68 – P68

Exploration du métabolome d'un individu

Cécile Martias[1], Raphaëlle Voisin , Sylvie Mavel [2], Antoine Lefèvre [3], Frédéric Montigny [4], Lydie Nadal-Desbarats [1,4], Patrick Emond [2]

[1] UMR 1253 iBrain, Université de Tours, Inserm, F-37000 Tours, France – Université de Tours – France [2] Université François-Rabelais, INSERM U930 "Imagerie et Cerveau", CHRU de Tours – Inserm : UMR930 – 10 Bv Tonnellé, 37044 Tours, France

[3] Université François-Rabelais, INSERM U930 "Imagerie et Cerveau", CHRU de Tours – Inserm : U930, Inserm : U930 – 10 Bv Tonnellé, 37044 Tours, France

[4] PST-ASB, Université de Tours, F-37000 Tours, France – Université de Tours – France

Contexte :
L'approche métabolomique se concentre classiquement sur l'étude d'un type de prélèvement par une seule plateforme analytique. Une couverture métabolique la plus large possible (multicompartiments et multi-plateformes) permettrait l'élaboration d'une carte d'identité métabolique d'un individu.
Objectif :
Standardiser pour la salive,les fèces et l'urine, le protocole d'extraction le plus adapté pour une exploration en RMN du 1H et en UHPLC-MS (HILIC et C18) afin d'avoir la couverture métabolique la plus exhaustive possible.
Matériels et Méthodes :
Pour la salive, les fèces et l'urine différents protocoles d'extraction ont été testés (précipitation des macromolécules par ACN, MeOH, dilution) et analysés en 1H-RMN, UHPLC-MS (HILIC, C18) en détection positif et négatif.
Le nombre de composés, le CV moyen a été calculé pour chaque méthode d'extraction et chaque plateforme analytique afin de choisir le protocole d'extraction le plus adapté. La complémentarité des plateformes a été évaluée par diagramme de Venn.
Résultats :
L'efficacité des protocoles d'extraction de métabolites pour chaque matrice est comparée par les analyses, en parallèle, 1H-RMN et UPLC-MS (HILIC, C18) en détection positif et négatif.
Pour la matrice salive, le solvant acétonitrile permet une plus grande extraction des métabolites soit 515 molécules potentielles. Une fois la redondance enlevée, la couverture métabolomique salivaire d'un individu est de 323 molécules.
Pour la matrice fèces, le solvant acétonitrile/methanol/eau permet l'extraction de 642 molécules potentielles. Une fois la redondance enlevée, la couverture métabolomique fécale d'un individu est de 391 molécules.
Pour la matrice urine, le solvant méthanol permet l'extraction de 585 métabolites potentielles. Une fois la redondance enlevée, la couverture métabolomique urinaire d'un individu est de 395 métabolites.
Conclusion :
Cette étude montre la complémentarité des plateformes 1H-RMN, UHPLC-MS (HILIC, C18) en détection positif et négatif pour l'exploration du métabolome d'un individu

Mots-Clés: Multi, matrice, Multi, plateforme, UHPLC, MS, RMN, carte d'identité métabolique, urine, salive, fèces

Poster 69 – P69

Développements analytiques pour la caractérisation non-ciblée de l'exposome chimique dans des matrices biologiques humaines

Jade Chaker [1], David M. Kristensen [2], Bernard Jégou [1], Arthur David [1]

[1] Univ Rennes, Inserm, EHESP, Irset (Institut de recherche en santé, environnement et travail) UMR$s1085,F-35000 Rennes, France--Univ Rennes--France$

[2] Department of Neurology, Danish Headache Center, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark – Danemark

L'exposition de l'homme aux mélanges complexes de xénobiotiques (i.e. exposome chimique) peut dans certains cas être un facteur environnemental d'importance contribuant à l'apparition de certaines maladies chroniques non-transmissibles. Il est donc primordial de pouvoir caractériser cet exposome chimique dans les matrices biologiques humaines afin d'évaluer son impact dans la survenue de ces maladies. Pour cela, les approches analytiques non-ciblées reposant sur la chromatographie liquide liée à la spectrométrie de masse à haute résolution (CL-ESI-SMHR) sont souvent utilisées afin de détecter sans à priori les mélanges de xénobiotiques accumulées dans ces matrices. L'utilisation de ces approches non-ciblées sur la matrice sang (plasma ou sérum) s'avère pertinent puisque cette matrice est couramment échantillonnée dans les études épidémiologiques ou de biosurveillance. Toutefois, ces méthodes non-ciblées reposant sur la CLSMHR sont souvent sujettes à des interférences analytiques importantes dues, entre autres, à la suppression d'ion. Elles nécessitent donc des développements analytiques afin d'améliorer leur sensibilité analytique mais également leur robustesse dans le but de pouvoir les appliquer à grande échelle. Afin de surmonter ces limites analytiques, onze méthodes de préparation d'échantillon reposant sur des méthodes de délipidation/déprotéination ou d'extraction en phase solide (SPE) ont été comparées avec une méthode traditionnellement utilisée pour les analyses non-ciblées (précipitation de protéine). Des critères quantitatifs (ex. recouvrement, effet de matrice) et qualitatifs (ex. facilité et rapidité d'implémentation) ont été utilisés pour l'évaluation de ces méthodes pour les matrices sérum et plasma. Ces résultats préliminaires ainsi que leur application sur des échantillons de cohorte mère-enfant seront présentés. Ces méthodes ont pour but d'être par la suite appliquées à grande échelle dans le cadre d'une étude épidémiologique (cohorte mèreenfant Danoise) pour renforcer la caractérisation de l'exposition humaine aux xénobiotiques et de mieux comprendre leurs impacts potentiels sur la santé humaine.

Mots-Clés: Exposome, Non ciblé, Xénobiotiques

Poster 70 – P70

Profiling and annotation of flavonoids using a product ion-dependent MS^n data acquisition method on a Tribrid Orbitrap mass spectrometer

Marie-Pierre Pavageau[1], Reiko Kiyonami[1], Iwao Sakane[2], Seema Sharma[1], Graeme Mcalister[1], Caroline Ding[1] and Andreas Huhmer[1]

[1]Thermo Fisher Scientific, San Jose, CA, USA, [2]ITO EN, LTD, Tokyo, Japan

The untargeted profiling of flavonoids provides insights into their biological functions and potential health benefits for humans. However, comprehensive identification of flavonoids from real samples remains challenging because of the limited availability of authentic standards and the structural diversity of this class of compounds. Previous studies relied upon extensive expert knowledge about fragmentation rules, a priori knowledge of the structures of flavonoids, and simple MS2 based analyses that are often not sufficient for complete structural characterization. Here we present a new flavonoid profiling workflow that uses comprehensive fragment ion information from HCD MS-MS and higher order $FTMS^n$ for rapid flavonoid identification and quantitation on a Tribrid Orbitrap mass spectrometer.

As the proof of concept of the workflow, flavonoid extracts from different types of natural products were tested. A C18 column was used for flavonoid separation, and a modified Orbitrap Fusion™ Tribrid™ mass spectrometer was used for collecting HRAM MS and MS^n (up to MS^5) data. The collected data were processed using Mass Frontier 8.0 and Compound Discoverer™ 3.0 software. A novel structure ranking algorithm included in the Compound Discoverer 3.0 software was applied to the MS and MS^n data for confident structure elucidation of the unknown flavonoids based on ChemSpider database and custom flavonoids database. The MS^n data were critical, especially for the identification of flavonoid glycocnjugates.

Brief summary : The new LC-MS^n workflow enables improved throughput, identification coverage and confidence for flavonoids profiling experiments

Communication Poster

Session Traitement du signal - Statistiques - Intégration des données

RÉSEAU FRANCOPHONE DE MÉTABOLOMIQUE ET FLUXOMIQUE

Poster 71 – P71

R FiBiCo script : In silico screening of bioactive compounds from extracts

Lucie Ory [1], El-Hassane Nazih [1], Yves-François Pouchus [1], Samuel Bertrand [1,2], Catherine Roullier [1,2]

[1] Mer, molécules et santé, MMS-EA 2160 – Université de Nantes – France
[2] Corsaire-ThalassOMICS Metabolomics Facility – Université de Nantes, Université de Nantes – France

In natural product drug discovery, several strategies have emerged to highlight specifically bioactive compound(s) within complex mixtures (fractions or crude extracts) using metabolomics tools. In this area, a great deal of interest has raised among the scientific community on strategies to link chemical profiles and associated biological data, leading to the new field called "biochemometrics". The project presented here falls into this emerging research by proposing a complete workflow, which was divided into three major steps. The first one consists in the fractionation of the same extract using four different chromatographic stationary phases and appropriated elution conditions to obtain five fractions for each column. The second step corresponds to the acquisition of chemical profiles using HPLC-HRMS analysis, and the biological evaluation of each fraction. The last step evaluates the links between the relative abundances of molecules present in fractions (peak area) and the global bioactivity level observed for each fraction. To this purpose, an original bioinformatics script (encoded with R Studio software) using the combination of four statistical models (Spearman, F-PCA, PLS, PLS-DA) was here developed leading to the generation of a "Super list" of potential bioactive compounds together with a predictive score. This strategy was validated by its application on a marine-derived *Penicillium chrysogenum* extract exhibiting antiproliferative activity on breast cancer cells (MCF-7 cells). After the three steps of the workflow, one main compound was highlighted as responsible for the bioactivity and identified as ergosterol. Its antiproliferative activity was confirmed with an IC50 of 0.10 μM on MCF-7 cells. The script efficiency was further demonstrated by comparing the results obtained with a different recently described approach based on NMR profiling and by virtually modifying the data to evaluate the computational tool behaviour. This approach represents a new and efficient tool to tackle some of the bottlenecks in natural product drug discovery programs.

Mots-Clés: metabolomics, biochemometrics, natural products, Liquid Chromatography, Mass Spectrometry, R script

Poster 72 – P72

Comparison of PLS-derived sparse methods to find accurate biomarkers in NMR spectra.

Manon Martin[1], Bernadette Govaerts [1]

[1] ISBA, UCLouvain – Belgique

The search for biomarkers in metabolomics is a vast topic with a multitude of methodological procedures that are suggested in the literature, and where some of them are rather intricate. Furthermore, the true biomarkers' location is usually unknown, and some uncertainty remains in the selected biomarkers' validity. This contribution compares the performances of common multivariate analysis methods, (O)PLS-DA, and their sparse extensions in a simulation study. The aim is to decipher pros and cons of the different approaches to help the researchers opt for the best method to apply, given their dataset attributes and the scope of their study. The dataset on which is based this simulation study consists in a two-class problem with simulated NMR spectra, where the exact location of biomarkers is known. Hence, the accuracy of the selected biomarkers can be precisely evaluated, in particular with ROC curves. Different dataset characteristics are stressed (size, degree of noise) to mimic the variability in the experimental datasets characteristics. Additionally, other performance metrics, such as feature stability and prediction accuracy, are also studied.

Mots-Clés: (O)PLS, sparse, simulated data, feature selection

Poster 73 – P73

” Less is More " : Analyse de données multivariées issues de plans d’expériences multifactorielles

Víctor González-Ruiz [1,2], Domitille Schvartz [2,3], Jenny Sandström [2,4], Julian Pezzati [1], Fabienne Jeanneret [1,2], David Tonoli [1,2], Julien Boccard [1,2], Florianne Monnet-Tschudi [2,4], Jean-Charles Sanchez [2,3], Serge Rudaz [1,2]

[5] Analytical Sciences, School of Pharmaceutical Sciences, University of Geneva – Suisse
[6] Swiss Centre for Applied Human Toxicology (SCAHT) – Suisse
[7] Translational Biomarker Group, Department of Internal Medicine Specialties, University of Geneva – Suisse
[8] Department of Physiology, University of Lausanne, Switzerland – Suisse

Les plans d’expériences (Design of Experiments, DOE) permettent l’évaluation systématique des différents effets potentiels lorsque plusieurs facteurs expérimentaux sont étudiés simultanément. Toutefois, plusieurs sources de variabilités influencent les signaux mesurés et la décomposition de la variance présente une difficulté supplémentaire dans le cadre de données métabolomiques complexes. Dans cette étude, nous appliquons l’algorithme ANOVA Multiblock OPLS (AMOPLS), à l’étude des processus neuro-inflammatoires dans des cultures de cellules souches neurales 3D de rats.
Les cultures cellulaires ont été exposées au trimethylétain (TMT) et trois facteurs expérimentaux ont été considérés dans le DOE, à savoir (i) la maturité des cellules, (ii) le temps d’exposition et (iii) la concentration en TMT. Les métabolites ont été mesurés et identifiés à l’aide de quatre analyses LC-MS (RP+/- et HILIC+/- avec Q-TOF). L’analyse des données a été effectuée à l’aide d’un modèle consensus-AMOPLS sous MATLAB.

La méthode AMOPLS permet la décomposition et la quantification des sources de variabilité provenant de chaque effet expérimental. Les contributions les plus importantes proviennent de l’état de maturation, du temps d’exposition et de leur interaction (22.3%, 15.3% et 13.5% de la variabilité totale). Bien que faible (6.3%), la contribution de la dose de TMT est très significative (p-value < 0.01%). Une étape originale de déconvolution s’appuyant sur les valeurs des VIP2 permet de dévoiler la variabilité de chaque métabolite en relation avec chaque effet étudié. Les métabolites avec les plus grandes valeurs de VIP2 pour l’effet de la dose ont été utilisés pour l’analyse de voies métaboliques et mettent en évidence des altérations de (i) la différentiation neuronale, (ii) la signalisation nicotinique et (iii) le métabolisme du GABA en conséquence de l’exposition au TMT.
Dans cette étude, l’algorithme AMOPLS a permis lánalyse des données incluant un DOE. À partir d’un modèle global, les métabolites reliés à chaque facteur expérimental ont été mis en évidence. Nos résultats ont montré une forte perturbation de la neurotransmission GABAergique après l’exposition au TMT.

Mots-Clés: analyse multivariée, toxicologie, décomposition d’effets

Poster 74 – P74

MIMOSA: A mass spectrometry application for processing and analysis of metabolomics data – Application to lipidomics

Joséphine Abi-Ghanem [1], Djomangan Adama Ouattara [1]

[1] BIOASTER – Omics Hub – France

MIMOSA (Mass spectrometry processIng of MetabOlomicS DatA) is a transversal computational framework for metabolomics. MIMOSA allows building simple to complex pipelines to process and analyze metabolomics data with a high reproducibility and traceability. MIMOSA is ready-to-use, fast and extendable. It is specially designed to handle large metabolomics data (up to hundreds of raw files) in a reasonable time laps on a classical laptop. It allows interfacing available software like ProteoWizard, OpenMS and built-in functions for data normalization, statistical analysis and the identification of metabolites using Sirius, Metfrag and LipidMatch. As well, other tools can easily be interfaced into the MIMOSA to build customized workflows. MIMOSA is available as open Matlab scripts and a standalone application to non-developer users. Its extension to more open programming languages is planned. MIMOSA is also presented in a workshop at the 12th RFMF. This workshop introduces the main fundamentals of the bioinformatics tools developed at BIOASTER for the processing and analysis of mass spectrometry data in metabolomics, with a focus on lipidomics.

Mots-Clés: MIMOSA, Lipidomics, Computational metabolomics, Analysis of metabolomics data, Metabolite identification, Workflow traceability

Poster 75 – P75

NMR-based metabolomic analysis of young St.John's Wort plants grown under different color LED lighting

Paul Shipley [1], Francois Miros [1]

[1] University of British Columbia – Canada

The use of metabolomics techniques to classify unrelated species, in particular for the detection of adulteration, has proven to be robust and relatively method-independent. In contrast, metabolomics analysis of more closely related samples, such as highly related species or different growing conditions, both classification and identification of significant metabolites can approach the false discovery rate. *Hypericum perforatum*, or St. John's wort, is a flowering plant native to parts of Asia and Europe, and invasive to several other regions including North America. St. John's wort has a long history of use in traditional medicine for the treatment of depression. In this study, clonally propagated St. John's wort were grown in culture with different light conditions. NMR data for the lyophilized and extracted plants were acquired and investigated using uni- and multivariate statistical methods. Two methods of feature reduction were used and compared, and metabolites that drove variance between plants grown in the different lighting conditions identified using VIP identification from multiple models, coupled with STOCSY analysis to identify peaks covarying with the identified peaks. It was found that primary metabolites varied most strongly in relationship to the ratio of red to blue light, corresponding to chlorophyll A and B absorbances.

Mots-Clés: Medicinal plants, NMR, magnetic resonance, model validation, significant metabolite identification, untargeted metabolomics

Poster 76 – P76

CANPA: Computer-Assisted Natural Products Anticipation

Alexander Enrique Fox Ramos , Coralie Pavesi , Marc Litaudon [1], Vincent Dumontet , Erwan Poupon [2], Pierre Champy [3], Grégory Genta-Jouve [4], Mehdi Beniddir [5]

[1] Institut de Chimie des Substances Naturelles – Centre National de la Recherche Scientifique : UPR2301 – France
[2] BioCIS – Université Paris Sud - Paris XI – France
[3] CNRS UMR 8076 BioCIS - BIOmolécules : Conception, Isolement et Synthèse (BioCIS) – CNRS : UMR8076 – CNRS UMR 8076 BioCIS - BIOmolécules : Conception, Isolement et Synthèse Bureau D5 309 - Bâtiment D5 - 5, rue J.B. Clément 92296 Châtenay-Malabry, France, France
[4] Université Paris Descartes (COMETE) – CNRS : UMR8638 – 4 Avenue de l'observatoire, France
[5] Université Paris Sud, Laboratoire de Pharmacognosie associé au CNRS (UPS, BioCIS UMR CNRS 8076) – Université Paris XI - Paris Sud – 5, rue Jean-Baptiste Clément, 92296 Châtenay-Malabry, France

Traditional natural products discovery workflows implying a combination of different targeting strategies including structure- and/or bioactivity-based approaches, afford no information about new compound structure until late in the discovery pipeline. By integrating a MS/MS prediction module and a collaborative library of (bio)chemical transformations, we have developed a new platform, coined MetWork, that is able of anticipating the structural identity of metabolites starting from any identified compound. In our quest to discover new monoterpene indole alkaloids, we demonstrate the utility of the MetWork platform by anticipating the structures of five previously undescribed sarpagine-like *N*-oxide alkaloids that have been targeted and isolated from the leaves of *Alstonia balansae* using a molecular networking-based dereplication strategy fueled by computer-generated annotations. This study constitutes the first example of a natural product discovery workflow, termed CANPA, in which the targeted structures were initially generated and therefore anticipated by a computer prior to their isolation.

Mots-Clés: chemical prospecting, natural products, alkaloids, structure elucidation, anticipation, in silico metabolization, MetWork

Poster 77 – P77

Overview of multi-organ lipid profile alteration in pre-diabetic mice through multiblock modeling

Florence Mehl [1], Hervé Lestunff [2], Kelly Meneyrol [3], Jessica Denom [3], Céline Cruciani-Guglielmacci [3], Leonore Wigger [1], Ana Rodriguez Sanchez-Archidona [4], Christian Klose [5], Mathias J. Gerl [5], Kai Simons [5], Bernard Thorens [4], Christophe Magnan [3], Mark Ibberson [1]

[1] Swiss Institute of Bioinformatics (SIB) – Suisse
[2] Institut des Neurosciences de Paris-Saclay – Université Paris-Sud - Paris 11, Centre National de la Recherche Scientifique : UMR9197 – France
[3] Biologie Fonctionnelle Adaptative" (BFA) UMR 8251 - – Université Paris Diderot - Paris 7 – France
[4] Center for Integrative Genomics - University of Lausanne – Suisse [5] Lipotype GmbH, Tatzberg 47, 01307 Dresden – Allemagne

Hyperlipidemia is a common feature of the metabolic syndrome and has been linked with type II diabetes and insulin resistance. It is thus of high importance to study how the lipid profile is affected when metabolism is subjected to high-energy dietary stress conditions leading to pre-diabetic phenotype.
In this work, three mice strains were involved. Although the mouse strain C57Bl/6 has been considered the strain of choice to study type II diabetes and the metabolic syndrome in mouse models, a recent comparison of the phenotypic and metabolic response to high fat (HF) diet of multiple strains in parallel through out different conclusions; highlighting not only C57Bl/6 but also DBA/2J and BALB/cJ as the strains that developed a clear pre-diabetic phenotype under high-energy dietary stress conditions with different features that may be relevant for the study of diabetes in both mouse models and human (Cruciani-Guglielmacci C, et al. 2017).

In order to find a common underlying mechanism regardless the different phenotypes of these genetically different strains under high fat diet; liver, adipose and soleus muscle tissues as well as plasma were sampled and shotgun lipidomic analyses were realized.

To better understand how lipidome reacts under high-energy dietary stress conditions it is mandatory that all lipid profiles from the different tissues and biofluid are integrated in a global model. A Common Component and Specific Weights Analysis (CCSWA) allowed us to integrate all lipid profiles from the 4 different biological matrices in a single model (Qannari, EM, et al. 2000). This unsupervised multiblock analysis showed that the most important and common source of variation of the dataset is the diet. This method enables the evaluation of consequences of a high fat diet on the lipid profiles of liver, muscle, adipose tissue and plasma.

Cruciani-Guglielmacci C, et al. Molecular phenotyping of multiple mouse strains under metabolic challenge uncovers a role for Elovl2 in glucose-induced insulin secretion. Mol Metab. 2017 Jan 26;6(4):340-351.Qannari EM, Wakeling I, Courcoux P, MacFie HJH. Defining the underlying sensory dimensions. Journal of Food Quality and Preference. 2000:11, 151–154.

data integration, lipidomics, diabetes, chemometrics, multiblock

Communication Poster

Session Développements bioinformatiques - Outils d'intégration

Poster 78 – Associé FP5

Analytic correlation filtration: A new tool to reduce analytical complexity of metabolomic datasets

Stéphanie Monnerie[1], Mélanie Pétéra [2], Bernard Lyan [2], Pierrette Gaudreau [3], Blandine Comte [1], Estelle Pujos-Guillot [2,1]

[4] Université Clermont Auvergne, INRA, UNH, Mapping – (INRA) : UMR1019 – France

[5] Université Clermont Auvergne, INRA, UNH, Plateforme d'Exploration du Métabolisme, MetaboHUB Clermont – INRA Clermont-Ferrand-Theix : UMR1019 – France

[6] Centre de Recherche du Centre hospitalier de l'Université de Montréal ; Département de médecine, Université de Montréal – Canada

Metabolomics generates massive and complex data that need dedicated workflows to extract the meaningful information and to enrich our knowledge of biological systems. For biological interpretation, experts are mainly focusing on metabolites rather than on the redundant different analytical species. Moreover, the high degree of correlation in datasets is a constraint for the use of data mining and statistical methods.
In this context, we developed a new tool to detect analytical correlation into datasets without confounding them with biological correlations that may exist within samples. The algorithm principle is to group features coming from the same analyte and to propose one single representative per group. The user can define grouping criteria with various options including correlation coefficient, retention time, mass information from a reference list of isotopes, adducts and fragments. Thresholds for all these criteria can be fixed and the representative feature can be determined following four methods according to the user needs and the analytical technology.

We chose to compare the present tool to one of the most commonly used free package proposing a grouping method: 'CAMERA', using its Galaxy version 'CAMERA.annotate' also available in Workflow4Metabolomics (W4M; http://workflow4metabolomics.org). To illustrate the 'Analytic correlation filtration tool' functionalities and the results obtainable on typical experimental data, a published dataset available on W4M was used as an example. This dataset named 'Sacurine' was obtained from human urine samples analysed by LC-HRMS in negative ionization mode (Thevenot *et al*., 2015).

Within the 3,120 ions of the urine dataset, the tool allowed creating 2,651 groups, meaning that 15% of ions are proposed to be filtered because of analytical redundancies. While CAMERA generated more than 20 groups of more than 10 ions, the proposed tool subdivided them into smaller ones corresponding to individual annotated metabolites, thus demonstrating the efficiency and relevance of the present approach.

As a key element in metabolomics data analysis, the tool will be available *via* the web-based galaxy platform W4M with different output files for vizualisation for further data analysis within workflows.

Mots-Clés: analytical redundancies, filtration, workflow

Poster 79 – P79

mlomic, an R package and Shiny application for automated reporting of metabolomics data analysis

Yasmina Adimy [1], Florence Castelli [1], Thaïs Hautbergue [1], François Fenaille [1], Benoit Colsch [1]

[1] Service de Pharmacologie et d'Immunoanalyse, Laboratoire d'Etude du Métabolisme des Médicaments, CEA, INRA, Université Paris Saclay, MetaboHUB, F-91191 Gif-sur-Yvette, France – CEA, CNRS, Université Paris-Saclay, CEA Saclay 91191 Gif sur Yvette France – France

Mass-spectrometry-based metabolomics is widely used in several clinical studies in order to correlate human, animal or microbial metabolic profiles with internal or external factors such as diseases or environmental parameters. However, large datasets produced by mass spectrometry are not directly readable and interpretable by medical and biological staff. Before sending metabolomics results, analytical data need to be translated into relevant biological information, presented under a clear and visual organization. In this context, we aim to automate the generation of analytical reports, integrating interactive visualizations of statistical analysis as well as annotations and providing guidelines to highlight pertinent results and guide further investigations.

We have implemented *mlomic,* a package usable from a shiny user-friendly interface, allowing users without programming experience to easily analyze their data. First steps of data analysis, including preprocessing, normalization and quality control, are done using XCMS implemented in the Workflow4Metabolomics platform. Then, in order to identify significant variables, package's workflow includes two steps: annotation and statistical analysis. Annotation consists in matching variables with either an in-house spectral database gathering more than one thousand compounds (essentially of human origin but also some xenobiotics) or with public databases. Then, we provide univariate and multivariate (PCA, PLS-DA) statistical analysis to identify significant features. All relevant and understandable results are gathered by automated reporting into HTML, PDF or Word documents, integrating interactive visualizations to give more direct insights into data. Finally, reports also include supporting information to help clinicians interpret their results. It allows reproducible and unbiased data treatment in a time-efficient manner with various relevant data visualization options.

This toolbox will afterward include metabolite projection into metabolic pathways and be extended to lipidomics data analysis. We could then make these tools available to the metabolomics community by implementing them in Workflow4Metabolomics.

Mots-Clés: Bioinformatics, shiny, Rmarkdown, Automated reporting, Annotation, Statistical analysis, Interactive visualizations

Poster 80 – P80

Intégration d'outils de LC-MS/MS et de visualisations interactives sur l'infrastructure Galaxy Workflow4Metabolomics

Julien Saint Vanne , Romain Dallet , Erwan Corre , Yann Guitton [1], Gildas Lecorguille

[1] Laberca, Oniris, INRA,Université Bretagne Loire, 44307, Nantes, France – Institut National de la Recherche Agronomique : UMR1329, Ecole Nationale Vétérinaire, Agroalimentaire et de l'alimentation Nantes-Atlantique : UMR1329 – France

L'analyse de données métabolomiques est un processus complexe, à multiples étapes, et en évolution constante avec le développement de nouvelles techniques d'analyse, de nouvelles méthodes mathématiques, de nouveaux outils bioinformatiques ainsi que de nouvelles bases de données. L'infrastructure Galaxy [1] de workflow4metabolomics.org [2,3] (W4M) fournit un environnement unique, centralisé, simple et performant pour créer, exécuter et partager des flux de travail métabolomique pour les technologies de LC-MS, GC-MS et de RMN.

Un des verrous actuels de l'approche métabolomique est l'identification des composés. La spectrométrie de masse en tandem (MS/MS) permet d'apporter une précision sur la structure des composés recherchés. Pour faciliter cette étape d'identification, cette technologie est de plus en plus utilisée par les laboratoires, mais l'analyse de ces données MS/MS reste complexe et fastidieuse. Afin de faciliter cette étape, différents outils d'aide à l'identification ont été développés par la communauté scientifique. Un workflow d'analyse de ces données MS/MS a été créé dans W4M en se basant sur 3 outils reconnus : un outil de sélection de données, msPurity [4], ainsi que deux outils d'identification, metFrag [5] et Sirius-CSI:FingerID [6].

Afin de faciliter l'utilisation des outils Galaxy, un ensemble de visualisations interactives ont été ajoutées à l'infrastructure W4M Galaxy. Le développement récent d'un ensemble d'applications Shiny exécutable dans des environnements interactifs ("Galaxy Interactive Environments") permet désormais une interaction (zoom, sélection d'échantillons, ...) avec des sorties graphiques telles que des chromatogrammes, des "heatmaps" ou des ACP. Toutes ces illustrations étaient classiquement figées au format pdf ou png, sans réelle possibilité d'interaction. Les utilisateurs de W4M peuvent à présent bénéficier de tous ces nouveaux développements.

Mots-Clés: Galaxy, Metabolomique, Interactivité, MS/MS, Identification, MS, NMR, Statistiques

Poster 81 – P81

Mapping lipidomics data on metabolic networks with MetExplore

Florence Vinson [1], Arthur Moreau [1], Pauline Le Faouder [2], Justine Bertrand-Michel [2], Fabien Jourdan [1], Nathalie Poupin [1]

[1] UMR1331 Toxalim – Université de Toulouse, INRA, ENVT, INP-Purpan, UPS – France
[2] Institut des Maladies Métaboliques et Cardiovasculaires – Hôpital de Rangueil, Université Paul Sabatier (UPS) - Toulouse III, Inserm : U1048 – France

Lipidomics has become a powerful tool for investigating alterations in lipid metabolism in various biological contexts (metabolic diseases, xenobiotics exposure ...). MS-based lipidomics analyses allow detecting changes in concentrations of lipids in tissues and cells. To go further in the interpretation of lipidomics profiles and identify the metabolic pathways and reactions that are affected, it is necessary to put lipids in their metabolic context. Genome-scale metabolic network reconstructions, such as the human metabolic network Recon2 [1], gather all the metabolic reactions and their associated metabolites that can occur in a cell or tissue. However, mapping lipids measured in lipidomics experiments on metabolic networks is challenging because of the differences in identifiers and in precision between lipidomics data and metabolic networks. Indeed, in metabolic networks, for many lipids (e.g., triacylglycerols), all specific molecular species are not detailed but only the generic class is represented. To help linking lipids from lipidomics datasets with lipids in metabolic networks, we developed a new mapping method, freely available on the MetExplore webserver (http://www.metexplore.fr) [2]. It allows performing matching based on identifiers from public databases, such as CHEBI and LipidMaps, and uses the ontology defined in those 2 databases to retrieve generic lipid "class" in network for specific lipid species in dataset when these species are not detailed in the network. We applied our method to the list of lipids that can be measured by the MetaboHub-MetaToul lipidomics platform [3],including lipids from different classes (fatty acids,phospholipids, cholesterol esters ...). Among these 611 detectable lipids, only 38 (mostly fatty acids and eicosanoids) could be retrieved in Recon2 metabolic network when performing usual exact mapping, but we were able to map 290 of these lipids when using our new method, additional mapped lipids being mostly glycerolipids and sphingolipids. This new method opens the way for better interpretation of lipidomics data in terms of lipid metabolism modulations. [1]Thiele I, *et al*. Nat Biotechnol. 2013;31: 419–425. [2] Cottret L, *et al*. Nucleic Acids Res. 2018. [3] Khoury S, *et al*. Biomolecules. 2018;8: 174.

Mots-Clés: metabolic networks, lipidomics

Poster 82 – P82

MetExploreViz : Nouvelles fonctionnalités et stratégie de visualisation

Maxime Chazalviel [1], Nathalie Poupin[2], Florence Vinson [2], Clement Frainay [2], Ludovic Cottret [3], Adrien Rohan [2], Fabien Jourdan[2]

[1] MedDay Pharmaceuticals – MedDay Pharmaceuticals – France
[2] UMR1331 Toxalim – Université de Toulouse, INRA, ENVT, INP-Purpan, UPS – France
[3] Laboratoire des interactions plantes micro-organismes (LIPM) – CNRS : UMR2594, Institut national de la recherche agronomique (INRA) : UMR441 – Chemin de Borde-Rouge - BP 27 31326 CASTANET TOLOSAN CEDEX, France

Le réseau métabolique d'un organisme regroupe toutes les réactions biochimiques qui peuvent se produire dans cet organisme, quelles que soient les conditions. Afin de visualiser ces réseaux, nous avons développé MetExploreViz, une bibliothèque javascript gratuite et open source. Nous avons implémenté de nouvelles fonctionnalités et proposons une stratégie améliorée pour interpréter des signatures métaboliques avec MetExploreViz.
Une visualisation plus complète et modifiable. MetExploreViz a été amélioré afin de produire des visualisations de réseaux métaboliques plus proches des visualisations conventionnelles des voies métaboliques et du format SBGN, un standard de visualisation de réseaux biochimiques. L'outil permet notamment de visualiser les cycles sous forme de cercles, de courber les arêtes et de prendre en compte les notions de substrats et de produits dans le dessin des réactions..

D'autres fonctionnalités permettent de mettre en évidence des éléments du réseau ou d'intégrer des données omics à la visualisation. Pour ce faire, MetExploreViz permet de modifier le style et la position des labels des nœuds de manière à les mettre en évidence ou d'éviter le chevauchement des nœuds. Il est également possible de mettre en évidence les voies métaboliques sur les arêtes pour apporter des informations fonctionnelles au réseau et d'importer et lier des images à un nœuds pour faire apparaître des informations sur les données.
Il est également possible de remplacer l'ensemble des métabolites et des réactions d'une voie métabolique par un seul nœud représentant la voie métabolique choisie de manière à alléger la visualisation. Cette fonctionnalité permet également de mettre en évidence les résultats du " pathway enrichment " de MetExplore.

Mots-Clés: Visualisation, Réseaux métaboliques, Interprétation

Poster 83 – P83

Annotation de nouvelles classes de glycérolipides par l'approche des réseaux moléculaires au sein du métabolome de la macroalgue brune Taonia atomaria

Nathan Carriot[1], Benoît Paix [1], Stéphane Greff [2], Jean-François Briand [1], Gérald Culioli [1]

[1] Laboratoire Matériaux Polymères Interfaces Environnement Marin (MAPIEM), EA 4323 – Université de Toulon : EA4323 – France

[2] Institut méditerranéen de biodiversité et d'écologie marine et continentale (IMBE) – Institut de recherche pour le développement [IRD] : UMR237, Aix Marseille Université, CNRS : UMR7263, Université d'Avignon – France

Dans le milieu marin, toute surface immergée est rapidement colonisée par des bactéries, ainsi que par d'autres micro-organismes, qui forment des structures tridimensionnelles appelées biofilms. L'algue cosmopolite *Taonia atomaria* est un modèle biologique peu colonisé et dont certains métabolites de surfaces présentent une activité anti-adhésion vis-à-vis de bactéries marines (Othmani et al 2016). Dans le but de réaliser des études en écologie chimique visant à mieux comprendre les mécanismes de défense chimique de cette algue, une annotation exhaustive de sa production métabolique est donc essentielle. Lorsqu'elle est couplée à une approche phytochimique " classique ", l'annotation par réseaux moléculaires (via des données obtenues par LC-HRMS/MS) constitue un outil puissant qui peut conduire à l'identification potentielle d'un grand nombre de métabolites et ce, même dans le cas d'organismes peu étudiés. Dans leur globalité, les réseaux moléculaires obtenus via le serveur GNPS s'articulent en *clusters* (groupements de métabolites) représentant des familles de molécules formées du fait de la similarité de leurs spectres de masse. L'identification d'un métabolite décrit lors de l'analyse phytochimique permet ensuite d'annoter les autres métabolites au sein d'un même *cluster*. Dans le cas de *T. atomaria*, la construction initiale d'un réseau simplifié a conduit à l'identification de plusieurs dizaines de métabolites répertoriés en 14 familles chimiques, dont certaines se sont avérées constituées de lipides originaux tels que des glycérolipides portant un groupement de type géranylgéraniol, farnésol ou acide méthacrylique. La construction de réseaux de plus en plus complexes a permis finalement de caractériser plus de 200 métabolites. Cette première annotation détaillée du métabolome de *T. atomaria* permettra par la suite de mieux appréhender, lors d'études menées par métabolomique, les mécanismes de défense chimique et d'adaptation métabolique de cette algue vis-à-vis de microorganismes colonisateurs.

Mots-Clés: Réseaux moléculaires, Lipidomique, Glycérolipides, Algues brunes, Biofilms marins

Poster 84 – P84

Development of an untargeted approach based on isotope profiling of metabolic networks by high resolution mass spectrometry

Noemie Butin[1,2], Maud Heuillet [1,2], Samuel Naudi-Fabra [1,2], Cécilia Bergès [1,2], Lindsay Peyriga [1,2], Jean-Charles Portais [1,2], Floriant Bellvert[1,2]

[1] MetaToul-MetaboHUB, National infrastructure of metabolomics and Fluxomics, Toulouse, France –
[2] LISBP, Université de Toulouse, CNRS, INRA, INSA, Toulouse, France – LISBP, Université de Toulouse, CNRS, INRA, INSA, Toulouse, France – France

The study of metabolism has become a major research challenge for understanding the operation of biological systems (cells, tissues, organisms). Relevant approaches to study the topology and operation of metabolic networks are based on 13C isotope labelling strategies. For this purpose, the MetaToul-MetaboHUB platform has developed different approaches for a wide range of applications, including biotechnology and human health, such as measuring metabolic fluxes and the isotopic profiling based to follow the incorporation of 13C atoms into metabolites through targeted analysis of central and energetic metabolisms and amino acid metabolism.
Based on the analysis of a limited number of metabolites, current targeted approaches are very effective, however, they do not allow to highlight untargeted and by extend unsuspected metabolic pathways. For this reason and to increase the coverage of metabolome and the understanding of the biological systems, the platform wishes to develop an untargeted approach based on isotopic profiling. This approach aims at the characterization of the metabolic networks of biological organisms without *a priori* knowledge.
The implementation of this workflow is based in particular on the development of a software for automated data integration adapted to non-targeted analyses with 13C labelling. For this purpose, the preliminary step consists of an evaluation of pre-existing tools allowing the establishment of specifications. Then, the software developed by the platform according to these specifications will be integrated in emzed, an open source toolbox for rapid and interactive development of LCMS data analysis workflows in Python language in collaboration with ETH Zürich.

Mots-Clés: untargeted, 13C isotope labelling, automation

Poster 85 – Associé FP4

MetGem Software for the Generation of Molecular Networks Based on the t-SNE Algorithm

Nicolas Elie [1], **Florent Olivon** [1], **Gwendal Grelier** [1], **Fanny Roussi** [1], **Marc Litaudon** [1], **David Touboul** [1]

[1] Institut de Chimie des Substances Naturelles – Centre National de la Recherche Scientifique : UPR2301 – France

Metabolomic studies based on MS produce large amount of data requiring dedicated chemo/bioinformatics tools to explore them. Whereas many algorithms are available for the data treatment at MS1level, methodologies for MS2 data are scarce. One of the most popular is the generation of molecular networks (MNs) through the calculation of cosine score on a collection of MS2 spectra available on the online GNPS platform. We introduce here dedicated software, called MetGem (https://metgem.github.io), allowing the generation of GNPS-like MNs together with a t-distributed stochastic neighbor embedding (t-SNE) based visualization of the cosine score matrix. Starting from the .mgf file, all spectra detected are compared to each other using the GNPSbased cosine score calculation system.
One way to represent these results is to conceive a square matrix gathering together highdimensional objects, i.e. MS2 spectra, whose dimensions contain the similarities (cosine score values), taken pairwise, between all the spectra of the dataset. As it is difficult to apprehend these high-dimensional objects and visualize them in a meaningful manner, several manifold learning algorithms have been developed for dimensionality reduction purposes and pattern recognition. The idea developed herein was to feed the t-SNE algorithm with the pairwise similarity matrix. Considering that only small distances are reliable in high-dimensional spaces, t-SNE aggregates local data points closer in the lower-dimensional space. By focusing preferentially on the local structure of the data, t-SNE tends to extract better clustered local groups of point. Thus, it allows distinguishing easily patterns lying in different manifolds by simple visual analysis and in an unsupervised way.

Performances and advantages of the t-SNE output have been evaluated on two datasets, i.e. LC-MS2 analyses of fractions from a supercritical CO2 extraction of S. lineata leaves and a bark extract of C. peltatum. In both cases, t-SNE allows us to annotate more nodes compared to the GNPS-based cosine score calculation system and thus a deeper exploration of the experimental datasets was demonstrated.
Finally, in terms of network calculation and visualization, our interface running on laptop computer is up to 20 times faster for small and medium datasets (< 1000 MS2 spectra) and competes equally with GNPS for larger files (up to 35000 MS2 spectra).

Mots-Clés: cosine, similarity, MS2, t, SNE, molecular networking, python, desktop, chemo, informatics, bioinformatics, mass spectrometry, algorithm, metgem, mgf, GNPS, manifold, machine learning