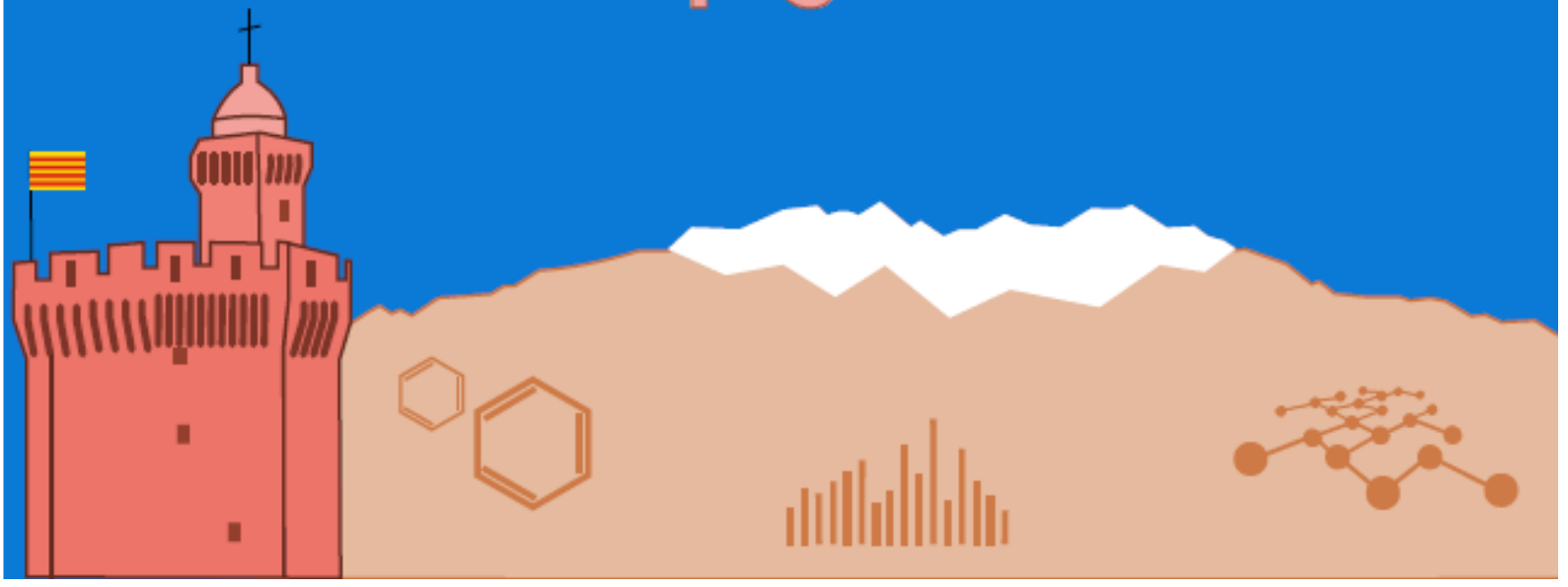
15e journées scientifiques

Réseau Francophone de Métabolomique et Fluxomique

24 > 26 Mai 2023

Perpignan



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- Hikmat Ghosson - (Chercheur PostDoc, Université de Perpignan) - UAR 3278 CRIOBE
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- Christian Espinoza (IR) - AkiNaO, Perpignan

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- Pascal de Tullio (Pr., Université de Liège) - Centre Interdisciplinaire de Recherche sur leMédicament (CIRM)
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- Yann Guitton (IR, Nantes) - Oniris, LABERCA - MetaboHUB
- Anne-Emmanuelle Hay, Chargée de Projet, MetaboHUB
- Audrey Le Gouellec (MCU-PH, Université Grenoble Alpes) - Plateforme GExiM - TIMC
- Florence Mehl (Biologiste computationnelle, Lausanne) - SIB Swiss Institute of Bioinformatics,Lausanne
- David Touboul (DR, CNRS) - ICSN, Gif-sur-Yvette / LCM (Ecole Polytechnique), Palaiseau

Les comités Scientifiques

Le comité scientifique des 15èmes JS du RFMF de Perpignan est composé des membres des CA et CA junior du RFMF ainsi qu'un conseilscientifique local

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- David Touboul (DR, CNRS) - ICSN, Gif-sur-Yvette / LCM (Ecole Polytechnique), Palaiseau

CONSEIL D'ADMINISTRATION JUNIOR

- Cécilia Bergès (IE), PlateformeO MetToul - Reseaux Metaboliques, Toulouse BiotechnologyInstitute, Université de Toulouse, CNRS 5504, Toulouse, France
- Loic Mervant (Post Doc), The Francis Crick Institute, Londres, UK
- Nathan Carriot (ATER), Université de Toulon, MAPIEM (EA 4323), Toulon (83), France
- Inès Le Mao (PhD student), ISVV, Université de Bordeaux, France
- Marine Letertre (Post Doc), CEISAM, Nantes, France
- Thomas Brunet (PhD student), Institut des Sciences Analytiques (ISA) UMR 5280, Universitéde Lyon, Villeurbanne, France
- Amandine Rocher (IE) Plateforme MetToul - Reseaux Metaboliques, Toulouse BiotechnologyInstitute, Université de Toulouse, CNRS 5504, Toulouse, France
- Ghina Hajjar (IR), Plateforme d'exploration du métabolisme (MetaboHUB Clermont-Ferrand),Unité de Nutrition Humaine, INRAE, Saint-Genès Champanelle
- Téo Hebra (Post Doc), Institute of Organic Chemistry and Biochemistry of the Czech Academyof Sciences, Czech Republic

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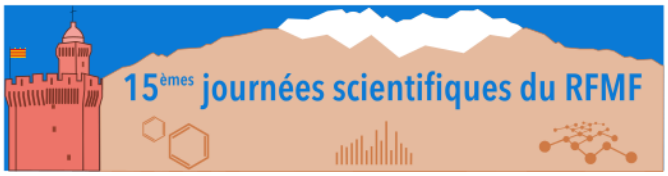
- Claire Vigor (MCU, UM, Faculté de Pharmacie) - IBMM
- Guillaume Marti (MCU, Université Paul Sabatier) - Université Paul Sabatier, Toulouse
- Loïc Legregam (IE, Plateforme MetaToul) - Université de Toulouse, CNRS

Programmes détaillés (Ateliers et 15e JS)



24 - 26 Mai 2023
Perpignan

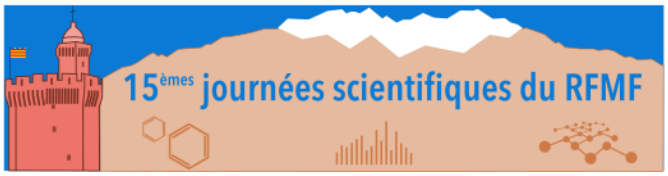
Ateliers - Mardi 23.05.2023

9h00 : Atelier Mentorat 1 : Cloture promotion 1 – **Audrey LE GOUELLEC**
11h00 : Atelier Junior – **Cécilia BERGES** et **Christelle PARCHEMIN**
13h00 : Repas Juniors (13h00 - 14h00)
14h00 : Atelier 1 : Empreinte ou profil RMN: Traitement interactif d'une série de spectres RMN 1D avec NMRProcFlow - **Catherine DEBORDE** et **David JACOB**

Atelier 2 : Métabolomique des écosystèmes microbiens en environnement, santé et biotechnologie - **Nicolas CREUSOT, Cyril JOUSSE** et **Binta DIEME**

Atelier 3 : Analyse métabolomique pas à pas pour l'identification des biomarqueurs à partir de données LC-HRMS - **Slimane CHAIB** et **Nathan CARRIOT**

17h00 : Pause (17h00 - 17h30)
17h30 : Atelier Mentorat 2 : Lancement promotion 2 – **Audrey LE GOUELLEC**
19h30 : Apéro Mentorat



24 - 26 Mai 2023
Perpignan

15èmes JS RFMF - Mercredi 24.05.2023

8h00 : Accueil des participants

9h00 : Allocution de bienvenue

9h30 : Prospective metabolomic studies in precision medicine: the Akribea project - **Oscar MILLET**

10h15 : Production of bioactive oxylipins in marine microalgae under stress conditions - **Amandine LINARES-MAURIZ**

10h35 : Shimadzu - Dissociation par attachement d'oxygène et logiciels de retraitements pour la localisation de liaison C=C de lipides non ciblés - **Bessem BRAHIM**

10h45 : Pause café

Modérateurs : Marine LETERTRE et Patrick GIRAUDEAU

11h15 : Immuno-Metabolite discovery: study of metabolic adaptation of *Pseudomonas aeruginosa* in Cystic Fibrosis patients - **Caroline PLAZY**

11h30 : Identification of chemical defenses involved in natural durability against lignivorous fungi of a tropical tree *Sextonia rubra* by targeted and untargeted metabolomics - **Marceau LEVASSEUR**

Evaluation of the environmental impact of a biopesticide using ana innovative approach coupling high-throughput methods (metabolomics and metabarcoding) - **Anouar MEJAIT**

Annotation of biomarkers in exhaled breath: combining real-time mass spectrometry and two-dimensional chromatography-mass spectrometry - **Elodie LAMY**

11h40 : Fast and sensitive 2D 1H-13C NMR for monitoring in vivo metabolism in *Daphnia Magna* in real time and without 13C enrichment - **Jonathan FARJON**

12h00 : Bruker - Driving Metabolomics solutions in Mass Spectrometry - **Sabine JOURDAIN**

12h10 : Repas

13h30 : Session Posters

Modérateurs : Cécilia BERGES et David TOUBOUL

14h30 : Chemical and biological analysis of plant and insect holobionts as a model for interaction and biosynthesis of specialized metabolites - **Véronique EPARVIER**

15h15 : Metabolic fluxes estimated by constraint-based modelling highlight specific response of susceptible tomato (Solanum lycopersicum) stems infected by *Botrytis cinerea* under gradual nitrogen nutrition - **Nathalie LACRAMPE**

15h35 : Métabolites dérivés du microbiome dans les maladies cardiométaboliques : de l'association à la causalité - **Marc-Emmanuel DUMAS**

15h55 : Thermo Fisher - Simultaneous Quantitation and Discovery (SQUAD) Analysis: Combining the Best of Targeted and Untargeted Mass Spectrometry-Based Metabolomics - **Marie-Pierre PAVAGEAU**

16h05 : Pause café

Modérateurs : Christelle PARCHEMIN et Samuel BERTRAND

16h45 : BioModTool: a python package to add biomass objective functions to genome-scale metabolic models from user data - **Clémence DUPONT-THIBERT**

17h00 : New method by Supercritical Fluid Chromatography with High Resolution Mass (Q-Tof) to characterize a complete profil of 230 Skin Ceramides on various samples - **Cyrielle CLEMENT**

17h20 : Establishment of a NMR-based metabolomics protocol for salivary samples - **Manon CAMPAS**

Interagir avec ses données de spectrométrie de masse avec Galaxy - **Julien SAINT VANNE**

Exploration des interactions dans l'holobionte algal pour la découverte de nouveaux antifoulings éco-compatibles - **Emilie ADOUANE**

17h30 : Metabolomics and dynamic molecular networking reveal the diversity and biosynthesis of pyran-2 ones in a mussel-derived Penicillium restrictum cultured on host-based medium - **Olivier GROVEL**

17h50 : AG RFMF senior

19h50 : Apéro

15èmes JS RFMF - Jeudi 25.05.2023

Modérateurs : Isabelle BONNARD et Christian ESPINOZA

9h00 : Metabolomics unravels Chemical Signaling in Aging, Rejuvenation and Interaction of Cells in the Plankton Microbiome – **Georg POHNERT**

9h45 : ^{13}C measurements and positional approaches with GC-MS: method validation and applications to plant ^{13}C-labeled experiments - **Younès DELLERO**

10h05 : Combining innovative NMR methods for improved environmental contaminants characterisation using metabolomics - **Marine LETERTRE**

10h25 : Proteigene – Biocrates - Impact of metabolism on therapeutic response - **Carlos MALPICA**

10h35 : Pause café

Modérateurs : Marie-Virginie SALVIA et Slimane CHAIB

11h05 : Intégration des approches omiques (métabolomique et protéogénomique) pour l'étude des effets de contaminants émergents sur la moule méditerranéenne *Mytilus galloprovincialis* - **Frédérique COURANT**

11h25 : Chemical diversity and chemotaxonomy of neo-tropical Xylariales fungi - **Juliette SEGRET**

11h40 : Untargeted LC-MS based metabolomics and molecular networking for the elucidation of ancient dyeing recipes - **Lyndsay MAS-NORMAND**

Metabolomics profiling of French artichoke leaves by NMR and Mass spectrometry **- Aurélien VIRET**

An introgression-based genetical metabolomics approach enables the diversification of the phytochemical repertoire of oilseed rape (*Brassica napus*) and highlights series of genomic factors involved in the chemical diversity in Brassica **- Pauline LE BOULCH**

11h50 : ^{13}C-labeled Mouse Urine Metabolomics by LC-HRMS: effectively improving metabolite identification - **Anaïs LEGRAND**

12h10 : Gencovery - Constellab™ : un écosystème numérique complet pour la digitalisation de projets complexes de R&D - **Adama OUATTARA**

12h20 : Repas

13h40 : Session Posters

Modérateurs : Corentine GOOSSENS et Nathan CARRIOT

14h40 : Pure Shift NMR of Aqueous Biofluids: Towards Quantitative Metabolic Profiling - **Nicolas GIRAUD**

15h00 : Invasive macroalgae shape the chemical and microbial composition of reef boundary layers - **Chloé POZAS SCHACRE**

15h20 : Prix de these : Analysis of the metabolic features of plant extremophile species from the Atacama Desert - **Thomas DUSSARRAT**

15h50 : Sciex - Maximising the power of the ZenoTOF7600 system for lipidomics and metabolomics - **Heather CHASSAING**

16h00 : Pause café

Modérateurs : Amandine Rocher et Cédric Bertrand

16h40 : New data normalisation methods for PTR-TOF-MS exhaled breath metabolomics - **Camille ROQUENCOURT**

17h00 : L'élucidation structurale de nouveaux biomarqueurs : un « Qui est-ce » grandeur métabolomique - **Chloé CLOTEAU**

17h15 : Expanding the chemical annotation of metabolites from biofluids by combining different LC-MS methodologies and strategies in untargeted metabolomics - **Romina PACHECO TAPIA**

17h35 : GC–MS metabolic profiling of Carignan and Grenache cultivars during grapevine berry development - **Domingo LORNI**

17h45 : RFMF d'honneur

18h30 : AG RFMF junior

20h00 : Dîner de gala

15èmes JS RFMF - Vendredi 26.05.2023

Modérateurs : Frédérique COURANT et Nathalie TAPISSIER

9h30 : Elucidating the role of specialized metabolite decorations regulated by warm temperatures in seeds - **Léa BARREDA**

9h50 : Improving comprehensive analysis and compound annotation in complex biological samples through molecular networking based on SWATH-MS and EAD fragmentation - **Valentina CALABRESE**

10h10 : Arteriovenous metabolomic approach reveals specific metabolite exchanges across organs and provides unique insights for understanding underlying metabolic perturbations in obese and insulin-resistant minipigs - **Imène BOUSAHBA**

10h25 : Pause café

Modérateurs : Anouar MEJAIT et Julien BOCCARD

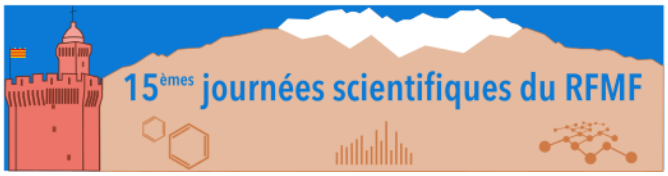
10h55 : The multiplicity of data analysis strategies in life sciences jeopardizes replicability: illustrations and solutions – **Anne - Laure BOULESTEIX**

11h40 : Exploration multi-omique et in silico des effets transgénérationels et sexe-spécifiques induits par le TBT, un composé obésogène, sur le métabolisme hépatique de la souris - **Nathalie POUPIN**

12h00 : Embarking on a delicious treasure hunt: exploring the brown and black world of fine dark chocolates with polyphenol metabolomics and molecular networking - **Aécio DE SOUSA DIAS**

12h20 : Allocution de cloture

Résumés des Ateliers



24 - 26 Mai 2023
Perpignan

Atelier junior 15èmes JS du RFMF le 23 mai de 11h à 13h

Personnes encadrant l'atelier :

RFMF junior berges@insa-toulouse.fr christelle.parchemin@univ-perp.fr

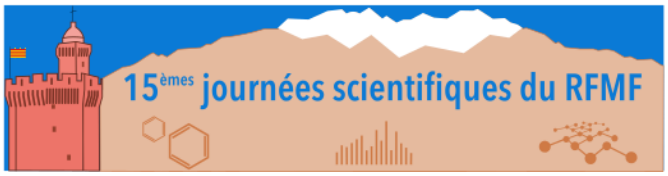
Public envisagé et les prérequis :

Public : tous les membres juniors du RFMF inscrits au congrès (personnes de moins de 33 ans) - Prérequis : aucun prérequis

Objectif de l'atelier :

L'objectif de cet atelier est de présenter les activités de la branche junior du RFMF et de promouvoir les interactions entre membres juniors. Pour cela, l'atelier proposé sera divisé en (1) une présentation rapide du RFMF junior (10 min) (2) le cœur de l'atelier et (3) un moment convivial autour d'un buffetpour clôturer la matinée.

Les juniors participant à l'atelier seront répartis dans des groupes correspondant à leur position actuelle (ex : doctorants, post-doctorants, techniciens, ingénieurs…). Ils seront ensuite invités à échanger entre eux sur leurs problématiques communes (rédaction d'un article, rédaction du mémoire de thèse, recherche d'un emploi…). Ensuite, les différents groupes seront amenés à se rencontrer successivement et à échanger autour de leur expérience (ex : les post-doctorants feront part de leur expérience pour trouver un emploi auprès des doctorants).



24 - 26 Mai 2023
Perpignan

Ateliers thématiques 15èmes JS du RFMF le 23 mai de 14h à 17h

Atelier 1

Empreinte ou profil RMN. Traitement interactif d'une série de spectres RMN 1D avec NMRProcFlow.

Personnes encadrant l'atelier :
Daniel JACOB daniel.jacob@inrae.fr
Catherine DEBORDE catherine.deborde@inrae.fr

Public envisagé et les prérequis :

- Public : intéressé par les approches de RMN métabolomique 1D ^{1}H (profil quantitatif ouempreintes).
- Prérequis : notion de lecture / retraitement d'un spectre RMN

Objectif de l'atelier :

Ex : L'objectif est de présenter les différentes approches de traitement d'une série de spectres RMN 1D ^{1}H pour les approches non-ciblée (empreintes) ou ciblées (quantification relative ou absolue) par intégration, bucketing/bining avec des exemples en métabolomique des plantes et en authenticité des vins.

La première partie de l'atelier présentera les potentialités de l'outil NMRProcFlow pour prétraiter et traiter de manière interactive une série de spectres RMN 1D : phasage, ligne debase (locale ou globale), réalignement local de pics, intégration pour les approches non-ciblée (empreinte) et ciblée (profil quantitatif).

La deuxième partie présentera les dernières options intégrées dans l'outil depuis sa publication en 2017.

L'atelier sera ponctué de prises en main de l'outil sur un jeu-test de spectres par les participants.

Atelier 2
La métabolomique des écosystèmes microbiens en environnement, santé et biotechnologie

Personnes encadrant l'atelier :
Nicolas Creusot, Cyril Jousse (visio) et Binta Dieme

Public envisagé et les prérequis :
- Public : tout public (étudiant-e, personnel technique, enseignant-e, chercheur-e)
- Prérequis : Bonne connaissance des worflows de métabolomique, en particulier dans l'étude des communautés microbiennes seules ou en interaction avec un hôte (holobionte)

Objectif de l'atelier :
1. Appréhender la métabolomique des communautés et ses objectifs.
    - La métabolomique des écosystèmes quésaco ? éco-métabo , méta-métabo ?
    - Les enjeux des systèmes microbiens (complexes)
    - L'initiative « réseau thématique » EcoMet.
    - Identification de questions clefs et priorisation

2. Concevoir le-s workflow-s idéal-aux afin de mener à bien une expérimentation preuve deconcept autour du « qui fait quoi » dans une communauté ?
    Cette partie se fera en sous-groupes avec une restitution commune.
    - Les outils et les méthodologies utiles, utilisés, ou en développement.
    - Les différentes étapes d'un worflow de métabolomique d'une communauté microbienne
    - Méta-omiques, multi-omiques et autres « méta-données » du système microbien.

N.B. Les groupes seront structuré en fonction de l'audience

3. Et la suite !? brainstorming autours des solutions proposées
    - Nous avons conçu un/des worflow/s (image parfaite ou puzzle incomplet?) – Quelssont les gaps qui existent ?
    - Nous pouvons réaliser ce-s worflow-s (quelles ressources, quels délais?).
    - Nous allons diffuser ce-s worflow-s (publication ? Enseignement?)

Atelier 3
Suivi d'une analyse métabolomique pas à pas permettant l'identification des biomarqueurs à partir de données LC-HRMS

Personnes encadrant l'atelier :
Chaïb Slimane : slimane.chaib@univ-perp.fr et Carriot Nathan : ncarriot@gmail.com

Public envisagé et les prérequis :
Public : Personnes débutantes en métabolomique. L'objectif de cet atelier est de proposer un workflow clair et utilisable pour les analyses métabolomiques. Un jeu de données sera fourni afin que chaque participant puisse effectuer en direct toutes les étapes. Les participants peu- vent également suivre l'atelier avec leurs propres données.

Prérequis : Des notions en métabolomique, statistiques et réseaux de similarités spectrales (néanmoins l'atelier vise les personnes débutantes en métabolomique). Et de la bonne humeur !

Objectif de l'atelier :
Cet atelier présentera un workflow post acquisition de données LC-HRMS visant à déterminer depotentiels biomarqueurs entre différents groupes. Plusieurs étapes de ce workflow seront développées :

Introduction des étapes clefs pour la création de matrice via le logiciel MZmine. Cette étape permettra de comprendre les différents paramètres à utiliser pour la création d'une matrice en métabolomique. Une description complète des paramètres permettra à l'utilisateur de les optimiser en fonction de son jeu de données.

La matrice générée sera, dans un second temps, utilisée pour la détermination de biomarqueurs à l'aide d'outils statistiques sous R et Metaboanalyst (PCA, PLS-DA, Heatmap, etc).

Une fois les biomarqueurs (ou VIPs) potentiellement déterminés, une troisième étape utilisant les réseaux de similarités spectrales aidera à les annoter (GNPS et Metgem). L'utilité et les paramètres déterminant la création de réseaux de similarités spectrales se- ront discutés. Ces derniers permettront d'explorer le métabolome des différents échantil- lons avec Cytoscape et de faciliter l'annotation des potentiels biomarqueurs. Enfin, une rapide description des banques de données en ligne sera faite. Les annotations seront confrontées à une analyse avec le logiciel Sirius afin de valider ou réfuter l'annotation obtenue.

Le but est d'obtenir un workflow général qui devra être ajusté en fonction du jeu de données de chacun et qui comprend les étapes clefs d'une analyse de métabolomique :

Création d'une matrice à partir des données LC-HRMS,
Utilisation d'outils statistiques pour l'interprétation de la matrice
Création de réseaux de similarités spectrales
Identification des biomarqueurs
Enfin, il est indispensable de mentionner que des formations sur la plateforme W4M sont à réaliser afin de mieux en comprendre les rouages pour optimiser le workflow ainsi que la créationde matrice.

Pour préparer l'atelier...
Logiciels spécifiques à télécharger en amont : R et Rstudio, MZmine (v2.53), Cytoscape, MetGem, Sirius.
Avoir un compte actif sur la plateforme GNPS et Workflow4Metabolomic

Communications des invités

Présentation des conférenciers invités

Anne-Laure Boulesteix (Ludwig-Maximilians University, Munich - Germany)

Anne-Laure Boulesteix obtained a diploma in engineering from the Ecole Centrale Paris, a diploma in mathematics from the University of Stuttgart (2001) and a PhD in statistics (2005) from the Ludwig Maximilian University (LMU) of Munich. After a postdoc phase in medical statistics, she joined the Medical School of the University of Munich as a junior professor (2009) and professor (2012). She is working at the interface between biostatistics, machine learning and medicine with a particular focus on metascience and evaluation of methods. She is a steering committee member of the STRATOS initiative, founding member of the LMU Open Science Center and president-elect of the German region of the International Biometric Society.

Véronique Eparvier (CNRS-ICSN, Gif-sur-Yvette - France)

Véronique Eparvier is graduated with two Master's degrees: Sciences of Agro-resources (National Polytechnic Institute of Toulouse), and Anthropology (Laboratory of Human Ecology and Anthropology, Aix-Marseille III University) and since 2005 with a PhD, specialty "Chemistry of Natural Substances" from the National Museum of Natural History. In 2006, she joined the CNRS-Guyane as a Research Engineer.
She joined the ICSN "Institut de Chimie des Substances Naturelles" in 2011 where she participates in the development of research programs on symbiotic microorganisms and developed a strain library. She obtained her HDR in October 2013 and she is CNRS research director since 2020;she leads the Functional Chemistry-Chemical Ecology team, and she is deputy coordonnator of "Natural Products and Medicinal Chemistry" department of ICSN. She is since 2021 a member of the CNRS national committee, section 16 CoCNRS. The team's research goals concern the broad field of natural substances chemistry. In this context, the team interest is to study the interactions

betweenmicroorganisms and plants and microorganisms and insects, in order to discover specialized metabolites with pharmacological and/or agrochemical interest but also to understand their ecological role. For this purpose, different methodological tools such as chemical ecology and functional chemo-diversity are used and developed. The aim of this research is to carry out integrated approaches for the study and valorization of specialized metabolites from original resources of natural substances such as symbiotic microorganisms.

Oscar Millet (Precision Medicine and Metabolism Lab, Bizkaia - Spain)

I obtained a Degree in Chemistry (Univ. Ramon Llull, 1994) and Chemical Engineering (IQS, 1995). After obtaining a PhD in Organic Chemistry (University of Barcelona, 1999) I joined the group of Lewis Kay in Toronto for a post-doctoralstay (University of Toronto, 2000-2004). I was thenrecipient of a Ramon y Cajal reincorporation contract at the Parc Científic de Barcelona (2004-2006) and I currently am the group leader of the Precision Medicine and Metabolism laboratory of CIC bioGUNE. My research line focuses on the use of nuclear magnetic resonance (NMR) to the study of biologically relevant proteins and enzymes, paying specialattention to the delicate balance existing between protein stability and dynamics. Such knowledge is applied for the development of new compounds with therapeutic activity in the field of rare diseases, and it has developed into the creation of a spin-off company, ATLAS Molecular Pharma. I have found a new pharmacological chaperone for the treatment of congenital erythropoietic porphyria, which is a repurposed drug and it has obtained the Orphan Drug Designation status. Additionally, I am also interested in the NMR-based metabolomics of biofluids for the diagnose of rare and prevalent diseases. I havepublished more than 100 papers with a total number of citations (1998-2017) of3500 and an h-index of 28. I have been awarded the prize of the Real Sociedad Española de Química (2004), the Spanish NMR group prize (2020), the Elhyyar- Goldschmidt award of the German Chemical Society (2022) and nominated Academic of the Academy of Medical Sciences of the Basque Country (2016). I am currently the president of the Spanish Chemical Biology group.

Georg Pohnert (Friedrich Schiller University Jena, Erfurt - Germany)

The current research of the Pohnert-group takes a highly interdisciplinary approach to understand the nature androle of chemical signals that shape complex communities inthe aquatic environment. A tool of fundamental importanceis comparative community metabolomics, to first identify the signals present in natural interactions. Isolation, spectroscopy and organic synthesis of natural products are combined with transcriptomics, biochemistry, and ecology, with the ultimate goal to understand the basis of community interactions in plankton and biofilm communities.

Georg Pohnert, studied Chemistry at the University of Karlsruhe. He then movedto the University of Bonn and the University of Washington where he pursued his doctoral studies in the Group of Prof. W. Boland. In 1997 he joined the groupsof Prof. Bruce Ganem and Prof. David B. Wilson at the Cornell University in Ithacaas a postdoc working on the biochemical and biophysical characterisation of *E. coli* receptors. He was then appointed to a group leader position at the Max- Planck-Institute for Chemical Ecology in Jena, Germany where he started his independent research carrier on algal defence reactions. In 2005, he joined the Institute of Chemical Sciences and Engineering of the Ecole Polytechnique Fédérale de Lausanne, Switzerland as assistant professor. A Lichtenberg Professorship was awarded to him by the Volkswagen Foundation. This broughthim to the Friedrich Schiller University where he holds a chair in Instrumental Analytics. Since 2015 Georg Pohnert is has been appointed as Max-Planck-Fellowat the Max-Planck-Institute for Chemical Ecology where he heads a research team focused on the chemical regulation of plankton population dynamics. Georg Pohnert currently serves as vice president for research at the Friedrich Schiller University.

Conf. invitée 1

Prospective metabolomic studies in precision medicine: the Akribea project

Oscar Millet
Precision Medicine and Metabolism Laboratory, CIC bioGUNE.

In conventional medicine, the analysis of biomolecules has, for a long time, served as a usefultool to diagnose diseases. Yet, this approach adds meaning to a limited number of metabolitesand often through a bijective relationship with the disease (i.e. glucose relationship with diabetes).

Nowadays, precision medicine emerges as an option to improve the prevention and/or treatment of numerous pathologies, focusing on the molecular mechanisms, acting in a patient-specific dimension, and leveraging multiple contributing factors such as genetic, environmental or lifestyle.

Metabolomics grasps the required subcellular complexity while being sensitive to all these factors, and it is a most suitable technique for precision medicine.

We will describe the NMR-based metabolomics database that is currently integrated in the Precision Medicine Initiative of the Basque Country (the Akribea project), to illustrate the procedures to be followed when conducting an NMR metabolomics study with a large cohortof individuals also emphasizing the critical points and discussing some relevant applications.

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Conf. invitée 2

Chemical and biological analysis of plant and insect holobionts as a model for interaction and biosynthesis of specialized metabolites

Véronique Eparvier
CNRS-ICSN

Insects and plants are symbiotic with a wide variety of microscopic life forms, including bacteria, fungi, protozoa, and nematodes. The study of these holobionts requires the isolationand identification of the microorganisms.

To this end, we first developed a tool for the identification and dereplication of environmental strains by MALDI-TOF MS fingerprint comparison. Then, in order to identify microorganisms naturally producing chemical weapons against different pathogens (such as entomo- or phytopathogens), the chemical diversity of microorganisms was explored based on liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) and molecular networks(with MetGem software based on t-SNE visualization developed in our institute). The search for biological activities is guided by the environmental function of these microorganisms.

The combination of different methods such as OSMAC (One Strain Many Compounds), metabolomics and genomics for the production and annotation of secondary metabolites implemented in this work allow to significantly accelerate the chemical study of extracts of microorganisms for the isolation of new bioactive compounds whose use is potentiallytransferable in human health.

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Conf. invitée 3

Metabolomics unravels Chemical Signaling in Aging, Rejuvenation and Interaction of Cells in the Plankton Microbiome

Georg Pohnert, Yun Deng, Marine Vallet

Friedrich Schiller University Jena, Bioorganic Analytics, Lessinstr. 8, Jena, Germany,

Email: Georg.Pohnert@uni-jena.de

In annually reoccurring patterns microalgae in the plankton form blooms that grow, persist and decline, thereby contributing massively to global biogeochemical cycles. These unicellularalgae interact with other members of the plankton microbiome by means of chemical signals.

By investigating algae in interaction situations, we demonstrate how these organisms respondto pathogen infection, interact with bacteria and are even rejuvenated after nutrient influx. We characterize the regulated pathways that mediate these processes using (single-cell) metabolomics and elaborate bioassays. Isotope labeling and fluxomics approaches further clarify the fluxes of metabolites within the complex communities.

Linking our laboratory findings with data from research cruises and mesocosms allows to construct a marine metabolic network that even explains global element cycles.

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Conf. invitée 4

The multiplicity of data analysis strategies in life sciences jeopardizes replicability: illustrations and solutions

Anne-Laure Boulesteix and Sabine Hoffmann
Ludwig-Maximilians University, Munich - Germany

For a given research question, there are usually a large variety of possible data analysis strategies acceptable according to the scientific standards of the field, and there are concernsthat this multiplicity of analysis strategies plays an important role in the non-replicability of research findings.

In this talk, I will outline the connection between the multiplicity of data analysis strategies (also referred to as data analyst's degree of freedom) and the so-called replication crisis in science based on metascientific studies from the field of life and health sciences. In particular, I will demonstrate the detrimental effect of result-dependent selective reporting on the validity of results in the context of regression modelling in epidemiology, prediction models (supervised learning) based on omics data, clustering and network analysis of microbiome data and differential expression analysis.

The second part of the talk will be devoted to potential (partial) solutions recently proposed in the literature, including our own recent studies in this field.

Communications orales

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Oral 1 - O1

Production of bioactive oxylipins in marine microalgae under stress conditions

Amandyne Linares-Maurizi*[1,2]

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[2]Microphyt – Microphyt, Microphyt – France

Microalgae are photosynthetic microscopic organisms that serve as the primary food source in aquatic environments. Microalgae have strong cellular plasticity and are able to adapt to different culture conditions. They can synthesize a wide range of molecules, such as polyunsaturated fatty acids (PUFAs) from the omega-3 and omega-6 series. Oxidative degradation of PUFAs by radical and/or enzyme conversion results in the formation of oxylipins, which are compounds known for their bioactive properties.

Our project aimed to investigate the production of bioactive oxylipins from marine microalgae grown in 1 L photobioreactors under stress conditions, such as the addition of reactive oxygen species (ROS), ethyl acetate, and NaCl. During their exponential phase, three different microalgae were harvested, extracted, and analyzed by LC-MS/MS to determine the qualitative and quantitative profiles of oxylipins for each species. The microalgae revealed a large diversity of metabolites, with about 50 oxylipins present in different concentrations depending on the growing conditions. Interestingly, the effect of stress on oxylipin production varies by species and type of stress.

This study shows that it is possible to direct the metabolism of microalgae towards the production of bioactive oxylipins by adjusting the culture conditions. Increasing the production of this rich mixture of oxylipins may provide human health benefits, including antioxidant, anti-inflammatory, neuroprotective, or immunomodulatory activities. Some oxylipins are also well known for their cardiovascular properties.

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Oral Constructeur 1 - C1
Shimadzu
Dissociation par attachement d'oxygène et logiciels de retraitements pour la localisation de liaison C=C de lipides non ciblés

Bessem Brahim

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Oral 2 - O2

Immuno-Metabolite discovery: study of metabolic adaptation of Pseudomonas aeruginosa in Cystic Fibrosis patients

Caroline Plazy*[1,2], Emilie Boucher[2], Razak Tidjani[2], Oriane Moyne[3], Max Maurin[4], Audrey Le Gouellec[2,4], Bertrand Toussaint[2,4], and Dalil Hannani[2]

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[4]CHU Grenoble – CHU Grenoble – France

During infection, bacteria interplay with immunity, especially with dendritic cells through producing/consuming immunometabolites, altering the polarization of subsequent immunity.
To identify new immunometabolites, we studied immune properties of metabolites derived from *Pseudomonas aeruginosa* clinical strains biobank, isolated from 32 chronically infected CF patients.
Bacterial supernatants of all strains (SN), containing excreted metabolites, were collected and their immunomodulatory capacities were evaluated on T cell polarization *in vitro*. Afterwards, we validated the immunomodulatory properties of the whole strains mediated by metabolites, *in vivo*, during a mouse infectious challenge. We have highlighted different immunomodulatory profiles depending on strains: some are immunostimulating and others immunosuppressive. Of note, infection of patients by immunosuppressive strains has been correlated with the decline of patients' respiratory functions. In parallel, the bacterial exometabolome was analyzed by LC-MS/MS. In line with previous results, untargeted metabolomics showed 2 distinct metabolic fingerprints in the strains, highlighting the importance of Pa strains metabolism in the modulation of immunity. To validate the role of metabolism, we analyzed the strains' endometabolome and conducted a transcriptomic analysis. All these characterizations allowed us to identify immunometabolites and associated metabolic pathways differentially expressed in the strains, and notably some metabolites involved in the induction of immunosuppression.
This study demonstrate that isolates display differential immune properties depending on secreted metabolites and their characterization by metabolomics highlight the importance of the strain's overall metabolism in its modulations. Some of the identified metabolites constitute potential therapeutic targets for restoring host immunity in chronic infectious diseases.

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Oral 3 - O3

Fast and sensitive 2D ^{1}H-^{13}C NMR for monitoringin vivo metabolism in Daphnia Magna in real time and without ^{13}C enrichment

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Daphnia Magna are freshwater crustaceans, commonly used for aquatic toxicity testing (1). Following their metabolism in-vivo is challenging due to spectral overlap and magnetic susceptibility distortions. Among analytical techniques, NMR is well suited to elucidate, identify and quantify mixtures. In particular, 2D 1H-13C NMR offers excellent spectral dispersion but is time-consuming and has intrinsic low sensitivity with 13C at natural abundance.
To date, Time-Resolved Non-Uniform Sampling (TR-NUS) HSQC have been used with 13C labelled Daphnia to monitor rapid response to pollutants (2). Here, in order to monitor Daphnia at natural abundance, we explored ASAP HSQC which is a faster 2D HSQC able to acquire a 2D map in seconds or to enhance the sensitivity per unit of time (3). In order to improve the sensitivity per unit of time, a lower NUS rate was implemented in the 13C dimension in combination with TR-NUS to monitor events at the minute scale. The new method termed TR-NUS ASAP HSQC allowed enhancing the sensitivity up to 3 as com- pared to the standard version. Thus, it was possible with this method to monitor anoxia and its recovery for the first time in unlabelled Daphnia using HSQC. TR-NUS ASAP HSQC opens the avenue to investigate environmental adaptability and exposure in living organisms in close to real time.

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Oral Constructeur 2 - C2

Bruker

Driving Metabolomics solutions in Mass Spectrometry

Sabine Jourdain

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Oral 4 - O4

Metabolic fluxes estimated by constraint-based modelling highlight specific response of susceptible tomato (*Solanum lycopersicum*) stems infected by *Botrytis cinerea* under gradual nitrogen nutrition

Nathalie Lacrampe*[1], Raphaël Lugan[1], Doriane Dumont[1], Philippe Nicot[2], François Lecompte[1], and Sophie Colombie†[3]

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[2]Unité de Pathologie Végétale – Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement – France

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Metabolic reprogramming is a strong determinant of the plant's response to necrotrophic fungi such as Botrytis cinerea, which determines its resistance levels in relation with the availability of nutrient resources. The metabolic fluxes were investigated upon B. cinerea attack in tomato stems under four nitrogen nutrition levels which modulated the plants susceptibility. Quantitative metabolomics and physiological data were implemented as constraints, for the resolution of a constraint-based model allowing to calculate metabolic fluxes for each N level in symptomless stem tissues adjacent to lesions. The metabolic network was constructed a priori to be finally constituted of 302 well-curated reactions representative of the central metabolism and of relevant blocks accounting for defence mechanisms. The results showed an unexpected increase in relative stem growth after inoculation, more pronounced at low N level, which was associated with an overall increase in the calculated fluxes in Botrytis-inoculated plants compared to control plants. Notably, there was an increase in cell wall synthesis with increased fluxes through the sugar-related, pentose-phosphate and shikimate pathways. Also, an increase in protein synthesis resulted in unexpected changes in TCA cycle fluxes, with an increase in one part and a reversal of another part, leading to non-cyclic functioning in Botrytis-inoculated plants. On the other hand, a decrease in starch synthesis was observed, highlighting a reallocation of carbon for defence. This work provided new insights for the comprehension of resistance to B. cinerea in tomato stems linked with N availability and could eventually help to enhance defence by tightly controlling nutrition.

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Oral 5 - O5

Métabolites dérivés du microbiome dans les maladies cardiométaboliques : de l'association à la causalité

Marc-Emmanuel Dumas*[1]

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Le microbiome intestinal - l'ensemble des gènes bactériens présents dans nos intestins est désormais reconnu comme un élément clé de la physiopathologie de l'obésité du diabète de type 2 et des maladies cardiométaboliques, ainsi que de leur composante d'inflammation chronique de basse intensité. Cependant, les signaux envoyés par les microbes intestinaux à l'hôte restent insaisissables. Grâce à l'apprentissage automatique et à l'analyse multivariée des métabolomes et des métagénomes, nous avons identifié des signatures métabolomiques et microbiomiques cliniquement pertinentes et non fondées sur des médicaments pour la progression du spectre des maladies cardiométaboliques, ouvrant la voie à de nouvelles hypothèses et à l'élucidation des mécanismes influencés par le microbiome intestinal, à l'aide d'une série de modèles précliniques.

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Oral Constructeur 3 - C3

ThermoFisher

Simultaneous Quantitation and Discovery (SQUAD) Analysis: Combining the Best of Targeted and Untargeted Mass Spectrometry-Based Metabolomics

Marie-Pierre Pavageau – Application Scientist, Thermo Fisher Scientific

A novel single injection simultaneous quantitation and discovery (SQUAD) metabolomics that combines targeted and untargeted workflows is described. It is used to identify and accurately quantify a targeted set of metabolites. It also allows data retro-mining to look for global metabolic changes that were not part of the original focus. This offers a way to strike the balance between targeted and untargeted approaches in one single experiment.

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Oral 6 - O6

BioModTool: a python package to add biomass objective functions to genome-scale metabolic modelsfrom user data.

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A genome-scale metabolic model (GEM) is a computational network representing all known enzymatic and spontaneous metabolic reactions and metabolic genes of a given organism. Flux Balance Analysis (FBA) is a widely used constraint-based modeling method for predicting metabolic fluxes using GEMs by optimizing an objective function. Biomass Objective Functions (BOFs), consuming all metabolites required to produce one gram of dryweight biomass, are commonly used as FBA objectives to simulate microorganisms' growth. Several studies have demonstrated the high impact of biomass composition on GEMs behavior and predictions. The requirement for accurate definition of the biomass composition has recently been addressed by experimental protocols and the BOFdat pipeline (1,2). BOFdat is a python workflow computing biomass stoichiometric coefficients from different datasets including genomics, transcriptomics, proteomics and lipidomics. However, omics data are not always available, therefore limiting the number of organisms to which BOFdat can be applied.

We developed BioModTool, a new python package, to easily create new BOFs in GEM from a single user dataset. BioModTool was applied to GEMs of two bacteria species Escherichia coli (iML1515) and Alicyclobacillus acidocaldarius (CNA Alicyclo), and one microalga Microchloropsis gaditana (iRJ1321 and unpublished iMgadit GEM) (1,3,4). In silico differential flux analysis confirmed the high influence of BOF definition on flux distributions.

By providing easy definition of new BOFs, BioModTool has the potential to support new GEM reconstructions, to improve the quality of existing GEMs and their capacity to predict flux distributions.

(1) https://doi.org/10.3390/pr6050038

(2) https://doi.org/10.1371/journal.pcbi.1006971

(3) https://doi.org/10.1038/nbt.3956
(4) https://doi.org/10.1016/j.algal.2017.08.014

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Oral 7 - O7

New method by Supercritical Fluid Chromatography with High Resolution Mass (Q-Tof) to characterize a complete profil of 230 Skin Ceramides on various samples

Cyrielle Clement*[1], Julia Soullier[1], Roselyne Gautier[2], Carla Orlandi[2], Emilien Jamin[2], Hélène Duplan[3], Carine Jacques-Jamin[3], Nuria Pell Vidal[4], Nathalie Jonca[4], Pauline Le Faouder[1], and Justine Bertrand Michel[1]

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Lipids are the main constituent of skin, and are mainly constituted by ceramides with prevalent very long acyl chains, free fatty acids, and cholesterol. In health context, the lipid composition of skin can be highly modified thus its full characterization is essential. The skin ceramides is a large and complex family of 12 sub-classes and their structural diversity, their wide range of concentration and the few standards available make this analysis very complicated.
The aim of this work was to develop an original profiling of the skin ceramides using SFC (UPC2 Waters), which is a technology especially suitable for the non-polar metabolites, and a Q-TOF (Xevo, Waters). The development and the validation of this work will be presenting in this presentation.
The SFC-HRMS data set obtained in such a large lipidomic profiling required a complex data treatment and unfortunately software solutions of suppliers are rarely adapted to our needs. A home-made data base was then created and was constituted with 230 species. MSDIAL was first used to reprocess these datas in semi targeted mode but new solutions such as MzMine3 proved to be better for our needs. A comparison between these two softwares will be developing.
Data collection for skin sample is complex: it can be biopsies, cigarette paper impregnated with sebum, but more often, samples are strip, or a solvent-in vivo skin extraction. Due to the wide variety of sampling, the lipid extraction has to be adapted. The ceramide profils obtained with these different samples will be presented.

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Oral 8 - O8

Metabolomics and dynamic molecular networking reveal the diversity and biosynthesis of pyran-2-ones in a mussel-derived Penicillium restrictum cultured on host-based medium

Olivier Grovel*†[1,2], Samuel Bertrand[2], Van Tuyen Le[2], Thibaut Robiou Du Pont[2], Fabrice Fleury[3], Emmanuel Gentil[2], Cédric Loge[4], and Grégory Genta-Jouve[5]

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Very little is known about chemical interactions between fungi and their mollusk host in marine environments. Here, we investigated the metabolome of a *Penicillium restrictum* strain isolated from the blue mussel *Mytilus edulis* collected on the Loire estuary, France. Following the OSMAC approach, the effect of salinity and of a mussel-derived medium on the metabolic expression of this strain were analysed using HPLC-UV/DAD-HRMS/MS. An untargeted metabolomics study was performed which highlighted various classes of compounds which were specifically induced in marine conditions. In particular, a high chemical diversity of pyran-2-ones was found related to the presence of mussel lyophilisate in the culture medium. MS- and UV-guided purification allowed to isolate thirteen pyran-2-ones including five new from the mussel-derived culture medium extract, whose complete structure was elucidated. A both untargeted and targeted time-scale metabolomics study has then been performed on the strain cultured for 11 days and extracted each day, which showed the dynamics of specialised metabolism with a variety of compounds produced at a very early, intermediate or late stage of growth. One compound appearing at the very late stage was successfully isolated and characterised as (2*E*)-5-acetoxy-3-methoxy-2-pentenoic acid, an unreported precursor of pyran-2-one biosynthesis. This last was further explored using a dynamic molecular networking approach, which highlighted the sequential biosynthetic steps leading to pyran-2-ones. These results illustrate the utility in using host-derived media for the discovery of new natural products and that combining metabolomics and dynamic molecular networking is a new approach to explore microbial biosynthesis and chemoversity.

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Oral 9 - O9

^{13}C measurements and positional approaches with GC-MS: method validation and applications to plant13C-labeled experiments

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Analysis of plant metabolite 13C-enrichments with gas-chromatography mass spectrometry (GC/MS) has gain interest over the last years, due to the ease of its implementation and the ability to combine information from multiple fragments of a same metabolite. However, measurement of carbon isotopologue distribution (CID) by GC-MS can be prone to analytical and matrix biases, that could be further enhanced with positional approaches (combination of multiples errors). Here, we took advantage of tailor-made 13C-Pascal Triangle standards, harbouring known CIDs and positional 13C-enrichments, to evaluate the reliability of GC-MS measurements and positional approaches for TMS-derivatives of organic and amino acids. Overall, we successfully validated the accuracy of the method, but some important biases were identified for proline, valine and threonine. Conversely, positional approaches were essentially validated for glycine and serine and questioned the wide application of such methodology without a preliminary validation step. Finally, we applied this method to explore the regulation of plant central carbon metabolism under dark and light conditions, by performing incorporation kinetics of U-13C-pyruvate and U-13C-glucose into *Brassica napus* leaf discs for up to 6 hours. The results showed interesting isotopic patterns related to the regulation of glycolysis, the tricarboxylic acid cycle and its phosphoenolpyruvate carboxylase anaplerotic pathway, and the photorespiratory-dependent biosynthesis of serine.

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Oral 10 - O10

Combining innovative NMR methods for improved environmental contaminants characterisation using metabolomics

Marine Letertre*[1], Virginie Silvestre[1], Marine Piou[1], Jonathan Farjon[1], Maykel Hernández-Mesa[2], Julien Boccard[3], Gaud Dervilly[4], and Patrick Giraudeau[1]

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While NMR is a well-established technique for metabolomics, it suffers from strong peak overlaps preventing biomarkers elucidation and quantification. Recently suggested fast 2D NMR methods better separate overlapped signals by spreading them along a second dimension while providing additional information for their identification. Here, we evaluate how these new techniques could be the foundation of more robust and trustworthy analytical strategies in the context of characterizing the exposure to environmental contaminants. Following a Bligh and Dyer extraction, both the metabolic and lipidic profiles of serum samples from mini-pigs exposed to the endocrine disruptor Bisphenol A (BPA) were determined using 4 NMR methods: quantitative 1H NMR, Ultrafast COSY, Non-Uniformly Sampled (NUS) TOCSY and NUS pure-shift HSQC. These experiments offer a good balance in terms of sensitivity, resolution, and experimental time. Although most of them have been applied to metabolomics, their complementarity remains unexplored.

Discriminant analysis models were built from each individual dataset to assess their ability to characterize metabolic alterations due to BPA exposure, and a multi-block strategy was implemented for data integration. Although challenges to process 2D NMR datasets had to be tackled, the preliminary results are encouraging for the use of fast 2D NMR in metabolomics. The 2D individual statistical models show similar performance to the 1D-based models, but are, at least for metabolomics, the models that exert the highest influence on sample discrimination across the multi-block model. Ongoing work includes the identification of potential biomarkers associated to BPA exposure, as well as the acquisition of complementary HRMS datasets.

Oral Constructeur 4 - C4
Proteigene-Biocrates
Impact of metabolism on therapeutic response

Carlos Malpica, PhD, MBA - Senior Business Consultant at biocrates Life Sciences

Large spectrum quantitative metabolomics by biocrates enables the study of the impact of metabolism on therapeutic response. Knowledge gathered on microbiome and cancer immunotherapy has led to new strategies for the modulation of chemotherapy. This short presentation will address how we can identify dietary supplements to overcome drug resistance.

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Oral 11 - O11

Intégration des approches omiques (métabolomique et protéogenomique) pour l'étude des effets de contaminants émergents sur la moule méditerranéenne Mytilus galloprovincialis

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Dans le cadre d'études en écotoxicologie, telle qu'une exposition à des contaminants émergents par exemple, les approches omiques jouent un rôle important dans l'identification d'effets moléculaires (mécanisme d'action notamment) qui peuvent être précurseurs d'effets physiopathologiques (Canzler et al., 2020). Toutefois, les modulations moléculaires sont plus informatives (et moins variables) lorsqu'elles sont définies au niveau d'une voie biochimique (plusieurs omiques ensemble), et non en tant que gènes, protéines ou métabolites individuels. Au cours de ce projet, des moules prélevées dans un étang autour de Montpellier ont été exposées (ou non) à un mélange de contaminants émergents. Après 3 jours d'exposition, les glandes digestives des moules mâles ont été prélevées pour réaliser à la fois des analyses métabolomiques et protéomiques (par LC-HRMS), sur les mêmes échantillons, considérés comme appariés. Suite à la fusion des données -omiques (MCUVE-PLS et consensus OPLSDA) (Boccard et al., 2013), nous avons mis en évidence des liens directs entre métabolites et protéines modulées et révélé des voies toxicologiques pouvant être activées par le mélange de contaminants émergents.
L'intégration de données multi-omiques réalisée dans ce projet a permis de documenter de façon plus solide la compréhension moléculaire des effets sur des organismes non modèles pour lesquels le génome n'est pas séquencé. De plus, les données omiques étant souvent fragmentaires (puisque toutes les protéines et tous les métabolites ne peuvent être d´détectés), l'acquisition d'information sur plusieurs niveaux d'expression du génome permet de combler les vides laissés par nos choix de protocoles et m´méthodes analytiques.

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Oral 12 - O12

Chemical diversity and chemotaxonomy ofneo-tropical Xylariales fungi.

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Fungi of the order Xylariales belong to the class Sordariomycete in the Ascomycete division. Most can form large fruiting bodies and produce a high diversity of specialized metabolites, including some with significant antibiotic activities. However, the majority of these compounds were extracted and identified from their microscopic form.(1) In this work, we will focus on the macroscopic form of Xylariales fungi to study their metabolic production and its potential relationship with taxonomy.

Thirty-eight fungal specimens were collected from French Guiana and morphologically identified. To further refine their identity, an environmental strain replication method developed at ICSN was employed, lipidic fingerprints were obtained by MALDI-ToF-MS, data processing was achieved using R(v4.1.2) and spectral comparison using MetGem.(2,3) Results showed a good match for replicates of six species from genus *Xylaria*, *Phylacia*, *Thamnomyces* and *Camillea.* However, no match was obtained within the *Kretzschmaria* genus despite several replicates of *K. clavus* and *K. deusta.*

To complete this chemotaxonomic study, specialized metabolite production was also explored. Ethyl acetate extracts of each specimen were analyzed by RP-LC-HRMS/MS. The data was used to generate a molecular network to annotate each metabolome. We detected compounds commonly produced by genera in the Xylariaceae family. Among these compounds, we focused on a family of metabolites abundant in genus *Thamnomyces* that could not be annotated using databases. Several compounds were isolated to characterize the structure of new or non-dereplicated molecules from the databases.

(1) Helaly,S.E. et *al. Nat.Prod.Rep.* **2018**

(2) Levasseur,M. et *al. Microorganisms* **2022**

(3) Olivon,F. et *al. Anal.Chem.* **2018**

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Oral 13 - O13

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

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The ability to identify metabolites with confidence is crucial to evaluate metabolic dysfunctions. Nonetheless, more than 97% of the estimated human metabolome remains unknown. Combining stable isotope tracers and sensitive analytical methods has boosted these investigations. However, most isotope-assisted studies are often limited to cellular models and observation of a restricted, known portion of the metabolome to answer specific metabolic questions. Here, the murine metabolome, close to the human one, was successfully labeled by feeding three mice a fully 13C-enriched diet for six weeks. Their urines were collected throughout this period and analyzed by LC-HRMS-based metabolomics using an Orbitrap FusionTM instrument (ThermoFisher Scientific). Results showed that the isotopic data provide improved metabolite identification: distinction of relevant signals, unambiguous assignment of chemical formula, better understanding of the fragmentation data, and greater confidence in the structural identification. More than 130 metabolites from the internal chemical library were identified with high confidence, and 120 metabolites were annotated as acylcarnitines, acylglycines, or acylglucuronides. Moreover, even if most metabolites exhibit an enrichment rate higher than 90% after 7 days, isotopic patterns and 13C-enrichement kinetics over 39 days depend on the metabolites structure and metabolic pathways.13C-enrichment profiles can then be used to differentiate different chemical classes of metabolites. Notably, metabolites composed of two building blocks exhibit a two-step 13C-enrichment process. For example, intermediate isotopologs composed of a fully labeled ribose unit and an unlabeled nucleobase were evidenced during the 13C-nucleoside biosynthesis. Thus, an overall presentation will highlight the value of *in vivo* 13C-labeling for metabolism investigations.

Oral Constructeur 5 - C5

Gencovery

Constellab™ : un écosystème numérique complet pour la digitalisation de projets complexes de R&D

Adama Ouattara

Gencovery est spécialisée dans la digitalisation des projets complexes de R&D dans la biotechnologie et dans l'industrie pharmaceutique. Nous développons et commercialisons la plateforme numérique Constellab™ qui est un écosystème numérique complet, simple d'accès pour les biologistes et bioinformticien.nes, et qui permet de digitaliser et accélérer de bout-en-bout toute la chaine de valeur de R&D : - Déploiement d'infrastructures de données et de calcul - Traçabilité des analyses et projets - Jumeaux numériques du métabolisme cellulaire - Analyse des données : bio-informatique, intelligence artificielle - Bases de données omiques structurées. Les applications de nos technologies, notamment de jumeaux numériques, sont l'étude des modes d'action, l'ingénierie métabolique, l'optimisation des bioprocédés.

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Oral 14 - O14

Pure Shift NMR of Aqueous Biofluids: Towards Quantitative Metabolic Profiling

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Accurate quantification of metabolites by nuclear magnetic resonance (NMR) is of prime importance in the field of health sciences for understanding the metabolic pathways of the investigated system, to address the mechanisms of action of diseases, and improving their diagnosis, treatment, and prognosis. Unfortunately, the absolute quantitative analysis of complex samples is still limited by sensitivity and resolution issues that are intrinsic to this technique. Ultrahigh-resolution *pure shift* methods have especially shown to be suitable for interpreting mixtures of metabolites in biological samples.
Here we will show how Pure Shift 1H NMR can be used to decipher the metabolism of DLBCL and understand the mechanism of action of antimetabolic drugs. We will present the methodological developments carried out in our group to acquire Pure Shift spectra with efficient suppression of the water signal on these biofluids. We will demonstrate a robust analytical protocol based on the use of a pure shift library of calibration reference spectra to fit the fingerprint of each metabolite of interest and determine its concentration. We will show for the first time a workflow for quantifying metabolites in extra-cellular media using Pure Shift data. We will describe how the analysis of the metabolic profiles allows for getting a unique insight into the metabolic pathways that are key to Lymphoma Cells.(1-2)

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Oral 15 - O15

Invasive macroalgae shape the chemical and microbial composition of reef boundary layers

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Shift of coral reefs towards macroalgal dominance posits several concerns on the persistence of coral communities. Studies demonstrated that benthic organisms could change the reef water chemistry influencing the microbial communities' structure and metabolism, yet how these changes operate spatially in the water column remains obscure. Here, we provide a comprehensive description of the chemical and microbial composition of reef boundary layers, the benthic and the momentum, from algal-dominated and algal-free bommies in Mo'orea, French Polynesia, with an emphasis on the two dominant macroalgal species *Dictyota bartayresiana* and *Turbinaria ornata.* By integrating untargeted metabolomics (LC-MS/MS) and 16s rDNA metabarcoding data, we showed that the multi-omic signature of the water column was specific of the underlying benthic communities and the boundary layer, reflecting the influence of ambient hydrodynamics and benthos exudates on the spatial distribution of metabolites and microbes. The taxonomic composition of the microbial communities gave insights on the spatialization of reef biogeochemical processes and revealed an enrichment of potential pathogens and opportunist copiotrophic bacteria in algal waters. Macroalgae released compounds of distinct chemical classes and their associated boundary layers were rich in potential infochemicals from diverse lipids classes (e.g. prenol lipids, glycerolipids) and labile organic matter (e.g. organooxygen compounds and organic acids). Our multiomic data allowed us to highlight co-variations of algal-associated metabolites and microbes in the momentum boundary layer. This study confirms that benthic community shift can modify the water chemistry and the microbial community composition which question about potential detrimental environments for the neighboring organisms, as corals.

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Prix de thèse RFMF

Analysis of the metabolic features of plant extremophile species from the Atacama Desert

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Biochemical adaptations of plants thriving in extreme lands have always attracted human curiosity. Previous studies targeted the analysis of a unique or a few extreme plants grown under controlled conditions, considering their adaptive mechanisms as mainly species-specific1. Here, we conducted a comprehensive approach from the ecosystem to the metabolite level to unveil convergent strategies employed by multiple plant species of the Atacama Desert, the driest nonpolar desert on Earth2,3. First, multi-platform metabolomics was used to characterise the primary and secondary metabolism of 24 Atacama species collected along an elevation gradient from 2500 to 4500 m. Generalised linear models unveiled a generic metabolic toolbox of 39 compounds predicting plant environment independently of plant species and sampling year. Next, we investigated the influence of facilitation processes on the survival of Atacama plant species as well as its metabolic consequences. In this context, we studied the nursing effect of the cactus *Maihueniopsis camachoi* across the elevation gradient. We combined cover and temperature measurements with predictive metabolomics to (i) highlight the driving role of facilitation processes on the structure of the Atacama ecosystem, and (ii) uncover a set of 93 markers predicting the interaction status with 83% accuracy. Overall, this study sheds light on the role of convergent evolutions in plant resilience to harsh climates and provides pioneer insights into our understanding of facilitation processes. Besides, our multi-species predictive metabolomics approach foreshadows promising studies that seek to discover predictive soft traits in agronomy and ecology.

Keywords: Predictive metabolomics, GLM, facilitation, Atacama Desert.

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Oral Constructeur 6 - C6

Sciex

Maximising the power of the ZenoTOF7600 system for lipidomics and metabolomics

Heather Chassaing - Accurate Mass Workflow Specialist, France and BeNeLux

With the introduction of the SCIEX ZenoTOF 7600 system, scientists can now achieve quantitative results at high speeds with high mass accuracy. The core innovation on the ZenoTOF 7600 system is the Zeno trap that when activated, provides significant improvements in duty cycle due to the optimization of ion transmission from the collision cell into the accelerator. This duty cycle improvement provides a substantial increase in MS/MS sensitivity and thus enables targeted high-resolution workflows. In addition, the ZenoTOF7600 offers an alternative electron based fragmentation technique, Electron Activated Dissociation (EAD). EAD combined with the sensitivity gains afforded by Zeno trapping, provides diagnostic ions which facilitate the differentiation of structural isomers and isobars. EAD also provides complete structural characterization of lipids in one experiment, providing the lipid species, the attachment of the fatty acid positions and localization of the double bond position. This makes the ZenoTOF perfectly adapted to provide complete and information rich MS data for metabolomic studies.

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Oral 16 - O16

New data normalisation methods for PTR-TOF-MS exhaled breath metabolomics

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Volatilomics is the branch of metabolomics dedicated to the analysis of volatile organic compounds (VOCs) in the exhaled breath for medical diagnosis or therapeutic monitoring purposes. Real-time mass spectrometry technologies such as proton transfer reaction - mass spectrometry (PTR-MS) are available to perform such studies. Data normalisation is an important step to discard unwanted variation from non-biological sources. Batch effects and loss of sensitivity over time may be observed with PTR-MS and have to be taken into account to avoid bias when comparing samples acquired over months. However, normalisation methods for real-time breath analysis were poorly explored.

We intended to benchmark known metabolomic data normalisation methods and transpose them to PTR-MS data analysis. We compared seven methods, five statistical-based methods and two using multiple standards metabolites, on two datasets from clinical studies on COVID-19 diagnosis. We assessed different means of feature selection to choose the standard metabolites, as well as the use of several repetition measurement of ambient air to train the normalisation methods. We show that normalisation tools allow an increase in the diagnostic performance of the machine learning models, while decreasing the dependency with time. The sensitivity increased from 65% to 77% for the cohort from emergency room patients and the global accuracy was improved from 93% to 96% for the cohort from intensive care patients.

Our results highlight the importance of adding an appropriate normalization step during the processing of PTR-MS data, which allows significant improvements in the predictive performance of statistical models.

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Oral 17 - O17

L'élucidation structurale de nouveaux biomarqueurs : un " Qui est-ce " grandeur métabolomique

Chloé Cloteau*†[1,2], Vivian Delcourt[2], Benoit Loup[2], Benjamin Chabot[2], Mylène Pescher[2], Elodie Susdorf[2], Zied Kaabia[2], Marie-Agnès Popot[2], Bruno Le Bizec[1], Ludovic Bailly-Chouriberry[2], and Gaud Dervilly[1]

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De par sa capacité à étudier l'impact du métabolisme sur l'empreinte chimique d'un fluide biologique, la métabolomique s'est avérée être une stratégie pertinente dans le cadre du contrôle de l'usage de substances interdites chez les chevaux de course. Les biomarqueurs d'effet révélés par ces approches, ont alors été intégrés dans des modèles de classification utiles pour le dépistage de l'administration de promoteurs de croissance. Toutefois ces biomarqueurs ne sont pas systématiquement élucidés alors même qu'il s'agit d'une étape clé pour l'interprétation biologique des résultats observés.
Suite à la mise en évidence de trois biomarqueurs urinaires signant l'administration d'anabolisants chez les chevaux de course, ces travaux ont eu pour ambition de réaliser l'élucidation structurale de ces composés en combinant différentes stratégies analytiques impliquant la LC- HRMS ainsi que la mobilité ionique, associées à des expérimentations de cationisation et de déconjugaison.

L'étude des spectres MS et MS/MS a conduit à l'identification d'un des métabolites comme l'hydroxy-tébuconazol glucuronide. Les différentes observations ont été confirmées par com- paraison des temps de rétention, des spectres MS et MS/MS mais également des valeurs de CCS avec les valeurs d'un standard de référence. Enfin, des études *in vitro* complémentaires ont démontrés que la présence de l'hydroxy-tébuconazole glucuronide dans les urines provenait de la métabolisation du tébuconazol par le système enzymatique équin.

Dans cette étude, un des métabolites mis en évidence lors d'une étude métabolomique a été identifié et la complémentarité des techniques utilisées a permis de mieux comprendre les perturbations observées après l'administration d'agents anabolisants à des chevaux.

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Oral 18 - O18

Expanding the chemical annotation of metabolitesfrom biofluids by combining different LC-MS methodologies and strategies in untargeted metabolomics

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Introduction

Host-associated samples subjected to untargeted metabolomics have provided valuable insights into how microbes influence health in a bidirectional way1. However, accurate metabolite annotation and identification remain to be a challenge2 along with ensuring analytical reproducibility and feature coverage for large cohorts of data3.

Technological and methodological innovation

Human serum samples were first assessed under a total of 22 UHPLC-HRMS methodologies from 6 metabolomic platforms (C18, C8 and HILIC modes in positive and negative mode) using a HPLC Vanquish Duo coupled to an Orbitrap Exploris™ 240 Mass Spectrometer. Iterative data acquisition workflow (Deep Scan) was implemented in addition to the DataDependent Acquisition (DDA) method and GNPS molecular networking approach (MN) was applied.

Results and impact

A total of 1390 unique metabolites were annotated and pure standards were used to validate some MS2 annotations. Deep Scan allowed us to increase the number of unique compounds with high-quality fragmentation spectra up to 50% regarding the DDA method. MN also allowed us to expand the chemical information of the unannotated metabolites. Our results will be essential for the implementation of a reproducible workflow for untargeted LCMS analysis of biofluids in the context of metabolomics in microbiome research.

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Oral 20 - O20

Elucidating the role of specialized metabolite decorations regulated by warm temperatures in seeds

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Specialized metabolites (SMs) play a crucial role in the interaction of plants with their environments. SMs are highly accumulated in plant and seeds of a wide range of Brassicaceae species, including the wild species *Arabidopsis thaliana*. While most studies have focused on SMs pathways regulation by stresses in vegetative tissues, the study of seed ability to produce these protective compounds in response to environmental stress has been neglected. Moreover, few information is known about SM decoration diversity and functions in seeds. We recently highlighted a strong environmentally-induced plasticity of SMs in seeds, suggesting a major impact of the environmental conditions on the accumulation of specific SMs. In this study, untargeted metabolomic analysis was conducted on *A. thaliana* seeds developed under warm temperatures or in control conditions. Overall, 38% of detected seed specialized metabolic features was affected by warm temperature stress, revealing a significant stimulation of seed SMs. Many of the stress-induced SMs were decorated cinnamic acids, tannins precursors and flavonols. While seed SM glycosylation, acylation and hydroxylation were activated by warm stress, SM methylation seems to be lesser affected by high temperatures. Transcriptomic analyses allowed the identification of many differentially expressed genes involved in SM decorations (*e.g.* glycosyltransferases, acyltransferases, hydroxylases) that are modulated by warm temperatures. Knock-out mutants for the most promising of these genes are being metabolically and functionally characterized.

We suggest an important role for metabolite decorations in seed tolerance response against warm temperature.

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Oral 21 - O21

Improving comprehensive analysis and compound annotation in complex biological samples through molecular networking based on SWATH-MS and EAD fragmentation

Valentina Calabrese*†[1], Thomas Brunet[1], Davide Degli Esposti[2], Arnaud Chaumot[2],Olivier Geffard[2], Arnaud Salvador[1], Yohann Clement[1], and Sophie Ayciriex‡[1]

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Untargeted high-resolution mass spectrometry (HRMS) for molecule profiling turns out to be decisive in the interpretation of complex biological systems, mapping molecules in biological pathways, and towards biomarker discovery (1). However, compound annotation remains a major challenge in metabolomics and lipidomics, especially for non-model organisms in environmental science, when reference data are partial or absent. Recently, molecular networking (MN) (2) together with unsupervised Machine Learning algorithm (*tSNE*) integrated on MetGem (3), have emerged as powerful bio-informatics tools for boosted compound annotation. Nevertheless, these MN representations are commonly obtained from product mass spectrum in Data-Dependent-Acquisition mode (DDA) under collision induced dissociation (CID), that results in a limited number of acquired fragmentation spectra and insufficient fragmentation of specific molecule classes. Here, we evaluated the use of MN and *t-SNE* for in-depth data interpretation and compound annotation from MS data collected in Data-Independent-Acquisition (DIA) through sequential window acquisition of all theoretical fragment ion spectra MS (SWATH-DIA). The use of the novel fragmentation process called electron activated dissociation (EAD), implemented on Q-ToF instruments, provides richer fragmentation spectra. We investigated DDA *versus* DIA acquisition mode supported with CID or EAD for multi-omics data set from *Gammarus fossarum* extracts, a freshwater amphipod used in ecotoxicology studies. The performances of each strategy towards number of detected/annotated features, resolution of isomers and semi-quantitative analysis will be discussed.

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Oral 22 - O22

Arteriovenous metabolomic approach reveals specific metabolite exchanges across organs and provides unique insights for understanding underlying metabolic perturbations in obese and insulin-resistant minipigs

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The specific role of organs on type 2 diabetes onset remains to be elucidated. Indeed, the available biomarkers are whole-body biomarkers that provide little information about organs' metabolism. We therefore developed an innovative arteriovenous (AV) metabolomics approach aiming to define early organ-specific signatures in insulin-resistant (IR) and obese minipigs. Minipigs were fed either a normal-fat (NFC) or a high-fat high-sugar diet during 2 (HF2M), 4 (HF4M) or 6 (HF6M) months. All HF groups developed similar IR and progressive obesity up to a morbid stage. By performing LC-HRMS metabolomics on 9 vessels and computing ratios of metabolites levels between blood going in and out of organs, we established the uptake and release profiles of 9 organs: head, muscle, intestine (small, large and total), liver, pancreas, kidney and spleen. Interestingly, regarding the 4 main organs involved in obesity and IR onset (liver, pancreas, muscle, intestine) we observed that the profiles of organ-specific metabolite exchanges shifted from release to uptake as early as in HF2M group for 5, 10, 7 and 1 metabolites respectively. Thus, several metabolites were actively up-taken in the HF2M animals, including methionine, cystathionine and 3-hydroxypentanoic acid by the liver, hydroxyoctadecadienoic acid and eicosapentaenoic acid by the pancreas and pyroglutamic acid by the muscle. Some of these metabolites are known to be related to oxidative stress potential and lipotoxicity and deserve more investigation. In conclusion, this organspecific AV approach is promising for the investigation of the metabolism regulation during obesity and IR onset at the organ and inter-organ exchanges levels.

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Oral 23 - O23

Exploration multi-omique et in silico des effets transgénérationels et sexe-spécifiques induits par le TBT, un composé obésogène, sur le métabolisme hépatique de la souris

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De nombreux contaminants chimiques, dont le tributylétain (TBT), sont suspectés être des perturbateurs métaboliques, contribuant à l´´étiologie de dérégulations métaboliques telles que l'obésité, avec des effets pouvant ˆêtre transmis sur plusieurs générations. Dans cette étude, nous avons exploré l'effet, sur le métabolisme hépatique, d'une exposition prénatale au TBT (génération F0) sur la génération F3. Les données phénotypiques démontrent un effet obésogène du TBT, après un challenge nutritionnel (régime "high fat"), uniquement chez les mˆ ales. La mise en place d'une approche multi-omique sur les foies de souris mâles et femelles de la génération F3 nous a permis d'explorer les effets hépatiques au niveau du transcriptome (RNAseq) et du métabolome, incluant l'analyse des métabolites polaires (RMN, LC-MS) et apolaires (lipidomique GC/LC-MS). Nous avons ainsi pu révéler un effet significatif du TBT sur le métabolome et le lipidome, à la fois chez les mâles et les femelles, malgré l'absence d'effet phénotypique observé chez ces dernières. L'implémentation d'une stratégie combinant des approches statistiques univariées et multivariées (AMOPLS) nous a permis de distinguer les effets globaux du TBT de ses effets sexe-spécifiques. Nous avons démontré qu'une partie du métabolome, et encore davantage du lipidome, est différemment affectée par le TBT en fonction du sexe, avec notamment l'augmentation de plusieurs classes de phospholipides chez les mâles et une diminution des triglycérides et diglycérides chez les femelles. L'analyse, dans le contexte du réseau métabolique, des signatures métaboliques et

transcriptomiques sexe-spécifiques de l'exposition ancestrale au TBT suggèrent des mécanismes d'action différents au niveau hépatique en fonction du sexe.

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Oral 24 - O24

Embarking on a delicious treasure hunt: exploringthe brown and black world of fine dark chocolateswith polyphenol metabolomics and molecular networking

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High-quality dark chocolates (70% cocoa content) have a dark brown color that is partially influenced by phenolic compounds. However, some chocolates show a light brown color, which is not desired for the conventional market, but could open new marketing opportunities. This work aimed at revealing compounds that discriminate black and brown chocolates. Among 37 fine chocolate samples provided by Valrhona and made from twenty different *Theobroma cacao* clones in years 2019 and 2020, eight dark black samples and eight light brown samples were selected. A non-targeted metabolomics study was performed based on ultra-high performance liquid chromatography-high resolution mass spectrometry/mass spectrometry experiments, univariate, multivariate, and feature-based molecular networking analyses. Twenty-seven overaccumulated compounds were found for black chocolates. Among them, glycosylated flavanols and oligomeric glycosylated A-type procyanidins (dimers and trimers) were highly representative. Fifty overaccumulated compounds were found for brown chocolates. Most of them were B-type procyanidins (from trimers to nonamers). Additionally, based on the ultra-high resolution (500k FWHM) profile of the M+2 isotopic peak, a sulphated glycosylated flavanol could be unambiguously identified. Phenolic and color profiles were mostly related to the sample genetic origins. This study provides new insights on the phenolic profiles of black and brown chocolates that may be useful to better understand the color variations of dark chocolates.

Communications flash

RÉSEAU FRANCOPHONE DE MÉTABOLOMIQUE ET FLUXOMIQUE

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Oral Flash 1 - FP1

Identification of chemical defenses involved in natural durability against lignivorous fungi of a tropical tree Sextonia rubra by targeted and untargeted metabolomics

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Sextonia rubra (Mez.) Van der Werff (Lauraceae) is a tropical tree endemic to the Guiana shield and the Brazilian Amazon (1). It is known for its durability and for its use as a building material. Its heartwood comprises numerous lactone derivatives of which the two main molecules are rubrynolide and rubrenolide. These two molecules have insecticidal and antifungal activities (2-3). Like any living organism, S. rubra is exposed to biotic and abiotic stresses during its lifetime, which trigger diverse defense mechanisms. Considering the tree as a system, explored through interdisciplinary methods, can help to decipher these complex processes.

Wood samples were obtained from central boards sawn at various stem heights of a mature individual (Paracou forest, French Guiana), and radially subdivided from the sapwood to the pith. Wood decay resistance was assessed using long-term soil bed tests. We applied untargeted and targeted metabolomics methods to annotate the different molecular families and quantify them, i.e., lactone derivatives and alkaloids. All the extracts were tested against 6 Glutathione-S-Transferase (GST) of Trametes versicolor, corollary of their anti-fungal activity (4).

Our results suggest that for pith and heartwood, the higher the concentration ratios of lactones towards alkaloids, the higher the reactivity of GSTs, and the more the natural durability against wood-decaying fungi is positively affected. Although the involvement of lactone derivatives in the natural durability of this species was known, the involvement of alkaloids is new and suggests that S. rubra specializes its chemical defenses according to the tissue.

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Oral Flash 2 - FP2

Evaluation of the environmental impact of a biopesticide using an innovative approach coupling high-throughput methods (metabolomics and metabarcoding)

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Biopesticides are complex substances that are derived from natural sources (e.g., plants, microorganisms), these substances offer a promising alternative to pesticides. Since biopesticides have recently been used, it remains unknown how long the biopesticides and their residues stay in the environment and how they impact organisms living in soil.

In this context, we conducted an experiment to evaluate the environmental fate and impact of Beloukha, a bioherbicide containing Pelargonic acid as active substance. A kinetics study was performed over 56 days in soil microcosms using treated and non-treated ("Controls") conditions. These samples were analyzed using metabolomics (UHPLC-HRMS) and metabarcoding (16S and 18S rRNA genes).

Firstly, metabolomics showed that we do not reach the resilience time; the latter evaluating both the dissipation of the xenometabolome (bioherbicide + by-products) and the impact of Beloukha on the soil endometabolome (soil metabolites). Thanks to the development of R scripts allowing the separation of the endometabolome and the xenometablome, we could find that transformation products still remained after 56 days. To test whether these products have an impact on soil biodiversity, we described bacterial communities using 16S metabarcoding. These analyses showed that bacteria were impacted at the beginning of the kinetic, and that this impact decreased eight days after the treatment and disappeared after 14 days.

Analyses on the eukaryotic communities using 18S metabarcoding, as well as correlations between the different datasets are in course. All the data will help to describe the effects of biopesticides on soil and to determine chemical and biologicals markers.

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Oral Flash 3 - FP3

Annotation of biomarkers in exhaled breath:combining real-time mass spectrometry and two-dimensional chromatography-mass spectrometry

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Background:
Exhaled breath analysis by Proton Transfer Reaction - Mass Spectrometry (PTR-MS) allows the on-line, real-time detection of biomarkers of pathologies, but the understanding of the pathophysiological mechanisms implies the annotation of the biomarker candidates with a high confidence level. Following the discovery of candidate VOC biomarkers for COVID-19 *(Grassin Delyle et al.)*, we implemented a combined PTR-MS and thermal desorption, two-dimensional chromatography coupled to mass spectrometry (TD-GCxGC-MS) analysis to enable their identification.

Methods:
Breath samples from intensive care unit patients and analytical standards of the candidate biomarkers (as obtained from library search) were analysed with both i) PTR-MS real-time analysis (PTR-Qi-TOF with liquid calibration unit, Innsbruck, Austria) and ii) TD-GCxGC- MS (BT4D, Leco, USA) from clinical samples collected on Tenax TA tubes (Markes, United Kingdom) or tubes spiked with the analytical standards. PTR-MS data analysis was performed with the ptairMS R-package.

Results:
Twenty-five analytical standards corresponding to 10 different *m/z* candidates (PTR-MS (M+H)+) were analysed. Signals were recorded at the expected *m/z* for PTR-MS for 22 standards. GCxGC-MS analysis allowed the detection of all compounds, adding the chromatographic separation of isomers and confirmation of VOC identification with comparison to NIST mass spectra library. The comparison of analytical standards and clinical samples was performed and allowed to increase the level of annotation of candidate biomarkers.

Conclusions:
Combining real-time and chromatographic analysis of breath samples may be useful for the rapid detection and the annotation of VOC candidate biomarkers, highlighting the interests of breath analysis for clinical use and pathophysiological studies.

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Oral Flash 4 - FP4

Establishment of a NMR-based metabolomicsprotocol for salivary samples

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In contrast to the most used biofluids such as blood and urine, saliva is rarely studied in metabolomics. However, this biofluid seems to be of great interest because of its non-invasive, simple and fast collection. Moreover, it is perfectly suited for self-sampling, well-adapted to a personalized approach of medicine and is expected to be complementary with other biofluids information. This is why we aim to develop and optimize a protocol to analyze salivary samples by NMR.

Therefore, this work focused on the identification of the best suitable protocol allowing to obtain the most pertinent and huge metabolomics information. For this purpose, four methods were selected according to the literature and to in-house processes and were compared. On the basis of several analytical criteria such as the number and the concentration of identified metabolites, the repeatability and the robustness, the best method was selected. Freeze- drying step followed by ultrafiltration led to the most informative and repeatable protocol. Finally, this optimized workflow was applied to a real-case study (fasting *vs* non-fasting volunteers) in order to prove the pertinence of the method and of the saliva's analysis in metabolomics. This study showed significant differences between the groups and discriminant metabolites were identified.

In conclusion, results obtained in this work are encouraging and highlight the real interest of using saliva samples in metabolomics. In the future, it will be interesting to apply the developed protocol to metabolomics studies and to combine it with the usual blood and urine's metabolomics analyses.

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Oral Flash 5 - FP5

Interagir avec ses données de spectrométrie de masse avec Galaxy

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Ces dernières années, une vaste gamme d'outils informatiques s'est développée, facilitant l'analyse des données de spectrométrie de masse générées dans les études de métabolomique.
Cependant, un manque d'informations et/ou de transparence sur leur fonctionnement est à déplorer, limitant la bonne prise en main par les utilisateurs. Dans un souci de clarté et d'accessibilité aux algorithmes et aux données, les principes du FAIR (Facile `a trouver, Accessible, Interopérable, Réutilisable) sont appliquées de manière croissante dans la plupart des laboratoires académiques favorisant leur intégration dans ces nouveaux outils. En dépit du fait que ces outils répondent à une grande partie des recommandations exigences du FAIR, des améliorations en termes d'interactivité, de visualisation des données au cours des différentes étapes de traitement restent encore à implémenter afin de faciliter leurs utilisations par la communauté. Des outils de visualisation interactive des données sont d'ores et déjà présent dans des logiciels " open-source " (openMS, MS-DIAL, mzMine) mais ils ne sont pas intégrés aux gestionnaires de workflow. Cependant, le gain en interactivité entre l'utilisateur et ses données entraine une perte en reproductibilité.
Le fait d'utiliser interactivement les données et les modifier fait partie intégrante des besoins des utilisateurs au quotidien. Idéalement, il faudrait pouvoir concilier cette interactivité avec le besoin de traiter un nombre croissant de données de manière automatisée tout en restant reproductible. Afin de répondre à ce double enjeu, nous présentons des applications en RShiny (HaloSeeker) qui permettent à la fois ces interactions avec la donnée et une bonne reproductibilité.

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Oral Flash 6 - FP6

Exploration des interactions dans l'holobionte algal pour la découverte de nouveaux antifoulings éco-compatibles

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Les biofilms sont des communautés complexes de micro-organismes en contact avec une surface et inclus dans une matrice qu'ils sécrètent. Ces biofilms microbiens posent de nombreux problèmes pour les activités marine militaires, industrielles et de plaisance. La plupart des composés antifouling utilisés sont des biocides particulièrement toxiques qui ont un impact significatif sur l'environnement naturel. Aucune alternative durable et respectueuse de l'environnement n'a encore été développée. Cependant, de tels composés chimiques existent dans la nature et de nombreuses espèces marines ont développé des stratégies pour se protéger des salissures biologiques. Par exemple, les macroalgues, telle que *Delisea pulchra*, produisent des molécules ciblant le quorum sensing (QS), un mode de communication intercellulaire impliqué dans la formation des biofilms. La recherche de composés de quorum quenching, inhibant ce mécanisme clé, est donc une stratégie innovante pour identifier de nouvelles substances antifouling. La connaissance de l'holobionte algal a révélé l'existence d'un microbiote associé aux macroalgues capable d'influencer l'état de santé de l'algue hôte mais également que la structuration des communautés microbiennes est intimement liée `a la production de médiateurs chimiques au sein du microbiote. Plus précisément, nous avons démontré que l'épimicrobiote de l'algue *Saccharina latissima*, une espèce clé des côtes nord européennes, est le siège de la production de signaux chimiques capables d'inhiber le QS bactérien et par extension d'inhiber la formation de biofilms. Dans ce contexte, nous développons une approche multi-omique alliant métabolomique et métabarcoding afin de décrypter ce dialogue moléculaire et de valoriser les molécules naturelles produites comme nouvel agent antifouling.

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Oral Flash 7 - FP7

Untargeted LC-MS based metabolomics and molecular networking for the elucidation of ancientdyeing recipes

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Until the end of the 19th century and the advent of synthetic dyes, the use of dyeing plants was one of the main way to dye textiles (1). Nowadays, chemical analysis of cultural heritage objects can help to identify the plants used in ancient dyeing processes and then to elucidate ancestral dyeing recipes (2,3). However, the characterization of the botanical origin of yellow dyes remains an analytical challenge due to the wide range of plants in nature providing this color. Metabolomics has been used in many fields such as health and environment, but is still uncommon in archaeometry.

To determine the botanical origin of yellow dyes present in cultural heritage objects, a metabolomic workflow was developed and applied to fourteen, local or commonly used, dyeing plants. After dyes extraction, plant extracts were analyzed by liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS/MS). Potential taxonomic chemomarkers were then selected by means of statistical analyses. Finally, the annotation of these metabolites was strengthened through the use of molecular networking.

Such an approach made it possible to identify chemical markers for each species and thus to chemically discriminate all these species between them.

In this study, metabolomics has proven to be a powerful tool for the investigation of plant dyes. This method will be applied to case studies to decipher ancient dyeing recipes.

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Oral Flash 8 - FP8

Metabolomics profiling of French artichoke leaves by NMR and Mass spectrometry

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Artichoke, or *Cynara cardunculus* subsp. *scolymus*, is a plant recognized for its fruit all around the world and known to contains compounds with various nutritive and therapeutic benefits. In this work, we intend to compare the metabolic profile of the leaves of different artichoke and cardoons varieties collected in France and investigate the variability of their composition. To allow a broader and easier identification of the metabolites, the analyses will be performed by combining NMR and Liquid Chromatography-Mass Spectrometry (LCMS) data. Firstly, the sample preparation had to be optimized because, for integrative methods, it is recommended to carry out the experimental analyses on a unique sample to avoid biases. The leaves were extracted in a first step with infusion, as usually performed by Evear Extraction, that allowed to obtain a cardoon or artichoke liquor containing most of the compounds known to have biological effects. Our first objective was to prepare the liquors for the two analytical techniques, NMR and LC-MS. Thus, different extraction methods were tested for several artichoke varieties and the ultrafiltration approach was retained as the more appropriate sample preparation for polar compounds in cardoon or artichoke liquors. Then, our second objective, currently undergoing, focused on the optimization of LC-MS acquisition parameters to allow the detection of a maximum of polar compounds by using a design of experiments. Once this objective achieved, it will be possible to analyse and combine NMR and LC-MS data and compare the metabolomic profiles.

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Oral Flash 9 - FP9

An introgression-based genetical metabolomics approach enables the diversification of the phytochemical repertoire of oilseed rape (Brassica napus) and highlights series of genomic factors involved in the chemical diversity in Brassica.

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Phytochemical diversity is central to shaping plant biotic interactions. Oilseed rape (*Bras- sica napus*) is an allopolyploid crop resulting from a small number of post-neolithic crosses between its two parental species *B. rapa* and *B. oleracea*. The strong genetic bottleneck at the origin of *B. napus* have deeply narrowed its genetic diversity compared to its two parental species. Here, the phytochemical diversity of a *B. rapa* and *B. oleracea* core-collection (10 accessions per species) was examined by LC-HRMS/MS-based untargeted metabolomics on leaf and root samples. This approach revealed series of contrasted metabolic features at both inter and intra-specific scales. Thereafter, untargeted metabolomic profiling was extended to samples from a genetically characterized collection of hyper-recombinant introgression lines (ILs), which derived from initial crosses between a pivotal oilseed rape (variety 'Aviso') and a core collection of *B. rapa* and *B. oleracea*. This allowed the identification of ILs harbouring different specialized metabolomic profiles compared to 'Aviso'. It notably enabled to identify two small regions controlling the accumulation of two compounds annotated in the LC-MSMS libraries as blumenol C malonylglucoside and dicoumaroyl spermidine. Interestingly, these regions included some genes with strong homologies to Arabidopsis genes involved in key biosynthetic steps for those two compounds. Similar approaches are underway to elucidate the genetic factors controlling the accumulation of various Brassica phytochemicals, whose biosynthetic pathways are less well understood. This work illustrates how introgression approaches can enable the enrichment of the metabolic repertoire of *B. napus* and provide novel insights into the elucidation of unexplored biosynthetic pathways.

Communications poster

RÉSEAU FRANCOPHONE DE MÉTABOLOMIQUE ET FLUXOMIQUE

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Poster 1 - P1

L'analyse du métabolome et lipidome de plaquettes de patients reçus aux urgences pour la COVID-19 permet de prédire l'évolution de la maladie pendant l'hospitalisation

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Introduction
L'activation anormale des plaquettes sanguines après infection au SARS-COV2 est un facteur important de morbimortalité des patients COVID-19. Nous recherchons une signature moléculaire dans ces organites capables de prédire l'évolution de la pathologie.

Méthodes
54 patients SARS-CoV-2 hospitalisés ont été inclus. Les patients ont été classés rétrospectivement selon le recourt (n=24) ou non (n=30) à la ventilation mécanique à 28 jours, représentant un pronostique d'atteintes sévères ou légères, respectivement. Le métabolome/lipidome des plaquettes prélevées `a l'inclusion ont été analysés par LC-HRMS. 10 échantillons de patients exempts de l'infection COVID-19 ont servi de contrôles externes.

Résultats
433 espèces lipidiques et 124 métabolites polaires ont été annotés dans les plaquettes. Différents modèles PLS itératifs excluant au hasard 1/10 patients ont permis de sélectionner une signature composée de 14 lipides (dont 8 céramides, 1 cholesteryl ester, 3 lysoPC et 1 phosphatidylethanolamine) et 13 métabolites polaires pouvant prédire le statut des patients à ~ 96% fiabilité. Combinés en un score composite par l'algorithme PLS, ces variables permettaient de discerner à 97% les formes modérées et à 92% les formes sévères de la COVID-19 (aire sous courbe ROC 0.97, $P < 0.002$), et de reconnaitre les témoins non covid avec 95% de fiabilité.

Conclusion
Cette étude est la première du genre examinant le métabolome/lipidome plaquettaire de patients COVID-19. Un biomarqueur composite comportant des lipides et des métabolites polaires permet de pronostiquer l'évolution des patients hospitalisés vers un stade modéré ou sévère. Il serait pertinent de consolider ce biomarqueur sur une cohorte de validation plus large.

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Poster 2 - P2

La métabolomique à 20000 lieues sous les mers: exploration du métabolome de plongeurs séjournant plusieurs jours consécutifs dans un habitat sous-marin

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Introduction. The CAPSULE is a prototype of underwater habitat, designed to accommodate teams of divers for several days for missions of ocean floor scientific observation. We used a metabolomics approach to evaluate the biological adaptation to this underwater environment.
Methods. We studied over 5 days the urine metabolome of 9 divers scattered in 3 capsules immerged at 20 m and breathing an atmosphere of Heliox (helium containing oxygen). Urine samples were collected at day-1, day1, day3 and day5 and analyzed by LC-MS/MS. The annotated metabolites were assembled into biological functions and scored using a PLS multiblock procedure.
Results. 1061 metabolites were annotated and distributed into 79 biological functions. Overall, 34 functions were sensitive to the underwater living conditions. We determined key functions having the greatest impact on the metabolic systems as determined in a partial correlation network analysis using the topological betweenness centrality coefficient. 24 key regulations out of the previous 34 were thus highlighted, corresponding to cell defense system, metabolic control, cardiovascular regulation, microbiota metabolism, detoxication system, primary metabolism, nervous system functioning and xenobiotics. A shift of these metabolic regulations occurred on day1, which progressively swung back until the exit day, when the metabolome almost catched back the initial status.
Conclusion. Around 1/3 of the metabolic regulations were involved in the adaptation to underwater habitat. Regulations occurred sharply and rapidly, but then returned progressively to the open-air conditions, highlighting adaptation to the underwater capsule environment. Metabolomics seems suitable to track the biological changes induced by such unusual extreme surroundings.

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Poster 3 - P3

Isotope-assisted metabolomics for the quantification of plasma metabolites: a preliminary inter-laboratory trial

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Human large-scale metabolomics analysis is facing important challenges to increase its robustness, reproducibility and interoperability. In this context, stable-isotope labeled compounds are increasingly used for identification and quantification of their unlabeled homologs. In this study, two laboratories were involved in an inter-laboratory absolute quantification trial of amino and organic acids in the reference human plasma sample (NIST-SRM-1950) using 24 commercially-available stable-isotope (13C/15N/D) labeled standards, among the 416 identified level-1 metabolites. A pool of the labeled compounds was prepared in concentrations similar to what is expected in human plasma (0.12-67.25mg/L). The labeled pool, the NIST-SRM-1950 and the spiked NIST-SRM-1950 were analyzed by LC-HRMS using HILIC and C18 chromatography along with QToF instruments in positive and negative modes.

Regardless of chromatography or detection mode, above 19 endogenous metabolites and their labeled homologs were detected without any effects on retention times of spiking and matrix. MS-peak areas of endogenous metabolites in spiked NIST-SRM-1950 were not impacted by the isotope addition. Compared to the analysis in buffer, the intensity of the labeled compounds was impacted (attenuation> 1 log for half of them) by the dilution in plasma. Calculated absolute concentration of endogenous metabolites were in accordance (±30%) with expected values for 35-58% of metabolites identified with C18 and 18-25% with HILIC. Results obtained for C18 in positive mode were consistent between the two laboratories. HILIC results complemented those from C18 by allowing quantification of metabolites not detected by C18.

This preliminary trial demonstrated that isotope-assisted metabolomics could provide comparable and reliable results across laboratories.

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Poster 4 - P4

Développement d'une méthode quantitative ciblée en LC-MS/MS des métabolites de la voie des kynurénines chez des patients transplantés rénaux

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Récemment, une étude métabolomique non supervisée sur des urines humaines a démontré un enrichissement en métabolites de la voie des kynurénines (acide kynurénique notamment) chez des patients transplantés rénaux qui présentent une tolérance opérationnelle spontanée à leur greffon après une transplantation rénale. Cela laisse suggérer l'activation de la voie IDO1 particulièrement associée à la tolérance immunitaire. A l'heure actuelle, peu de données sont disponibles concernant le lien entre la tolérance allo-immune et les changements métaboliques de la voie des kynurénines suite à l'activation de la voie IDO1.

Afin de mieux investiguer ces changements métaboliques, une méthode quantitative ciblée en LC-MS/MS est développée dans le but de quantifier 17 métabolites de la voie des kynurénines sur des matrices humaines d'intérêt (sérum, plasma, urine). En amont, la préparation d'échantillons a été optimisée afin d'obtenir des étapes simples, rapides et efficaces pour extraire les métabolites d'intérêt des matrices étudiées.

Après validation de cette m´méthode, celle-ci permettra un suivi longitudinal de la voie des kynurénines au cours de la transplantation en parallèle d'un calcul du score clinique de risque de perte du greffon développé au sein du CR2TI. *In fine*, cela permettra de discriminer ou non les patients avec un très faible risque de perte du greffon afin d'adapter leur traitement au cours de leur suivi après la transplantation rénale.

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Poster 5 - P5

Development of a miniaturised workflow for the extraction and the analysis of enzymatic oxylipins

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Oxylipins are important lipid mediators from the metabolism of polyunsaturated fatty acids such as archidonic acid, linoleic acid, alpha-linoleic acid, eicosapentanoic acid and docosahexanoic acid through enzymatic reactions. In mammals, oxylipins have an essential role in many physiological processes such as the regulation of the cardiac, renal or vascular system. These compounds are also involved in the development of chronic diseases when their biosynthetic pathways are disrupted.

Due to their very low concentration, a specific sample preparation and a highly sensitive analytical method are required to quantify them in biological samples. They are therefore analysed with a new highly sensitive analytical method on a micro-liquid chromatography coupled to tandem mass spectrometry with a micro-electrospray ionisation source (NexeraX2- LCMS8060, Shimadzu). Moreover, research is progressing towards smaller quantities of matrices, for example a decreasing quantity by 10 for tissues or biological fluids.

In this context, we have developed a miniaturized solid phase extraction (SPE) protocol using 2 mg SPE plate (Oasis HLB, Waters). This method allows to reduce by 10 solvent volumes of the extraction and to eliminate the drying and recovery steps compared to the 30 mg SPE plate (Oasis HLB, Waters). Therefore, this miniaturization allows to gain in sensitivity by reducing the loss of analytes. Finally, we will present the results obtained on standard solutions and on plasma samples and will compare the recoveries with results obtained from 30 mg SPE plate.

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Poster 6 - P6

Predictive metabolomics presumes the mycorrhiza and plants traits from soil metabolome

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Mycorrhizal associations vary with different functional types of plants, thus it is assumed that the soil chemical composition varies accordingly. Since soil plays a major role in plantsoil interactions, it is important to profile soil chemistry in relation to the different plant traits, including mycorrhizae. Here, we aimed explicitly at associating soil composition and diversity to mycorrhizal interaction such as arbuscular and ectomycorrhizal associations, with a range of tree species and other traits (family, deciduousness, type and planting site). Untargeted metabolomics based on LC-MS/MS was conducted on 51 soils samples from 8 tree families, planted on 3 different arboreta. LC-MS signals was processed using MS-DIAL resulting in 9729 features and annotation was performed via an internal plant database. After curation, 2144 variables were used for descriptive and predictive analyses. PCA showed that the planting site was the most important trait to distinguish the soil metabolomic profiles, explaining 68.4% of the total variance. Predictive metabolomics based on LASSO, Ridge, and Elastic-Net models showed excellent results with prediction accuracy above 70% for all tested phenotypes. A perfect accuracy (100%) was obtained with planting sites and revealed 535 variables markers important for the prediction. Deciduousness was predicted at 82% accuracy, family at 77%, type (angiosperms/gymnosperms) at 80%, and mycorrhizae at 86%. Metabolic markers are being annotated for future biological interpretation. Overall, we demonstrate that soil metabolome is a good predictor of plant functional types, including mycorrhizal partnerships. These results can help elucidate associations between metabolic profiles and future applications using different plant-soil combinations.

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Poster 7 - P7

Biomphalaria glabrata, l'escargot cosmopolite, livre ses secrets de résistance/sensibilité grâce à une approche métabolomique : découvrez les biomarqueurs de cet organisme qui en sait plus que les autres pour parer le parasite Schistosoma mansoni

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La schistosomiase est une maladie parasitaire affectant plus de 200 millions de personnes dans le monde. La forme intestinale de cette maladie est causée par le parasite *Schistosoma mansoni* dont l'hôte intermédiaire est un mollusque d'eau douce du genre *Biomphalaria.* La résistance du mollusque vis-à-vis de son parasite est un trait complexe et multigénique qui reste encore à caractériser. Les recherches sur la résistance de *B. glabrata* et sur les mécanismes de l'infection par *S. mansoni* sont donc d'une grande importance pour la compréhension et la lutte contre la schistosomiase.

A partir de données LC-HRMS obtenues de plasma de *Biomphalaria glabrata*, une première étude métabolomique est menée afin d'identifier des biomarqueurs de la Résistance/Sensibilité sur deux lignées de mollusques sélectionnées sur la capacité à éliminer le parasite. En complément, une seconde étude métabolomique a été réalisée sur des souches de mollusques d'origine géographique différentes et présentant un gradient de sensibilité à l'infestation par le parasite. Pour chacune de ces deux études, des réseaux de similarités spectrales sont réalisés pour faciliter l'exploration du métabolome et l'annotation des biomarqueurs. Les logiciels R, Sirius, MzMine et Cytoscape ainsi que les plateformes GNPS et W4M ont été utilisés pour traiter les résultats. Enfin, les 2 matrices obtenues sont concaténées afin de déterminer des indices de corrélations entre les populations Résistantes/Sensibles et leur origine géographique.

Ce poster présente les résultats permettant de mettre en avant les biomarqueurs de Résistance/Sensibilité de *B. glabrata* face à *S. mansoni* et de prédire sa pré-disponibilité potentielle à être parasité.

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Poster 8 - P8

Characterization and monitoring, by GC-MS and molecular networking, of the essential oil Cananga odorata biomarkers towards the revendication of a specific terroir in Mayotte (CosMahora-Innov)

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Canaga odorata is cultivated to extract the essential oil of ylang-ylang. The three main producers of this essential oil are the Union of the Comoros, Madagascar and Mayotte (PAFR, 1998). The main constituents of ylang-ylang essential oil are organised in 5 differents classes of chemical constituents (AFNOR). However, only this three parameters are used to differentiate them: density, rotatory power and refractive index.
CosMahora-Innov project aims to create cosmetic products with fragrances derived from ylang-ylang. One of the claims is to demonstrate the presence of a terroir effect of the Mayotte essential oil. For this, 42 ylang-ylang essential oils were analysed by GC-MS. A first batch (containing 18 samples) with the essential oils produced in 2020 was carried out. A matrix and molecular networks were generated with GNPS and MzMine. Statistical analyses were performed with R. Biomarkers specific to Mayotte essential oil were identified and characterised with the NIST list and Kovats index. A second batch (containing 24 samples), with essential oils produced in 2022, and analyses were carried out in order to monitor the Mayotte biomarkers. This allowed to confirm the presence of a significant differences in the Mayotte essential oil. The main results show an authenticity of Mayotte essential oil (contrary to what is expressed in the AFNOR standard) and a potential Mahoran terroir effect. Finally, other studies are underway to demonstrate genetic differences between Mayotte and Comoros.
This communication presents the results highlighting the biomarkers tracking and the potential terroir effect of Mayotte essential oil.

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Poster 9 - P9

Environmental DNA and metabolomics reveal the influence of the invasive plant Miconia calvescens on the soil diversity in the island of Moorea (French Polynesia)

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Miconia calvescens est une espèce végétale originaire d'Amérique du Sud, qui est considérée comme l'une des plantes invasives les plus importantes en Polynésie française, en particulier à Tahiti. Elle a été introduite pour la première fois dans les années 1930 et s'est rapidement propagée, principalement en raison de son taux de croissance élevé, de sa capacité à s'adapter à une variété de conditions environnementales et de son manque de prédateurs naturels. La plante peut atteindre une hauteur de plus de 10 mètres et former des sousbois denses qui empêchent la croissance des plantes indigènes. Les autorités de Polynésie française mènent aujourd'hui des efforts pour contrôler et éradiquer cette espèce envahissante. C'est dans ce cadre que le projet RHIZINV a permis de mener une première étude expérimentale sur la biodiversité rhizosphérique de la plante ainsi que sur la recherche de métabolites biomarqueurs de cette espèce. Dans un premier temps, l'étude métabolomique a permis de mettre en avant certains biomarqueurs spécifiques à *M. calvescens*. En parallèle, l'étude metabarcoding de la rhizosphère a permis de mettre en lumière des taxons spécifiques de *M. calvescens.* A partir de l'ensemble des résultats générés, l'analyse multi-Omics a mis en exergue la présence de corrélation significative entre certains taxons appartenant aux Cryptomycota et à des terpénoïdes dont un méroterpénoide, métabolites favorables à la communication plantes-microorganismes au sein de la rhizosphère.
Ce poster présentera les résultats de ces travaux publiés récemment dans le journal Microorganisms.

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Poster 10 - P10

Study of gut-brain interactions in Parkinson's disease modeled in Drosophila : metabolomic analysis by NMR

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The gut-brain axis is a major bidirectional communication system now recognized as being involved in the initiation and propagation of neuronal pathologies, including Parkinson's disease. Our NMR metabolomic study aims to provide a better understanding of brain-gut interactions in Parkinson's disease.

The fruit fly *Drosophila melanogaster* is a model of choice for the study of human disorders. This organism possesses homolog genes for about 70% of known human genes causing diseases. It is relatively cost and time effective, and the manipulation of gene expression in the fly is easily achieved.

Here we studied Parkinson's disease models developed in *Drosophila*, by the *in vivo* expression of a mutant pathogenic form of human *α*-synuclein (*α*-synA30P). In order to uncover reciprocal influences of these two organs during the development of the pathology, we expressed *α*-synA30P either in neurons and/or the gut in *Drosophila*.

We analyzed by NMR metabolomic modifications in the brain and gut, at early (10 day-old) and late (30 day-old) stages of the disease. Metabolites were extracted from the head and body of *Drosophila*. 1D 1H-NMR spectra were acquired on a 700 MHz spectrometer equipped with a cryoprobe. Statistical analyzes were performed to differentiate diseased *Drosophila* from their controls and detect reliable biomarkers.

Our preliminary results demonstrate interestingly that the two organs interact and influence each other at both stages of the disease. *α*-synA30P expression in the brain leads to rapid metabolic defects, visible in both organs, while the effects of its expression in the gut appear to be slower.

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Poster 11- P11

Annotation de métabolites bioactifs provenant d'extraits de plantes issues de la cosmétopée traditionnelle Mahoraise par couplage LC-HRMS, méta-BIO-guidage et les réseaux de similarités spectrales

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Située dans l'archipel des Comores au sein de l'Océan Indien, l'île aux parfums se trouve entre Madagascar et la côte du Mozambique. A l'exemple des nombreux territoires avoisinants, Mayotte possède de nombreuses richesses aussi bien terrestre que marine sans compter une culture, des traditions et un savoir-faire qui se transmettent de manière trans-générationnelle. Ces dernières sont observables dans de nombreux domaines : médecine, cosmétique, etc. Malheureusement, comme dans beaucoup de régions du monde, ces savoirs sont menacés. Le projet " CoCoMay " consiste à recenser un maximum de connaissance sur la cosmétopée mahoraise et les usages traditionnels des plantes. Ces données permettront d'évaluer leurs potentiels et de les valoriser dans divers domaines.

Pour réaliser cela, un criblage basé sur la littérature et des entretiens avec des tradipraticiens a permis de sélectionner une trentaine de plantes. Différents types d'extraits ont été générés puis testés pour évaluer trois types de propriété classiquement recherché en cosmétique : l'activité anti-tyrosinase, anti-élastase et anti-collagénase. A partir des résultats obtenus, ` trois extraits avec une activité significative dans chacun des tests ont été sélectionnées. Des empreintes chimiques en LC-HRMS ont été effectués puis les extraits ont ´été soumis à du méta-BIO-guidage dans le but de déterminer le/s métabolite(s) responsable de l'activité. Enfin, ces derniers seront annotés via le logiciel SIRIUS, la base de données LOTUS ainsi qu'un outil innovant : les réseaux de similarités spectrales.

Ce poster présente les résultats obtenus au cours de ces travaux ainsi que la validation des revendications traditionnels des plantes.

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Poster 12 - P12

Mise en place d'une méthode de profilage quantitatif des carnitines par LC-HRMS

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La L-carnitine joue un rôle essentiel au niveau du métabolisme énergétique des tissus via le transport des acides gras (AG) à chaîne longue à travers la membrane interne de la mitochondrie. En effet, cette dernière est imperméable aux AG à chaîne longue (12 `a 20C) et la L-carnitine est l'unique transporteur leur permettant de pénétrer au sein de la mitochondrie. Les AG, activés en Acyl-CoA, sont convertis en acylcarnitines et traversent ainsi la membrane mitochondriale, avec l'action de trois enzymes. Une fois dans la mitochondrie, les AG, à nouveau sous forme d'Acyl-CoA, vont subir la *β* - oxydation, dont le rôle majeur est la fourniture d'ATP aux organes à forts besoins d'énergie. Cette voie métabolique est un acteur clé en métabolisme.
Ce poster présente la mise en place d'une méthode d'extraction et d'analyse quantitative spécifiques de la famille des carnitines : de la L-carnitine jusqu'à l'acylcarntine C18:1, par chromatographie liquide couplée à un spectromètre de masse de type orbitrap (Thermo Exploris 240). Des premiers tests d'extraction ont été réalisés sur tissus et plasma ainsi qu'une s´séparation sur colonne Waters Acquity HSS T3. Différents étalons internes deutérés (de longueurs de chaines variés) ont été utilisés pour faire les quantifications absolues. Une validation de la m´méthode sera effectuée.
Ce nouveau profilage sera proposé en prestations sur MetaToul mais il sera surtout décliné pour suivre ces composés partiellement marqués au 13C dans le cadre d'expérimentation de marquage métabolique. Il sera un complément important au profil d'AG marqués déjà proposé.

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Poster 13 - P13

New insights from the application of metabolomics to vitis vinifera cell cultures studies

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Vitis vinifera cell cultures are well recognised for their ability to produce a wide range of secondary metabolites such as stilbenes, flavanols and, in the case of *Gamay Teinturier* cultivar, anthocyanins. These metabolites are well appreciated in nutraceuticals and cosmetic industries because of their antioxidant properties. Recent developments in MS-metabolomics can offer a wider description of the secondary metabolites produced in these plant cell cultures and a better understanding of their metabolic pathways regulation.

The main highlights of our current research include: 1) the detailed quantification thanks to the sensitivity of UHPLC-MS/MS approaches of 13 anthocyanins and 35 other polyphenols in the *Gamay Teinturier* cells, 2) the application of HRMS/MS for the detection and annotation of additional minor compounds of interest such as stilbene oligomer glucosides and aroma precursors, 3) the integration of targeted and untargeted HRMS-metabolomics to compare the potentiality of different plant cell materials from *Vitis* and non-*Vitis* cultivar callus to produce metabolites of interest; 4) the use of these approaches for studying the effects of different elicitors (phytohormones, cyclodextrins, sorbitol) on the growth kinetics, sugar metabolism and polyphenols production in *Gamay Teinturier* cell suspensions.

These results are a first step to conducting additional fluxomics studies to determine the dynamics of secondary metabolite production under various stress conditions. The data obtained will facilitate more efficient productions of enriched extracts of interest.

This work was supported by the Département des Sciences de l'Environnement of the Bordeaux University (IsoDeVim projet), the Bordeaux Metabolome Facility and the MetaboHUB (ANR11-INBS-0010) project.

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Poster 14 - P14

Profils métaboliques spécifiques d'une cystite radique dans le cadre du traitement du cancer de la prostate

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Malgré l'amélioration des techniques d'irradiation, la radiothérapie pelvienne est responsable d'effets indésirables pouvant être précoces ou tardifs au niveau de la vessie et qui sont définis comme une cystite radique (CysR). Les premiers symptômes de lésions vésicales sont susceptibles de survenir pendant le traitement ou durant les 3 mois suivant la radiothérapie chez environ 50 % des patients irradiés. Ces lésions précoces peuvent conduire au développement d'une fibrose, entraînant alors une perte de la fonction vésicale et une importante dégradation de la qualité de vie des patients. La physiopathologie de la CysR reste méconnue, en partie à cause des risques de complications causées par l'accès au tissu vésical après irradiation. D'où l'objectif de ce travail consistant à étudier le profil métabolique de patients irradiés suite à un cancer localisé de la prostate et ayant développés ou non une CysR précoce.
Pour cette étude, 10 patients ayant un cancer de la prostate ont été suivis pendant 3 mois. Des prélèvements de sérum et d'urine ont été réalisés à W0 (avant irradiation), W4 (après 4 semaines de radiothérapie) et W12 (3 mois après le début de la radiothérapie). Des questionnaires sur la qualité de vie ont également été collectés. Tous les échantillons ont été analysés sur un appareil UPLC-QToF en phase inverse et phase normale ainsi qu'en ionisation positive et négative. Les données ont été extraites sur Galaxy-Workflow4Metabolomics. Des variables d'intérêt dont le taux diffère au cours du temps en fonction du développement de CysR ont été mises en évidence.

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Poster 15 - P15

ChromAnnot -a webserver for deep LC-HRMS/MS chromatogram annotation

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One main challenge in Metabolomics remains the annotation of features extracted from LC-HRMS/MS data, to be able to interpret observed metabolic alteration. In real such annotation is generally performed only on features of interest. Still having information on all detected features from a LC-HRMS/MS chromatogram remains challenging. At ISOMer laboratory, we developed an entire workflow taking into consideration each step of the general annotation strategy that was particularly adapted for dereplication of specialized metabolites in LC-MS profiles. This automated approach based on R (Cran), XCMS (3), CAMERA (4), SIRIUS (5), database search, Taxize (6) and CFMID (7). Biological origin and MS2*in silico* fragmentation comparison were added into the workflow to improve compound discrimination.
The workflow, which analyses directly raw LC-HRMS/MS data (in open format), was integrated into a web-based interface to ease exploring the annotation result. The ChromAnnot webserver is accessible at https://chromannot.univ-nantes.fr/.
This deep annotation of LC-MS data strategy is currently applied to characterize and highlight known compounds produced various *Penicillium* strains.

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Poster 16 - P16

Étude métabolomique par RMN de l'exposition chronique à faible dose de composés génotoxiques

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Parmi les toxiques, les agents génotoxiques doivent être identifiés car ils présentent un risque pour la santé humaine/animale et les biosystèmes. Le test du micronoyau est reconnu comme l'un des tests génotoxiques le plus abouti et peut être effectué *in vitro*, mais ne permet pas de différencier les mécanismes d'action. Les techniques omiques pourraient apporter des informations complémentaires afin de mieux caractériser les mécanismes de toxicité de ces substances. Dans le cadre du projet ANR Genoshift (ANR-20-CE34-0016-01) qui étudie les effets génotoxiques après exposition à des substances toxiques, une approche de modélisation des effets et classification des génotoxiques à partir de données multi critères a été mise en place.

Des cellules hépatiques humaines (HepaRP) ont été exposées à de faibles doses de composés génotoxiques (12 composés) quotidiennement pendant cinq jours. Après extraction des culots cellulaires, les extraits ont été analysés par RMN du proton à l'aide d'un spectromètre RMN 600 MHz.

Les analyses statistiques multivariées réalisées sur les données RMN à l'aide de l'interface web Workflowformetabolomics (W4M) ont pu mettre en évidence des signatures métaboliques différentes entre les cellules témoins et les cellules exposées pour chaque composé génotoxique. Le nombre et la nature des métabolites dont la concentration est significativement différente entre les cellules témoins et les cellules exposées dépend du composé toxique. Ces premiers résultats montrent que les voies métaboliques perturbées sont spécifiques du composé toxique, suggérant des mécanismes d'action différents. Cette méthode pourrait donc permettre de classer ces composés toxiques en fonction de leurs effets métaboliques.

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Poster 17-P17

Projet ID-SharK

Identification of biomarkers of sharka disease in peach for the development of an early detection tool based on roots

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La Sharka est une maladie virale touchant les arbres du genre Prunus. Elle est causée par le Plum Pox Virus (PPV). Cette maladie est responsable d'énormes pertes économiques pour le secteur de la production de fruits, le moyen de lutte utilisé en France est l'arrachage systématique des plants contaminés. L'identification des arbres malades est réalisée par une inspection visuelle afin d'observer les symptômes de la maladie, notamment sur les feuilles et les fruits. Des analyses en laboratoires sont aussi possibles, notamment par analyse moléculaires (RT PCR) et par analyse sérologique (ELISA). Des études précédentes en métabolomique ont démontré qu'il était aussi possible de discriminer les feuilles des arbres sains des feuilles symptomatiques et asymptomatiques d'arbres contaminés par analyse de leur métabolome. Ces différences tendent également à démontrer qu'il est possible d'identifier des arbres contaminés `a un stade précoce de l'infection, avant même l'apparition des symptômes. Ainsi, nous proposons la même approche sur les racines de jeunes plants afin de déterminer si le métabolome des racines était également impactés par le virus. Ainsi, en conditions contrôlés sous serres les racines d'arbres sains (n=7) et contaminés (n=5) ont été prélevés. Des analyses par approche métabolomique non-ciblée (UHPLC-HRMS) et des analyses moléculaires, par RT-PCR (Olmos et al. en 2005), ont été réalisées afin de déterminer si le virus à la capacité de migrer dans l'appareil racinaire des plantes. Les premiers résultats en ACP montrent une séparation des groupes basée sur l'analyses de l'occurrence de certains métabolites en cours d'annotation.

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Poster 18-P18

Towards a diagnosis of non-celiac gluten sensitivity: the contribution of metabolomics for monitoring metabolites produced by in vitro digestates of bread

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Body fluid metabolomics is a large-scale approach allowing exploring the mechanisms that might underlie specific diseases or sensitivity to processed foods, and identifying associated biomarkers for diagnostics or stratification. Over the past decade, the non-celiac gluten sensitivity (NCGS) is more and more self-diagnosed, which makes the gluten-free diet more frequent, without objective clinical criteria. In fact, because of a lack of clinical indicators, NCGS is poorly understood and challenging to diagnose in contrast to celiac disease. Therefore, finding biomarkers associated with this phenotype is critical for an accurate diagnosis and innovative patient management.

To understand the relationship between bread digestion mechanisms and the occurrence of NCGS, a recent approach with *in vitro* investigation was applied to study the overall digestive process of different breads, combining tools from the oral step thanks to the AM2 masticator apparatus, until the end of digestion thanks to a dynamic digester (DIDGI©) mimicking the physiology of the adult gastrointestinal tract "GIT". One objective in this study was to monitor metabolites produced by *in vitro* digestates using an untargeted metabolomics approach.

In this study, we will outline the methodological strategy taken from preparation of the stomach and intestinal digestates, to acquisition, processing, and annotation of the LCHRMS data.

Interestingly, the first results show fluctuations in certain metabolites identified according to the type of bread digested. This reveals the impact of type of bread on the digestibility and allowed us to emphasize the contribution of metabolomic approach for monitoring the metabolites produced by *in vitro* digestates.

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Poster 19-P19

Cinétique temporelle de la réponse métabolomique d'une microalgue verte (Scenedesmus costatus) à deux facteurs environnementaux combinés : lumière et température.

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Face aux changements globaux, les milieux aquatiques sont soumis à une augmentation de la température et de l'intensité lumineuse pouvant nuire au fonctionnement de ces écosystèmes. En particulier, des études récentes ont mis en évidence l'impact de ces facteurs sur la physiologie des micro-algues vertes qui jouent un rôle clef dans le réseau trophique des écosystèmes aquatiques. Toutefois, il subsiste un manque de connaissance sur les mécanismes moléculaires/biochimiques adaptatifs en jeux et sur l'effet de ces facteurs en combinaison sur le métabolisme de ces organismes. Ainsi, cette étude a visé à caractériser dans le temps l'effet combiné de la lumière et de la température sur le métabolome de la microalgue verte *Scenedesmus costatus*. Pour ce faire, une culture de *S. costatus* a été exposée en condition contrôlée durant 7 jours à une combinaison de deux températures (18, 23 °C) et intensités lumineuses (72, 189 μmol.m^{-2}.s^{-1}). Un échantillonnage a été réalisé à J0, J4 et J7 et le métabolome a été caractérisé par une approche en métabolomique non-ciblée basée sur la spectrométrie de masse haute résolution en couplage avec la chromatographie liquide (UPLC-TOF). Les données ainsi acquises ont été explorées avec différents outils chimiométriques (PCA, HCA, PLS-DA, ASCA). En particulier, l'ASCA a permis de mettre en évidence un effet très marqué du temps (39% de l'effet total) en comparaison de la température (4%) et de la lumière (13%) sur le métabolome mais également l'effet de leurs interactions. L'annotation des signaux en lien avec chacun des facteurs et leurs interactions sera prochainement entreprise.

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Poster 20-P20

Diversity and environmental plasticity of the seed specialized metabolome in camelina species

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Specialized metabolites (SMs) contribute to seed interactions with its environments and impact their agronomic quality. Thanks to their antioxidant and antipathogenic properties, accumulation of many phenylpropanoids SMs have been associated with stress resistance in seeds. SMs are widely accumulated in seeds of Brassicaceae such as camelina (*Camelina sativa* L.). This crop has gained a lot of interest in the past few years as a rustic oilseed crop.

In a first work, we analysed SMs profiles of six camelina genotypes grown in the field and harvested over five consecutive years (Boutet, Barreda et al., 2022, doi:10.1111/tpj.15662). Untargeted metabolomic analyses highlighted that many phenylpropanoids, including cinnamic acids and flavonols, showed a strong environmentally-induced plasticity, which was higher with respect to most of the primary metabolites, including fatty acids, proteins and lipids. We highlighted major effects of the environment on the stimulation of the seed specialized metabolome suggesting that seeds show a very dynamic and inducible metabolism.

In a second work, we aim at gaining a deeper knowledge on the impact of biotic stresses on seed SM accumulation in camelina. An in-depth investigation of available chemical and transcriptional diversity in domesticated and wild camelina species was performed. The analyses allowed the identification of a large number of decorated cinnamic acids that might play a role in seed-pathogen interaction and that are being validated.

Characterizing seed SM pathways regulated by stresses will help the development of crops better adapted to the environment and the identification of new agro-ecological solutions with potential biocontrol applications.

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Poster 21-P21

Nitrogen-mediated metabolic patterns of susceptibility to Botrytis cinerea infection in tomato (Solanum lycopersicum) stems

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Botrytis cinerea, a necrotrophic fungus, forms potentially lethal lesions on the stems of infected plants. Contrasted levels of *B. cinerea* susceptibility were obtained in tomato grown on a range of nitrate concentration. Metabolic deviations and physiological traits resulting from both infection and nitrogen limitation were investigated in symptomless stem surrounding the necrotic lesion. Prior to infection, nitrogen-deficient plants showed reduced levels of nitrogen-based compounds such as amino acids, proteins, and glutathione and elevated levels of carbon-based and defence compounds such as α-tomatine and chlorogenic acid. After *B. cinerea* inoculation, all plants displayed a few common responses, mainly alanine accumulation and galactinol depletion. The metabolome of resistant plants grown under high N supply showed no significant change after inoculation. On the contrary, the metabolome of susceptible plants grown under low N supply showed massive metabolic changes in central metabolism around glutamate and respiratory pathways, suggesting active resource mobilization and production of energy and reducing power. Redox and defence metabolisms were also stimulated by the infection in susceptible plants; glutathione and chlorogenic acid accumulated, as well as metabolites with more controversial defensive roles, such as polyamines, GABA, branched-chain amino acids and phytosterols. Taken together, the results showed that nitrogen deficiency, although leading to an increase in secondary metabolites even before the pathogen attack, must have compromised the constitutive levels of defence proteins and delayed or attenuated the induced responses. The involvement of galactinol, alanine, cycloartenol and citramalate in the tomato stem response to *B. cinerea* is reported here for the first time.

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Poster 22-P22

PhysioFit v3

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Quantification of microbial growth rates and extracellular uptake and production fluxes is an essential task to address fundamental and applied questions in the fields of systems and synthetic biology, biotechnology, and health. Fluxes can be estimated using various mathematical models by fitting time-course measurements of the concentration of cells and extracellular substrates and products. A few tools are available to calculate extracellular fluxes, but they are hardly interoperable and include specific, hard-coded growth models. We present an updated version of our open-source flux calculation software, PhysioFit, which can be used with any growth model and is interoperable by design. PhysioFit v3 includes by default the most common growth models, and additional models can be implemented by users to calculate fluxes and other growth parameters for metabolic systems or experimental setups that follow alternative kinetics. PhysioFit can be used via a Graphical User Interface, a Command-Line Interface or directly as a Python module, thereby maximizing its interoperability with other computational tools. For demonstration purpose, we have implemented PhysioFit on Workflow4Metabolomics (Giacomoni *et al.*, 2015), a collaborative portal for the metabolomics and fluxomics community, where it can be used as a standalone tool and be easily integrated into user-made workflows.

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Poster 23-P23

Curation of drug signals in metabolomic datasets to unveil the metabolic response induced by treatment in cellular models.

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Background

The influence of exogenous compounds in experimental models is not taken into account during metabolomics analysis. Therefore, untargeted metabolomics profiling of cellular models treated (or not) with pharmacological agents leads to a statistical separation driven by the presence of drug signals, thereby concealing the drug-induced metabolic response. We aimed at developing a tool to remove exogenous signals in such experiments.

Methods

We used a metabolomics dataset acquired on human lung epithelial cells treated or not with the CFTR modulator triple combination elexacaftor/ivacaftor/tezacaftor (cystic fibrosis treatment). We designed a specific function to eliminate the drugs' signals: (1) the drug compounds are detected based on their specific m/z and retention time (Rt); (2) features highly correlated to them are identified; (3) amongst these, features with a 10-fold intensity in the treated samples compared to the controls are deleted, as well as features with a Rt similar to drugs. The function was appended to our LC-MS data processing workflow.

Results

From an initial dataset of ~5000 spectral features, the workflow eliminated 30-40 drugrelated features. Using this function, data acquired from cell-free culture media spiked with drugs or with vehicle could not be discriminated, suggesting that drug signals have been exhaustively eliminated. Nevertheless, there was a discrimination between treated and control cellular samples, indicating an underlying metabolic impact of the treatment.

Conclusions

This function allows to eliminate drug-related features in experimental models. The use of isotopically labelled drugs may help to identify eliminated features and further validate this pre-processing function in future experiments.

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Poster 24-P24

Iterative exclusion strategy for improved lipidome coverage of human plasma using SFC-HRMS/MS

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Lipidomics has seen significant advancements thanks to the development of separation techniques, especially reverse phase liquid chromatography (RPLC) coupled to HRMS/MS which provides intraclass separation based on the acyl chain length and number of double bonds (1). One of the major drawbacks of RPLC concerns the co-elution of different lipids belonging to different classes, causing signal suppression.

To overcome this issue, Supercritical Fluid Chromatography (SFC) enables interclass separation (2). While the class separation facilitates the overview of lipid species diversity within a time retention interval, the lipidome characterization is usually achieved by DDA MS/MS approach, limiting the fragmentation to the most intense signals (3).

To improve the human plasma lipidome coverage and structural characterization, an iterative exclusion strategy was employed to obtain more meaningful MS/MS data. Experiments were conducted on a SFC-Q-TOF (Agilent Technologies), while data annotation was achieved using the MS-DIAL software and *in-silico* databases (4).

Enrichment of the exclusion list during iterative injections in positive/negative ionization modes considerably improved lipid annotation compared to the traditional DDA approach. Although structural elucidation remains challenging due to the differences in lipid concentrations and ionization efficiencies, this study provides annotation for over 400 lipid species and gives access to low-abundance potential biomarkers in complex matrices thanks to SFCHRMS/MS.

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Poster 25-P25

Reaching metabolomic niches through miniaturization of 1H-NMR experiments

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NMR spectroscopy is used in metabolomics notably for the wealth of information it provides, its robustness and its quantitative nature. On the other hand, its lack of sensitivity is a hindrance to its use for samples limited in quantity. This challenges its use for e.g. kinetic studies on mouse blood, extractions of small organisms, and especially if multi-omics analysis is expected. Very often these samples must be diluted and the experiments must be lengthened to accumulate a sufficient amount of signal, which is not desirable for high throughput analysis. The use of microprobes with 1.7 mm NMR tubes could allow the high throughput analysis of small quantities of samples with limited dilution and also to perform multi-omics studies.
NMR experiments lasting less than 10 minutes on different biological matrices have shown an increase in sensitivity for limited amounts of plasma (15 μL) and freeze-dried liver extract (1 to 5 mg) with 1.7 mm tubes used in microprobes compared to conventional 3 mm tubes used in 5 mm probes.
The results of these experiments confirm that when sample quantities are limited, miniaturization offers real advantages which furthermore open the possibility to analyze small organisms and to perform spatial analysis of organs with NMR spectroscopy.

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Poster 26-P26

Influence of FUT2/FUT3 polymorphism on human breastmilk oligosaccharide composition in the EDEN mother-child cohort

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Human Milk Oligosaccharides (HMO) are highly diverse and abundant components of human breastmilk, known to play a key role in the development of the neonate microbiota and immunity. HMO composition depends mainly on genetic polymorphisms of the mother, notably on genes encoding *FUT2* and *FUT3* fucosyltransferase: as reported in the litterature, FUT2 (secretor status, Se) induces synthesis of HMOs such as 2-fucosyllactose (2'FL) and lacto-N-fucopentaose I (LNFPI), whereas FUT3 (Lewis status, Le) is associated with presence of lacto-N-fucopentaose II (LNFPII) and lacto-N-difucohexaose I (LNDFHI). In the present work, we aim to provide a comprehensive characterization of the global HMO composition depending on Se/Le status.

Early BM samples collected within the EDEN mother-child cohort (n=317) were analysed using liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS, QTOF instrument, Bruker) following a previously optimized protocol. A dedicated R annotation workflow was developed, and led to the detection of 130 potential HMOs (among them, 2'FL, LNFPI, LNFPII and LNDFHI). Single Nucleotide Polymorphisms (SNP) in the *FUT2* and *FUT3* genes were available (n=62) for 220 out the 317 mothers.

Comparison of HMO composition and *FUT2/FUT3* genotypes showed that, except for one mother, 2'FL and LNFPI contents were associated with the *FUT2* rs516246 SNP. In contrast, *FUT3* SNPs were not associated with specific HMO composition. Interestingly, the Se status was also associated with a particular HMO composition, far beyond the presence/absence of 2'FL and LNFPI. Corresponding signature will then be related to EDEN children health outcome available up to their 5th birthday (ex: food allergy).

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Poster 27-P27

Development of a root exudate collection protocol to monitor changes in exudation in response to stress by NMR metabolomics analysis

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Root exudates are composed of a wide range of compounds including primary and secondary metabolites. They are involved in plant growth and responses to environmental stresses. Although root exudates are important in root-microorganisms interactions, few data exist on the metabolite's composition of this highly sophisticated molecular mixture and most of them are still unknown. In addition, the impact of biotic or abiotic stresses on their precise composition remains to be clarified.

Here, using pea *(Pisum sativum)* as a plant model, we have developed an easy and reproducible protocol to follow modifications of root exudation in response to both biotic (elicitors, pathogens) and abiotic stresses (drought, salinity, extreme temperatures...) using an NMR untargeted approach. Pea seedlings were hydroponically grown, and root exudates were collected in distilled water or calcium chloride solution for 15 or 120 minutes. NMR analysis showed that root immersion during 15 minutes in calcium chloride limits seedling stress without modifying exudate diversity. Using these "basal" collection conditions, the protocol was applied to faba bean *(Vicia faba)*, a closed related member of the Fabaceae family. NMR metabolic profiles revealed that the two species could be discriminated according to their exudate composition (Fortier et al., 2023).

Using this protocol, we are currently investigating the impact of elicitors on pea root exudate composition.

Fortier et al. (2023) Plant Science. 331 : 111694.

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Poster 28-P28

Lactate Metabolism and Plasticity of Adipose Tissues

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For many years, lactate has been considered as a metabolic waste product but is now viewed as a critical regulator of metabolism by acting as both a carbon and electron carrier and a signalling molecule between cells and tissues. It has been shown to activate energy-dissipating brown / beige adipocytes, which represents an attractive therapeutic strategy against metabolic disorders. The role of lactate transport and metabolism in beige adipocytes metabolic activity remains poorly understood. In this work, we have combined molecular studies, metabolomics, and 13C-fluxomics to show that the lactate transporter MCT1 is specifically expressed in inducible beige adipocytes and mediates bidirectional and simultaneous inward and outward lactate fluxes in these cells. While lactate release favours glycolysis, the imported lactate feeds the oxidative metabolism of beige adipocytes. Lactate fluxes play a key role in finely tuning beige adipocytes metabolic activity according to extracellular metabolic conditions, hence reinforcing the emerging role of lactate metabolism in the control of energy homeostasis.

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Poster 29-P29

Metabolic profile of patients at ultra high risk of psychosis

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Schizophrenia is a progressive psychiatric illness with several described clinical stages. There would be metabolic abnormalities in patients at ultra high risk of psychosis (UHR) and during the first episode of psychosis (FEP). The measurements currently available make it possible to study a limited number of biological parameters or prove to be very costly and with limited replicability. Nuclear magnetic resonance (NMR) implemented within the framework of IVDr (In Vitro Diagnostic for research) methods applies a standardization of data adopted at the international level for studies of human biological fluids. It allows precise, fast and replicable measurement of more than 150 variables in blood, including accurate lipoprotein parameters.
Our objective is to evaluate the interest of an NMR approach for the metabolic profiling of UHR patients.
Despite a small number of subjects, we were able to highlight an evolution of the metabolic profile of the patients over time. These preliminary results show that antipsychotic treatments largely modify lipid metabolism and could be used to identify early biomarkers capable of predicting the side effects of these treatments in young patients with early psychosis.

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Poster 30-P30

High-throughput separation of chiral molecules using non-covalent complexes and SIM2 analysis on a TIMS-ToF

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Chiral molecules are commonly found throughout the living world and frequently encountered in metabolomics. Enantiomers are particularly challenging in analytical and life sciences, since they may possess various biological activity and/or toxicity while having similar physical and chemical properties. Chiral analysis using mass spectrometry (MS) provides sensitive and specific detection but relies mostly on the derivatization or formation of non-covalent complexes in the gas phase. Direct-Introduction-MS coupled with ion-mobility spectrometry (IM-MS) has emerged as a promising tool for the rapid analysis of isomeric compounds by being able to separate ions based on their *m/z* ratios and collision cross section (CCS) values. In this work, chiral analyses were conducted on a trapped ion mobility spectrometry time-of-flight (TIMS-ToF) instrument, using serial acquisitions by applying single ion mobility monitoring (SIM2) at high mobility resolving power and at different ion mobility ranges. Our approach successfully allowed chiral distinction of most of D- and Lamino acids (AAs) as non-covalent CuII complexes with phenylalanine (Phe) and proline (Pro) as chiral references. Other biologically relevant chiral molecules such as flavonoids and drugs were also investigated, demonstrating the potential of our SIM2 approach for rapid chiral analysis.

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Poster 31 - P31

Search for markers involved in lymph node remodeling in the pre-metastatic stage by metabolomic analysis.

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Background

Our laboratory has provided evidence for the existence of a premetastatic niche in the sentinel lymph node (LN) draining a human cervical neoplasm, which is characterized by a specific lymphangiogenic, immune and extracellular matrix profile. Metabolomics is a promising approach that provides an opportunity to link the metabolome with physiological or pathological status. The aim of this project is to identify additional markers of the arrival of metastases in the LN by applying a metabolomic approach to human and murine samples.

Methods

To identify metabolic pathways modulated in LN, we benefit from a preclinical mouse model, the "ear sponge assay", which reproduces each step of the metastatic cascade. The mice's lymph nodes are analyzed by NMR-based metabolomics and histological procedure. Furthermore, we will use a cohort of patients suffering from advanced cervical cancer and we will analyze different samples issued from the same patient.

Results

Different analyses performed on mice LNs highlight a clear discrimination between the cervical LN (draining the tumor) and the other LNs (mandibular, sub-draining LN, and axillary/inguinal, control LN) issued from mice bearing a tumor. Our data show that the tumor development impacts the environment of the draining LN, but not that of distant LNs.

Conclusion

Our results highlight metabolomics's interest in investigating LN remodeling during the metastatic process. Indeed, the tumor microenvironment impacts the cervical lymph node at a metabolite level. These results will be confirmed by additional experiments in order to perform further analysis on the discriminating metabolites.

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Poster 32 - P32

Metachromatic leukodystrophy diagnosis in Morocco: exploration of sulfatides diversity in urine samples of infantile patients using molecular networks

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Artichoke, or *Cynara cardunculus* subsp. *scolymus*, is a plant recognized for its fruit all around the world and known to contains compounds with various nutritive and therapeutic benefits. In this work, we intend to compare the metabolic profile of the leaves of different artichoke and cardoons varieties collected in France and investigate the variability of their composition. To allow a broader and easier identification of the metabolites, the analyses will be performed by combining NMR and Liquid Chromatography-Mass Spectrometry (LCMS) data. Firstly, the sample preparation had to be optimized because, for integrative methods, it is recommended to carry out the experimental analyses on a unique sample to avoid biases. The leaves were extracted in a first step with infusion, as usually performed by Evear Extraction, that allowed to obtain a cardoon or artichoke liquor containing most of the compounds known to have biological effects. Our first objective was to prepare the liquors for the two analytical techniques, NMR and LC-MS. Thus, different extraction methods were tested for several artichoke varieties and the ultrafiltration approach was retained as the more appropriate sample preparation for polar compounds in cardoon or artichoke liquors. Then, our second objective, currently undergoing, focused on the optimization of LC-MS acquisition parameters to allow the detection of a maximum of polar compounds by using a design of experiments. Once this objective achieved, it will be possible to analyse and combine NMR and LC-MS data and compare the metabolomic profiles.

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Poster 33-P33

Vers une meilleure efficacité et rapidité dans l'identification des métabolites dans le cadre d'études de métabolomique non ciblées ?

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En métabolomique, l'identification de métabolites est une étape clé pour comprendre les mécanismes biologiques étudiés. Pour répondre à cette étape, combiner dans un même workflow d'annotation des sorties de MZmine avec SIRIUS est particulièrement adapté. L'objectif ici, est non seulement de vous montrer l'efficacité de la combinaison systématique de MZ mine et Sirius dans l'annotation de métabolites d'intérêts dans des projets impliquant la métabolomique non ciblée par LC-HRMS, mais aussi de vous démontrer que l'utilisation des données de "pseudoMS2 " provenant d'acquisition MS simples combinées à Sirius est particulièrement adaptée dans l'identification d'ions difficiles à isoler. Ces 2 approches ont été réalisées comme preuve de concept sur des échantillons plasmatiques de différents projets afin d'identifier de manière fiable les métabolites d'intérêts. Ainsi, des données issues d'analyses par UHPLC MS de type électrospray – ToF (Impact II - autoMSMS) ou Orbitrap (pseudoMS2) ont été utilisées.

Concernant la première approche, l'utilisation du workflow lors d'études scientifiques permet de créer des bases de données temporaires propres à chaque étude, facilitant ainsi l'annotation des métabolites.

En seconde approche, des données de " pseudoMS2 " acquises en mode ESI+ sur LC-LTQOrbitrap Velos, Et LC-Q-Exactive (Laberca) dans le cadre d'anciens projets scientifiques ont permis de montrer son efficacité. Cette approche a été appliquée lors d'études scientifiques, sur des ions difficilement identifiables.

En conclusion, l'utilisation systématique du workflow MzMine Sirius combinée à des modes d'acquisition data-dépendant, et l'approche " pseudoMS2 " ouvrent la porte à une plus grande efficacité dans l'étape d'identification des métabolites lors d'études de métabolomique non-ciblée.

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Poster 34 - P34

Exposition de moules méditerranéennes à un anti-dépresseur : recherche des métabolites de la fluoxétine formés suite à l'exposition

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Il est admis que l'environnement est exposé et soumis à différentes pollutions induites par les activités humaines. L'amélioration des instruments analytiques et des limites de détection a permis la mise en évidence de nouvelles molécules dites " contaminants émergents ", parmi lesquels les produits pharmaceutiques font partie. Le rejet direct des eaux usées dans l'environnement, via des émissaires en mer par exemple, et les traitements en station d'épuration non adaptés aux contaminants émergents, exposent les organismes non-cibles des milieux aquatiques à ce type de produits. Notre étude a pour objectif de déterminer et caractériser les métabolites de la fluoxétine (FLX) produits par la moule *Mytilus galloprovincialis*, organisme sentinelle de l'environnement et facilement adaptable aux expérimentations en laboratoire, après une exposition en conditions contrôlées. Les moules ont été exposées pendant 28 jours à 3,1 μg/L de fluoxétine et un échantillonnage a été réalisé à la fin de l'exposition. La recherche des métabolites est effectuée par LC-HRMS avec une approche non-ciblée. L'apport de ce type d'approche est qu'il permet d'identifier d'une part les métabolites connus de la FLX, et d'autre part des métabolites minoritaires et/ou non reportés jusqu'à présent dans la littérature. Un premier travail a été réalisé sur le protocole d'extraction et son optimisation pour la détection de la FLX et de ses métabolites connus. Par la suite, la méthode développée sera appliquée à la quantification de la fluoxétine dans les moules exposées et à la recherche de ses métabolites connus ou inconnus dans les empreintes LC-HRMS qui seront acquises.

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Poster 35 - P35

Secondary metabolites from the interaction between plants and disease-suppressive soils: useful biomarkers to monitor plant physiological responses in a context of climate change?

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Climate change leads to modified agriculture systems. One consequence is a higher prevalence of crops like wheat. The European project SuppressSoil, involving partners in France, Switzerland and Germany, aims to decipher whether soils whose microbiota contributes to protect current crops from root diseases (termed disease-suppressive soils) will also protect crops like wheat in a context of global change. Thus, we want to determine to which extent soils suppressive to black root rot disease caused by the fungus *Thielaviopsis basicola* on dicots such as tobacco, beans etc., will grow healthy wheat despite the emergence of the wheat fungal pathogen *Fusarium graminearum*. To this end, two different locations where suppressive soils occur were used in the greenhouse with wheat plants that were inoculated or not with the pathogen *Fusarium graminearum*. Here, we focus on the physiological response of wheat in suppressive soils vs non-suppressive (= conductive) soils, as an important task of this project is to analyse plant's secondary metabolism. Roots and leaves from different conditions (plants inoculated or not, from suppressive or conducive soils) were analysed separately with the UHPLC-UV/DAD-MS-QTOF non-target technique to identify possible suppressiveness biomarkers and profile differences. Differences found in the preliminary data will be presented. This information is important to understand the contribution of plant physiology in the tripartite interaction between soil microbiota, phytopathogen and plant host. New knowledge from this project will help to set up management strategies to improve crop health in soils with poor or no suppressiveness properties.

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Poster 36 - P36

Metabolic changes in poplar roots under N and Fe deficiency

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Poplar (*Populus x canescens*) is a fast-growing tree that combines rapid maturation and wide geographic distribution with economic relevance for wood and biomass production. Poplar is an important model to study wood formation. In contrast, poplar responses to environmental stresses and especially nutrient deficiencies have been scarcely studied. Thus, the aim of this work is to determine how metabolomics profiles of poplar roots are affected by N and Fe deficiency. This is expected to highlight metabolic strategies acquired by the plant to cope with nutritional deficiencies. Poplar cuttings were propagated in vitro, then transferred to hydroponic solution and finally submitted to N or Fe starvation. Plants presented deficiency symptoms as iron chlorosis, decrease in biomass weight, SPAD value and water uptake. Metabolites from poplar roots were obtained by methanolic extraction and then analyzed by LC MS/MS. As preliminary results, poplar roots accumulated specific flavonoids, salicylates, stilbenes and coumarins under N or Fe starvation. Future studies will aim at investigating the effect of combined N limitation and Fe deficiencies.

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Poster 37 - P37

Development of a targeted metabolomics method for automated analysis of general metabolites using GC-MS/MS

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We have developed a targeted metabolomics method based on multiple reaction monitoring (MRM) for the detection of general metabolites using gas chromatography coupled with triple quadrupole mass spectrometer (GC-MS/MS). The selected panel of metabolites was chosen based on their relevance to studies of mammalian biological systems and compiles 62 metabolites from different classes of chemical families such as amino acids, steroids, amines, alcohols, sugars, fatty acids and other organic acids. Derivatization of hydrophilic functional groups using methoxyamination and trimethylsilylation (TMS) is required to increase volatility. To enhance the feasibility and reproducibility of the sample preparation process, we implemented an automated derivatization step using a robotic auto-sampler system. We validated the method using a set of quality control samples, demonstrating its accuracy, precision, and sensitivity. This targeted metabolomics method, based on the GC-MS technique and MRM approach in combination with automated derivatization, has the potential to significantly improve the sensitivity and specificity of general metabolites quantifications in both clinical and research settings.

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Poster 38 - P38

Capturing growth, physiology and quality traits of ten fruit species using machine learning-based predictive metabolomics

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A better understanding of the developmental basis of fruit growth, physiology and quality traits would allow the identification of molecular targets for their improvement. Metabolites are integral descriptors of cell and tissue physiology, as their abundance is determined by complex regulatory networks that involve transcriptional and enzymatic scales as well as the levels of other metabolites. Thus, profiling the fruit metabolome during development offers the potential to examine the key physiochemical changes contributing to fruit growth and ripening. In the framework of *GLOMICAVE* project (*Global Omic Data Integration on Animal, Vegetal and Environment Sectors*, EU H2020 No. 952908), we collected the metabolic profiles for an allometric series of ten fruit species at nine different developmental stages (from early fruit establishment to maturity). We first applied various imputation methods of missing values to address the challenge of the high number of missing values that arise mainly from the presence or absence of metabolites in different species. Next, we deployed predictive metabolomics to capture fruit growth, physiology and quality traits via machine learning-based models, yielding overall good prediction accuracies. Besides, thanks to a large phytochemical database developed by MetaboHUB research infrastructure, we annotated the predictive metabolic markers that were further used to identify biochemical pathways/metabolites important for the predicted traits. These pathways/metabolites represent molecular targets for future breeding efforts to improve fruit traits. Dr Melandri and Barros-Santos should be considered as co-first authors.

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Poster 39 – P39

Métabolomique non ciblée sur gouttes de sang séché

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Les micro-prélèvements de sang ont initialement été employés en milieu hospitalier chez le nouveau-né dans le cadre de dépistage de maladies congénitales ; aujourd'hui ce type de prélèvement se développe de plus en plus dans les études cliniques. Analysés principalement en méthodes ciblées, peu de travaux rapportent des résultats dans le cadre d'approche en métabolomique ouverte. L'objectif du travail présenté est d'évaluer des supports de prélèvements, des méthodes d'extraction de ces échantillons ainsi que la couverture analytique obtenue par LCHRMS.

Des papiers de collecte 903 ® Whatman, principalement utilisés pour ces prélèvements ainsi que des supports Mitra® Neoteryx possédant un volume fixe de prélèvement de 20μL, ont été testés dans différentes conditions de stockage et d'extraction. Les analyses ont été réalisées à partir de plasma hépariné et sang total sur LC-QToF puis retraitées sur W4M.

Les premiers résultats obtenus ont permis une comparaison qualitative des échantillons de plasma hépariné versus ces mêmes échantillons déposés sur les supports étudiés dans les différentes conditions étudiées. Après optimisation de la préparation de ce type d'échantillons, les échantillons de sang total ont pu être caractérisés.

En conclusion, ces travaux préliminaires en métabolomique ouverte vont permettre d'investiguer l'analyse de micro-prélèvements permettant un mode de collecte peu invasif, facile d'emploi à domicile, pour répondre à des questions liées aux études cliniques et au développement de la science participative.

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Poster 40 - P40

Impact of perinatal exposure to the food additive TiO2 on local and systemic metabolome in the progeny

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Titanium dioxide (TiO2) is a common food additive containing nanoparticles (NPs). In 2022, its use has been banned in EU, based on its potential genotoxicity, while Food Safety Authorities encouraged additional experimentations for further risk assessment. Perinatal window is a period of high susceptibility to environmental factors that can then have long term-effect on progeny health. This study aims to assess the impact of perinatal exposure to TiO2 in a mother-child mouse model, looking at potential metabolism perturbation. Female mice were fed with pellets containing TiO2 at human relevant doses (ornot, control group), from 30 days beforemating until the end of lactation. After weaning, progeny was fed with the same diet as their mothers for 10 days and plasma and faeces were sampled. Nontargeted metabolomic analysis was carried out using a robust analytical workflow based on liquid chromatography coupled to high-resolution mass spectrometry. Metabolite annotation was performed using an in-house spectral database containing 1200 metabolites. Statistical analyses were performed using omics-dedicated R packages and the MetaboAnalyst software.

Annotation process led to identification of 296 and 240 metabolites in faeces and plasma respectively. In male, 33 faecal and 94 plasma metabolites were significantly different between groups. Pathways enrichment analysis evidenced that purine and pyrimidine metabolisms, two major components of cellular energy systems, signalling, and RNA/DNA production, were particularly impacted by TiO2 exposure, both at the local and systemic levels. Our study highlights the potential of non-targeted metabolomics analysis to detect subtle effects of perinatal exposure to NPs-containing food additives.

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Poster 41 - P41

METACLOUD Project: Highlighting the biological activity of Clouds under different conditions (day VS night) with 13C isotope profiling by high resolution mass spectrometry

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Metabolically active microorganisms in clouds have been highlighted as potential cata- lysts of biochemical reactions, especially in the use of small organic compounds (organic acids or aldehydes) (1). There are still many uncertainties regarding the potential bio-activities occurring during the day and night, and their impacts on cloud chemistry. Indeed, the bio- logical activities may dominate the chemical reactivity during night while abiotic processes would be dominant during the day due to the photochemical production of hydroxyl radicals (2). The METACLOUD project (ANR-19-CE-0004) proposes to investigate the metabolic functioning of cloud microorganisms in two contrasted situations simulating a summer day (17 C, light, H2O2) and a winter night (5 C, dark). A focus is made on formaldehyde dueto its key role both in cloud radical chemistry and in many C1 biological pathways. First experiments were performed with an artificial consortium designed from microbial strains isolated from cloud water sampled at puy de Dˆome Station and suspended in an artificial medium mimicking marine cloud water chemical composition (major inorganic and organic compounds). Then freshly sampled cloud water from the top of the puy de Dˆome was incu- bated with 13C-formaldehyde to evaluate its bio-assimilation with 13C-metametabolomics by HRMS. Global metabolic adaptations between those two conditions were highlighted *via* this approach and first results with 13C-formaldehyde showed incorporation of this molecule in several classes of metabolites (e.g. nucleotides, amino acids, central metabolites) since 2h and until 192h of incubation.

(1) Vaïtilingom M. et al. (2013). doi: 10.1073/pnas.1205743110 (2) Vaïtilingom M. et al. (2011). doi: 10.5194/acp-11-8721-2011

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Poster 42 - P42

Linking blood oxylipin profiles to internal POPs exposure levels in women undergoing in vitro fertilization

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The impact of environmental pollutants on gynecological disorders is mediated by inflammation and oxidative stress, but little is known about the interaction of oxylipins, bioactive lipids derived from polyunsaturated fatty acids that play an important upstream role in regulating of these biological processes. The aim of this study was to explore factors influencing the levels of oxylipins and their precursor fatty acids in the serum of women undergoing *in vitro* fertilization (IVF), including internal exposure to chemical pollutants and underlying gynecological disorders in infertile women. We analyzed the serum of 100 women recruited at the Nantes University Hospital with female infertility related endometriosis, polycystic ovaries, ovarian insufficiency or unexplained. Fertile women consulting for male infertility or being oocyte donors were also included as a control group. The concentrations of ten oxylipins were determined in serum using negative electrospray liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), after protein precipitation and SPE-based extraction. Twenty-six free fatty acids were subjected to liquid-liquid extraction and derivatization before detection by gas chromatography coupled to a flame ionization detector and mass spectrometry system (GC-FID/MS). Negative associations were found between the fluorinated industrial pollutant, perfluoroundecanoic acid (PFUnA) and levels of prostaglandin E2, 15-hydroxyeicosatetraenoic acid (15HETE) and 5-HETE. Levels of 5-HETE and 8,9-epoxyeicosatrienoic acid were found to be at lower levels in women with endometriosis-related infertility. Further research will be conducted to better understand the potential role of oxylipins on the functional associations between environmental pollutants and gynecological disorders.

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Poster 43 - P43

The link between external microbiota, metabolome, and monogenean ectoparasites of Sparids (Teleostei)

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While teleost fishes represent two thirds of marine vertebrates, the role of their external microbiota in relationship with their environment remains poorly studied. Hence, the interaction of their microbiota with ectoparasites is largely unknown. Microbiota can act as a protective barrier against pathogens, and/or be involved in host recognition by parasites. Monogeneans (Platyhelminthes) are direct life cycle ectoparasites commonly found on teleost skin and gills. Their larvae actively swim towards their host based on chemical stimuli. Bacterial communities are suspected to contribute to these cues and could thus play a significant role in host-parasite specificity mechanisms. We are exploring these mechanisms through the characterization of teleost external mucus microbiota and metabolites.

We focused on a well-known association between Sparidae, a family of Mediterranean fishes, and monogeneans of the Lamellodiscus genus which exhibit various patterns of host specificity and species richness for their different fish hosts species.

To understand the role of fish external mucus, we collected two groups of sparid fishes, the first one contains bogues which are never parasitized by *Lamellodiscus* and the second one contains different sparid species all known to be parasitized by *Lamellodiscus*.

For all individuals we characterized bacterial communities (high-throughput sequencing of the V3-V4 regions of the 16S ribosomal gene) and chemical composition of the external mucus (liquid chromatography coupled to mass spectrometry - LC-MS). We aim to identify correlations between metabolome and the abundance of particular bacterial genera and determine metabolites influencing (or influenced by) the parasitic load of sparids (dissecting microscope).

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Poster 44 - P44

Untargeted metabolomics reveals that specialized metabolite diversity of rapeseed spermosphere is influenced by the environment and aging

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With a growing world population and the major environmental problems caused by climate change and bioagressors, providing enough food for everyone may become challenging.
Synthetic pesticides are used to protect seeds and plants to cope with biotic stresses, but these chemicals can have negative effects on the environment. SUCSEED ("Stop the Use of pestiCides on Seeds by proposing alternatives") project aims to find alternatives biobased molecules that can serve as protectants for seeds to cope with pathogens. Part of the project focus on rapeseed, and their main pathogens involved in damping-off.
The spermosphere is the zone surrounding germinating seeds that is composed of specialized metabolites (SMs) and other molecules that are exuded by the seeds, along with beneficial or pathogen microorganisms that are attracted by these molecules.
In this work, seed spermosphere of 10 rapeseed genotypes cultivated in various environments were characterized by untargeted metabolomics, which was integrated with other omic techniques. To mimic seed alteration during storage, artificial aging was also performed to find which metabolites are impacted. To identify SMs implied in defences mechanisms and impacting seed physiology, untargeted metabolomic analyses (LC-MS/MS) were correlated with germination to analyse seed exudates composition. Most of annotated SMs found in seed exudates belong to amino acids derivatives, cinnamic acids, flavonols, terpenoids and glucosinolates. The analyses revealed that SMs composition of rapeseed spermosphere is strongly influenced by the environment and seed aging. Few, selected SMs will be tested against seed pathogens and some effective molecules will be proposed for coating formulation.

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Poster 45 - P45

How epigenetic modifiers modulate mycotoxins production of Fusarium verticilloides

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Toxigenic fungi are capable of producing toxic metabolites, called mycotoxins. But the presence of silent and lowly expressed genes represents a main challenge for the discovery of novel mycotoxins, especially the lesser-known forms of mycotoxins, commonly referred to as 'emerging mycotoxins'. Epigenetic modifiers are compounds able to alter the production of metabolites through the induction of silent biosynthetic pathways leading to an enhanced chemical diversity. The aim of this study was to assess the effects of different chemical modulators on the metabolic profiles of a toxigenic fungal strain, *Fusarium verticilloides*, know to produce Fumonisins mycotoxins, in order to unreveal the toxigenic potential of this strain. Four epigenetic modifiers, 5-azacytidine (AZA), sodium butyrate (SB), nicotinamide (NIC) and sodium valproate (SV), were used. The metabolic profiles were analyzed by UHPLCHRMS/MS under targeted and untargeted metabolomics approaches. Our results show that the treatment with sodium valproate and 5-azacytidine induced the most important alteration of the secondary metabolic profile of *Fusarium verticillioides.*

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Poster 46 - P46

Réponse métabolique de la plante aux infections microbiennes : mêmes armes pour se défendre ou coopérer ?

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Dès lors que les plantes détectent dans leur environnement des motifs moléculaires microbiens ou des effecteurs, elles mettent en place des mécanismes de défense. La reconnaissance de ces éléments par les cellules végétales déclenche le système immunitaire de l'hôte végétal. Cette réponse inclut notamment la production de Dérivés Réactifs de l'Oxygène (DRO), de protéines antimicrobiennes ou de métabolites secondaires antimicrobiens. Dans le cas de l'interaction avec un symbiote, le système de défense serait également induit lors de la reconnaissance par la plante mais rapidement modéré pour permettre l'entrée du symbiote dans les tissus racinaires. Cette étude a pour objectif de confronter les systèmes de défense déployés par la plante face aux microorganismes pathogènes et symbiotiques. Notre hypothèse de travail propose que certains mécanismes seraient communs, mais la plante posséderait la capacité de moduler son statut immunitaire selon le microorganisme. Des différences dans la composition ou la concentration des molécules de défense pourraient influencer l'intensité, la qualité et le délai de la réponse immunitaire. Dans cette étude, nous nous sommes focalisés sur le modèle *Alnus glutinosa*, pour rechercher des variations dans son métabolisme après inoculation par un microorganisme symbiotique (*Frankia alni*) ou pathogène (*Phytophthora alni*), à deux temps très courts après inoculation (24h et 72h, étape de reconnaissance du microorganisme). Les empreintes métaboliques des métabolites secondaires de racines et de feuilles de plantes infectées par *F. alni* et/ou *P. alni* et de plantes non-infectées ont été comparés par LC/HRMS (approche non-ciblée). Les acides aminés ont été dosés par HPLC/fluo (approche ciblée).

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Poster 47 - P47

Regulation of leaf primary metabolism and sink/source relationships during acclimation to drought stress in Brassicaceae (PRIMABRA)

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Winter Oilseed Rape (WOSR) is the second most cultivated oleaginous crop in the world. However, its grain yield potential is threatened by climate change. Indeed, water shortage negatively impacts WOSR growth by reducing photosynthesis and accelerating leaf senescence (chloroplast degradation). In this context, the tricarboxylic acid cycle (TCA cycle) is supposed to centralize the main drought acclimation adjustments by allocating and optimizing fluxes directed to: i) compatible osmolyte accumulation for osmotic potential homeostasis, ii) mitochondrial oxidative processes for energy production, iii) carbon and nitrogen nutrient recycling processes for source-to-sink trophic relationships. To investigate these metabolic regulations, we applied 15 days of progressive water shortage and analyzed both sink and source leaves of WOSR during vegetative growth. At different time points, we evaluated both senescence and water status of WOSR leaves, combined to absolute quantifications of leaf primary metabolites (amino acids, organic acids, sugars, polyols). Overall, our results confirmed accelerated dehydration and senescence processes in older source leaves while younger sink leaves maintained their relative water contents and chlorophyll levels. Interestingly, sink leaves strongly accumulated the compatible osmolyte proline but also glucose. Both sink/source leaves accumulated branched chain amino acids (BCAAs) and glutamine in response to drought. Regarding TCA cycle metabolites, drought stress decreased citrate content while malate content only increased in sink leaves. The results were discussed with respect to the metabolic origin of BCAAs, proline in sink leaves and TCA cycle flux modes. 13C-labelled experiments and measurements of TCA cycle enzymatic activities are currently performed to address these questions.

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Poster 48 - P48

Évolution métabolique entre 2 temps de gestation (G13-G19) chez la rate : métabolomique du placenta, du liquide amniotique et du sang.

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Le concept DOHaD propose que les facteurs environnementaux aux stades précoces du développement peuvent être impliqués dans la survenue de maladies à distance. L'objectif de ce travail est de décrire l'évolution du métabolome de la rate gestante à partir de prélèvements de sang maternel, de placenta et de liquide amniotique à 2 temps (13 et 19 jours de gestation : G13, G19). Les prélèvements ont été analysés par LC-MS (Orbitrap) par des approches ciblées et non ciblées. La complémentarité et l'évolution métabolique de ces 3 matrices ont été étudiées. L'approche non ciblée donne 27% (placenta), 48,4% (liquide amniotique), et 24% (sang) des ions, détectés de façon robuste, statiquement différents (p(FDR)< 0,05) entre ces 2 stades de gestation. En cumulant les 3 matrices obtenues par approche ciblée, les résultats obtenus donnent 78 métabolites (14%) augmentés au stade G13 (Fold Change (E13/E19)> 2 et p-value< 0.05) alors que 138 métabolites (24%) le sont à G19. La matrice biologique qui évolue le plus entre G13 et G19 est le placenta (41,2% de ses métabolites ciblés), puis le liquide amniotique (34,3%) et enfin le plasma (24,5%). Ces métabolites les plus différentiels dans l'évolution entre G13 et G19 par analyse Volcano appartiennent à la voie des " Aminoacyl-tRNA biosynthesis " dont " Alanine, aspartate and glutamate metabolism " et " Cysteine and methionine metabolism ", mais aussi à la voie " Pyrimidine metabolism ". Les effets métaboliques dynamiques sont à prendre en compte lors d'analyses prénatales, le placenta et liquide amniotique montrant des modifications plus importantes que le sang maternel.

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Poster 49 - P49

Imagerie par spectrométrie de masse appliquée à la conception de nouveaux outils pour le traitement de glioblastomes

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Le glioblastome est la tumeur cérébrale la plus fréquente et la plus agressive chez l'adulte. Des stratégies locales à l'aide de biomatériaux et des innovations thérapeutiques basées sur la chimie sont développées dans le cadre du projet NeurOncochimie, projet régional transdisciplinaire. Le devenir de ces molécules et la réponse thérapeutique du glioblastome sont objectivés par l'imagerie par spectrométrie de masse (MSI) de coupes de cerveaux de souris xénogreffées par une lignée tumorale, en l'absence et présence de traitement. Ainsi, l'objectif de la MSI est de mettre en évidence l'efficacité du traitement et de localiser et d'identifier les métabolites présents au sein de la tumeur traitée, et en périphérie pour évaluer l'effet de la thérapie et la plasticité cérébrale. Les développements présentés sont réalisés sous ionisation par désorption laser assistée par matrice (MALDI) couplée à un analyseur hybride quadrupôle-temps de vol équipé d'une cellule de mobilité ionique TIMS (TIMS-TOF Flex, Bruker). Les méthodes ont été développées sur des coupes de cerveaux de souris en mode d'ionisation positif et négatif avec et sans mobilité ionique, de façon à obtenir la couverture métabolique la plus large possible. La résolution spatiale choisie est de l'ordre de 10 μm. Le traitement de données a permis de mettre en évidence des marqueurs spécifiques des tissus étudiés et des traitements utilisés. L'investigation du contenu moléculaire permettra l'étude de la distribution spatiale des molécules exogènes et d'améliorer la compréhensions des mécanismes moléculaires lors de la récidive du glioblastome ou lors du traitement de la tumeur.

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Poster 50 - P50

Assessment of A. muciniphila nutritional needs for the development of next-generation probiotics

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Many diseases have been proven to be closely related to gut microbiota. Therefore, modulation of intestinal bacteria could lead to health improvement. *Akkermansia muciniphila* (*A. muciniphila*), a strict anaerobic gut bacterium, has been inversely associated with obesity, diabetes, inflammation, and metabolic disorders. For these reasons, *A. muciniphila* is an interesting candidate for next-generation therapeutic probiotics.
In order to maximize *A. muciniphila* biomass production, the aim of this study was to assess its nutritional needs. This knowledge could lead to the development of specific yeastbased nutrients adapted to the industrial production of this microorganism.
For that, fermentations of *A. muciniphila* were carried out using different yeast-based nutrients. Performance was assessed as a function of biomass production *via* O.D measurements. Then, an LC-MS based metabolomic study was performed. By comparing the composition of the culture broth before inoculation to that of the spent culture medium, molecules preferentially consumed by *A. muciniphila* could be identified.
Results pointed to essential nitrogen-containing compounds, as *A. muciniphila* consumes preferentially small peptides (di- to tetrapeptides). Regardless of which yeast-based nutrients were added to the culture medium, *A. muciniphila* nutritional needs remained the same.

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Poster 51 - P51

Biomarkers identification of severity and treatment-resistance in Major Depressive Disorder

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Major Depression Disorder (MDD) is one of the most frequent and severe mental disorders. Practical guidelines recommend treating MDD with antidepressant (AD) therapy. However, about 30 to 50% of MDD patients do not respond to the first-line AD. Currently, the diagnosis and the therapeutic follow-up remain based on the clinical evaluation. Developing molecular biomarkers should thus improve patient's care. We conducted a metabolomic study to identify blood and fecal biomarkers associated with MDD.

Fifty adult patients were included when the MDD diagnosis was realized according to the DSM-5 criteria with a MADRS score (> 20). Blood and feces samples were collected before the treatment was set up with an AD (Escitalopram). The MADRS score was reevaluated over 8-week treatment to distinguish responders from non-responders. Metabolites were extracted and analyzed by LC-MS. Then, data were analyzed using univariate (GraphPad Prism9®) and multivariate (SIMCA17®) analysis.

When we compared the MDD severe group with the MDD moderate group, we observed in blood, a decrease of phosphatidyl choline and in several metabolites of tryptophan's metabolism, and in feces samples, we observed an increase of fumarate and malate, and in several B-vitamins. In addition, OPLS-DA analysis showed the possibility of estimating the response to treatment based on the analysis of the 21 VIP metabolites found in the stool. These pilot results show that metabolomics can help in the diagnosis of depression by bringing molecular elements to complete a clinical diagnosis of the severity of the disease and potentially estimate the response to an AD treatment.

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Poster 52 - P52

Identification of metabolites as biomarkers of mastitis in goat's plasma and milk using 1H-NMR and UHPLC-HRMS

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Mastitis is an inflammation of the udder, affecting dairy goats, often caused by bacterial infection; with or without external signs (clinical or subclinical mastitis, respectively). Subclinical mastitis is difficult to diagnose and are currently based on the SCC (Somatic Cell Count) level in milk even if this not specific to mastitis, making the diagnosis unreliable. In this context, identify molecular biomarkers with metabolomics studies can be interesting to improve mastitis diagnosis.

Milk and blood samples were collected once a week for 4 weeks on fifty Alpine goats right after parturition. Goats were divided in two groups based on the milk SCC level: the infected group with a SCC level > 1.106 cells/mL on the 4 samples and the control group with a SCC level < 750 000 cells/mL on the 4 milk samples.

Samples were analyzed by LC-MS and NMR. Then data were analyzed by univariate and multivariate analyses using GraphPad Prism9® and SIMCA 17® to determine discriminant metabolites (VIP) between the two groups.

OPLS-DA have shown that it is possible to discriminate the two groups in both matrices with the two analytical platforms at each time-point. In blood, lactose is a VIP metabolite and significantly increased in INF group at the 4 time-point. In milk, 11 metabolites are discriminant between both group (phosphocreatine, proline, methionine, choline, methyltryptamine, leucine, lactic acid, propylene glycol, guanosine monophosphate, inosine & thiamine). This novative study including multi-matrices and multi-platforms analyzes, shows the possibility to identify molecular biomarkers for subclinical mastitis diagnosis in dairy goats.

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Poster 53 - P53

1H-NMR metabolomics modification induced by low purine diet in combination with Sida acuta Burm.f in slow growing chicken.

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Chicken meat is classified as a purine-rich food, which limits its consumption by those susceptible to gout and hyperuricemia, pathologies of growing incidence. Dietary strategies decreasing the content of purine in animal's diet or supplying natural products interfering with purine metabolism would thus be valuable to improve the nutritional quality of premium chickens. Sida acuta Burm.f., a plant containing flavonoids acting as inhibitors of the xanthine oxidase enzyme was chosen. Two groups of slow-growing Korat Chicken (KC), developed for Thai smallholder farmers, were bred from hatch until 63 days of age and fed a control (CON) or a Low Purine diet (LPD). The LPD contained - 30% purine, and 0.6% SA. At 63 days of age, the chickens were slaughtered. The muscle samples were collected and analyzed by high-performance liquid chromatography (HPLC) and 1H NMR spectroscopy. In the muscle of female chickens, HPLC detected significantly lower levels of hypoxanthine (-10%) and total purine (-9%) in LPD vs control ($P < 0.05$, $n = 6$). 1H-NMR identified and quantified 75 metabolites, twenty-three of them allowed a metabolic profile discrimination between CON and LPD in a PLS-DA model (R2Ycum = 0.993, Q2cum = 0.955, CV-ANOVA p = 1.6E-4). Twenty-one were up-regulated and two were down-regulated in LPD. The alterations concerned mainly the amino-acid and carbohydrate metabolic pathway and the nucleotide metabolic pathway (purine metabolism). Although the tested dietary strategy led to reduced purine content, a full understanding of the respective contribution of reduced dietary purine and SA supplementation will require further study.

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Poster 54 - P54

Study of tomato leaves (Solanum lycopersicum) metabolism by different pest attacks

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The tomato plant, *Solanun lycopersicum* L. (Solanaceae), is one of the most widely consumed vegetables in the world and plays an important role in human diet. Tomato cultivars are hosts for diverse types of pests, implying diverse chemical defense strategies.

This work aimed to study the tomato defense metabolites when plant was attacked by different kind of pests at different plant location (leaves, roots). The pest attacks studied were *Macrosiphum euphorbiae*, *Helicoverpa armigera*, *Oidium neolycopersici*, *Meloidogyne incognita*. And the study focused on the upper and lower systemic leaves and the local leaves in contact with the pests. For this study, a metabolomic workflow was set up. 700 leaf samples were analyzed by UHPLC/HRMS to obtain the metabolomic fingerprint of tomato leaves infested or not by pests. Given the large number of samples analyzed and small difference in chromatographic profile observed, chemometric tools were necessary to highlight the metabolites that vary after the infestation of tomato plants. These analyzes were preprocessed by W4M, the matrices were reduced, and statistical analyzes were performed by metaboanalyst.

It was highlighted a differentiation of the leaves belonging or not to plants which have been in contact with a pest. The metabolites differ according to the type of pest and according to the location in the plant.

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Poster 55 - P55

3-NPH derivatization of SCFAs in human biofluids for longitudinal and multi-matrix exploration using LC-MS²

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Short-chain fatty acids (SCFAs) are involved in many metabolisms and deregulations are suspected in diseases of the digestive system such as colon cancer, Crohn's disease but also in neurodegenerative diseases such as Alzheimer's disease. One of the reference methodologies for the analysis of SCFAs uses GC-MS because of its reproducibility and its sensitivity although its implementation is technically tricky. Currently, analysis by LC-MS after chemical derivatization is becoming the preferred method, in particular because of its ease of implementation. Our goal is to develop a method for the absolute quantification of SCFAs for future use in a clinical setting for all biological samples such as faeces, urine, serum, saliva, CSF and DBS (dried blood spot). The chemical derivatization that we've selected is 3-nitrophenylhydrazine (3NPH) which increases the sensitivity and the selectivity of the method by improving the ionization of the target compounds. Moreover, by introducing a recognizable molecular pattern by MS identical to all the compounds, the detection of these chemical species is facilitated. After validation of the method (intra-daily and inter-daily repeatability, matrix effect, carryover, lower limit of quantification LLOQ), we analysed samples in order to propose reference values for SCFAs concentrations for each type of sample. Longitudinal monitoring of SCFA concentrations was also carried out over 24 hours in saliva. The results show that salivary production of SCFAs follows a circadian rhythm. The proposed methodologies make it possible to explore the metabolism of SCFAs at the scale of an individual and in a longitudinal manner (absolute quantitative data).

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Poster 56 - P56

Élucidation du rôle des glutathion transférases de peuplier au cours de l'interaction hôte-pathogène entre le peuplier et le champignon Melampsora larici-populina

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Les glutathion transférases (GSTs) constituent une superfamille d'enzymes multifonctionnelles notamment impliquées dans la détoxication des xénobiotiques, la synthèse et/ou le transport de métabolites spécialisés. Les fonctions de ces enzymes reposent sur leurs propriétés catalytiques ou sur leur propriété non catalytique dédiée au transport ou au stockage de ces molécules. Lors du stress biotique généré par le champignon pathogène *Melampsora larici-populina* (*Mlp*) sur le peuplier, des analyses transcriptomiques ont montré que l'expression de certains gènes de peuplier codant des GSTs est fortement régulée.

Afin de préciser les rôles et fonctions des GSTs de peuplier au cours du processus d'infection, les objectifs sont i) d'inventorier, par des approches métabolomiques, les molécules présentes dans des extraits de feuilles de peuplier infectées ou non par *Mlp*, ii) d'identifier, à partir de ces extraits, les ligands et substrats des GSTs de peuplier dont l'expression est régulée au cours du processus d'infection et enfin, iii) de caractériser aux niveaux biochimique et structural les interactions GST-substrat/ligand identifiées.

Des extraits de feuilles de peuplier infectées par *Mlp* ont été analysés par chromatographie liquide couplée à la spectrométrie de masse. Le réseau moléculaire, généré par le logiciel *MetGem*, a conduit à l'identification des métabolites produits au cours du processus d'infection. Tout en se servant de réseau moléculaire précédemment établi, l'étape suivante consistera à identifier puis à caractériser, par des approches de biochimie et de biologie structurale, les molécules présentes dans ces extraits qui interagissent avec les GSTs d'intérêt.

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Poster 57 - P57

Revisiting a protocol for 1H-NMR profiling of wine samples before an interlaboratory comparison.

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The main objective of this work was to revisit several steps of a wine profiling protocol based on 1H-NMR to insure its deployment on all spectrometers of WAP-NMR consortium. The previous protocol1 required manual steps in front of the spectrometer and needed to be adapted to automation used in the future for high-throughput authentication studies. Wine samples were pH-adjusted (BTpH, Bruker). NMR spectra were acquired with automation at 500 MHz and manually at 600 MHz. Two pulse sequences were used, a single (zgpr) and a multi-solvent (noesy) suppression. NMR spectra were processed with MestreNova for targeted analysis, and with NMRProcFlow2 (nmrprocflow.org) for targeted and untargeted analysis. The explored steps concerned sample preparation taking into account wine type, calibration for compound quantitation, NMR acquisition parameters including iconnmr and spectra processing.

Two deuterated-water concentrations and three buffer-solution strengths were tested. Effects on duration of gradient shimming adjustment and of pH-adjustment as well as on NMR spectral quality were checked. Repetition times depending on longitudinal relaxation time and field strength were also determined. Wine sample stability was verified during two weeks to set the time span allowed between sample preparation and acquisition and to minimize uncontrolled variability.

We report on our first results of these interlaboratory comparisons of wine profiling by 1D 1H-NMR spectroscopy across two different spectrometers.

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Poster 58 - P58

1H-NMR profiling of tomato samples with benchtop spectrometer

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Metabolic phenotyping or metabolomics of tomato fruit is well documented and easily monitored for fruit sampling at a given developmental stage especially for whole fruit or for pericarp tissue. A detailed characterization of fruit development by qNMR metabolomics of cultivated tomato tissue**1** and pericarp of tomato mutant lines**2** has been published recently at high field NMR. To widen the interest of such an NMR-based approach, decreasing the analytical cost and increasing the analytical throughput are of interest. Therefore, the main objective of this work was to test the ability of benchtop NMR to discriminate two tissues of tomato (pericarp and locular tissue) at four stages (Mature-green, Breaker, Orange, Redripe) and to quantify the major soluble sugars and organic acids.

1H-NMR spectra of the same tomato hydromethanolic extracts were acquired with waterpresaturation at 80 MHz (Magritek, Aachen, Germany) and at 500 MHz (Bruker, Wissembourg, France). They were processed with NMRProcFlow**3** (nmrprocflow.org) for targeted and untargeted analysis (ERVA**4** method), and the data were analyzed with univariate or multivariate statistical analyses.

We report on our first results of these comparisons of tomato tissue profiling by 1H-NMR at 80 and 500 MHz. Benchtop NMR analysis of fruit tissue extracts could be proposed to biologists studying tomato or other fleshy fruits, to characterize fruit development of wild-types and mutants in a greenhouse, or to phenotype large series of genotypes.

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Poster 59 - P59

Impact de l'´erythropoïétine (EPO) sur le profil métabolique de fèces de souris

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L'EPO est une hormone qui agit sur la moelle osseuse afin de produire des globules rouges et améliorer le transport de l'oxygène. Dans certaines situations comme l'anémie et l'hypoxie certaines cellules sont capables de détecter la baisse d'oxygène et ainsi augmenter la production de l'EPO et permettre un bon transport de l'oxygène. Les études sur l'EPO se tournent généralement vers des analyses sanguines ou urinaires. Cette étude a pour but d'observer l'impact de l'EPO sur le profil métabolique de fèces de rongeur. Pour cela, nous avons analysé des échantillons de fèces de souris " normales " aka Wild-Type (WT), des fèces de souris double-mutant sous exprimant l'EPO (EPO-TAgh) " -/- " ainsi que des fèces de rat. Objectifs : 1/ mettre en évidence des différences métaboliques entre les différents groupes. 2/ identifier les métabolites qui permettent de discriminer les échantillons. Matériels et méthodes : nous avons réalisé l'extraction de métabolites en broyant 300 mg de fèces dans 1,2 mL de tampon phosphate (D2O, pH=7,4). On ajoute au surnageant du TSP afin de permettre la quantification des métabolites par résonance magnétique nucléaire (RMN) du proton 1H. Les matrices de données obtenues à partir des spectres RMN sont ensuite utilisées pour des analyses multivariées (ACP, PLS-DA et OPLS-DA) sur R grâce au package ropls. Résultats : les analyses multivariées ont permis de mettre en évidence des différences métaboliques entre les trois groupes. Les métabolites discriminants ont été identifiés en utilisant la base de données HMDB.

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Poster 60 - P60

Miniaturization of a metabolomics and fluxomics sample preparation workflow.

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One of the current challenges in metabolomics and fluxomics is to gain access to the cellular heterogeneity to have access to subpopulations of cells or even of a single cell metabolism. In its second component, the national metabolomics and fluxomics infrastructure MetaboHUB is developing a work package with the objective of being able to perform single cell experiments in metabolomics and fluxomics. Unlike other omics like transcriptomics, Singlecell metabolomics is still in its infancy and only very few attempts of single cell fluxomics have been reported so far. In this poster, focus will be done on the developments realized for metabolomics and fluxomic sample preparation step. Indeed, methods conventionally used involve working with several hundred microliters or even several milliliters. An intermediate step of this workpackage is to be able to work on small volumes of samples in order to analyze matrices for which we have few amounts available or to concentrate classical samples and have access to minority metabolites. For this purpose, we adapted our current robotic systems in order to allow precision work with smaller volumes. The developments made allow us today to aspirate and dispense 2 μL with a CV less than 10%. In a second hand, developments of new robotic system protocols were done in order to prepare miniaturized NMR samples. With the use of new capillary needles, we can automatically filled NMR tubes of 1.7 and 1mm inner diameters. This opens up new perspectives, notably by reducing the volume of samples required for NMR analysis.

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Poster 61 - P61

Deciphering chemical interactions within the holobiont of the brown alga Ascophyllum nodosum

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Among the algal products developed to alleviate abiotic and biotic stresses in plants, many contain extracts of brown algae, and *Ascophyllum nodosum* is one of the main species exploited. However, the chemical nature of the bioactive compounds present in *A. nodosum* extracts has not been full characterised, even with regard to secondary metabolites. According to the recent literature, seaweeds live in close relationship with endophytic and epiphytic microorganisms, forming with their host algae a functional dynamic entity called an holobiont. Within this holobiont, the microorganisms and their host produce secondary metabolites that are often involved in the health and stability of this entity, raising questions about the biosynthetic origin of the compounds found in *A. nodosum* extracts.
Thus, in order to understand the entire metabolome of *A. nodosum* extracts, we isolated and characterised the cultivable microbiota associated with the brown alga and developed chemical dereplication based on molecular networking from LC-MS and LC-MS/MS data. This work is also part of the ANR SEABIOZ project.

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Poster 62 - P62

A metabolomic investigation of the effects of corrosion products of galvanic anodes on the metabolome of Crassostrea Gigas

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Cathodic protection is widely used to protect metal structures from corrosion in marine applications. It is commonly applied on marine metal structures using galvanic anodes. Zinc has been a common material for them but nowadays Al-Zn-In alloys are considered to offer the best performance in seawater.
The protection of seaports structures may require the use of hundreds of tons of anodes, which are expected to corrode instead of the metal constituting the structure to be protected. This process leads to the formation of dissolved species, Zn2+ and Al3+, and solid phases like Al(OH)3, which are released in water. It is necessary to assess the environmental impact of galvanic anodes. Various studies were conducted and concluded that further investigations are needed to analyze in controlled conditions other potential effects of anodes on marine organisms and to compare Zn and Al-Zn-In anodes.
We therefore propose an experimental approach to evaluate on oysters stabulated in tanks, the comparative effects of Zn and Al-Zn-In anodes, operating under similar conditions to those used in the ports of La Rochelle. To study the effects of these anodes on the entire oyster metabolome with a high sensitivity, without any *a priori*, we chose a non-targeted metabolomic approach using ultra-high performance liquid chromatography coupled to high resolution mass spectrometry.
We identified 16 and 2 metabolites modulated by Zn- and Al-Zn-In-anodes exposure respectively, which are involved in many biological functions, such as energy metabolism, osmoregulation, oxidative stress, lipid, nucleotide and nucleoside, amino acid metabolisms and defense and signaling pathways.

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Poster 63 - P63

Comparaison de profils métaboliques de mutants dans la voie de Biosynthèse des flavonoïdes par métabolomique non ciblée LC-QTOF.

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L'agriculture a besoin de nouvelles variétés plus productives en huile ou en protéines. Dans ce contexte, une bibliothèque de mutants d'*Arabidopsis thaliana* a été criblée afin d'identifier des mutants affectés dans la teneur en huile/protéines de la graine. Le mutant *HEM115* présentant une teneur en huile réduite par rapport au sauvage, possède une mutation dans le gène *TT7*, connu pour être impliqué dans la biosynthèse des flavonoïdes. Ces composés présents dans le tégument et l'embryon de la graine, assurent de nombreux rôles dont la viabilité des semences.

Pour comprendre le lien entre teneur en huile et composition en flavonoïdes nous avons choisi d'étudier trois allèles du gène *TT7* et d'autres mutants de cette voie (*tt4, tt3* et *fls1*). Des analyses proche infrarouge confirment que tous les mutants *tt7* présentent une réduction de teneur en huile alors que les autres mutants présentent une teneur supérieure ou similaire au sauvage. De plus, des analyses préliminaires montrent que *tt7* accumule plus de kaempférol (dérivés di/tri-glycosylés), mais pas de quercétine et que le mutant *tt4*, dépourvu de quercétine et kaempférol n'a pas de défaut de teneur en huile. Une analyse métabolomique LC-QTof des différents mutants et doubles mutants nous permet d'avoir une vue d'ensemble sur 940 features métaboliques, nous aidant à comprendre si la teneur en kaempférol est responsable de la diminution en huile chez *tt7*.

Cette analyse métabolomique montre aussi sa force dans la mise en évidence d'une erreur de génotypage sur une plante par un simple test statistique de clusterization.

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Poster 64 - P64

The CHaMP web-app: an interactive tool to infer biotransformation pathways from your MS peaks

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The investigation of exogenous compounds' fate within an organism has significant outreach, encompassing fields such as drug design (biotransformation, pharmacokinetics), hazards identification (toxicology) and the development of bioremediation strategies. One effective strategy to study compound biotransformation is to use metabolically competent systems (like human liver sub-cellular fractions with cofactors) incubated with a mix of both the native and deuterated studied molecule (Twin-Peaks approach). Metabolites produced by such systems can be easily detected using non-targeted high-resolution mass spectrometry based on the diagnostic native/deuterated mass difference. Pending the development of adequate tools, the resulting large datasets could be automatically processed to support elucidation of the metabolic pathways undergone by the molecule.

We introduce CHaMP, a tool for automated search of putative biotransformation reactions from MS data. CHaMP utilizes 122 generic metabolic transformations, with expected mass shifts, to identify potential metabolization pathways. Complementary to existing tools, CHaMP integrates an interactive network visualization and offers the opportunity to use expert knowledge to manually add non-generic biotransformations. The CHaMP interface relies on RShiny and can be freely accessed online without requiring any registration or installation nor programming skill (source code available under open license). On the server side, the mass difference network is handled using the MetNet and igraph R packages. CHaMP was evaluated using acetaminophen, which metabolic pathways are well described, and proved to be an efficient tool to retrieve the metabolic pathways from generated MS data. CHaMP is now applied to organic pollutants whose metabolic pathways are yet partly or entirely unknown.

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Poster 65 - P65

Chemical interactions between fungal endophytes isolated from Bixa orellana L. (Bixaceae)

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Fungal endophytes chemical study has increased this last decade, since many secondary metabolites have been isolated from this hidden source (1). As they live within a real ecosystem, constantly interacting either with their host-plant and its microbiome, they may represent a source of bioactive metabolites. An equilibrium between the partners of this ecosystem is established through chemical communication, including the biosynthesis of specialized metabolites. An endophytic strain of Cophinforma mamane isolated from Bixa orellana L. (Peru) leaves has been studied in our lab for its production of secondary metabolites, especially the thiodiketopiperazines botryosulfuranols A-C (2). As conventional cultures in laboratory limit the huge potential of novelty of these microorganisms, different methods including epigenetics, "One-Strain Many Compounds" approach and co-cultures have been carried out to modulate its metabolome with two goals: to search for new compounds and to increase the production of botryosulfuranols (3-5). Here, C. mamane was co-cultured with nine endophytic fungal strains also isolated from B. orellana L.. As they live naturally in the same ecological habitat, they will likely compete for similar nutrient resources, resulting to interactions that may activate biosynthetic gene clusters encoding for metabolites to overcome those interactions. Phenotypic study showed interaction zones, including pigmentation and inhibition zone. Extracts of axenic strains and co-cultures were analyzed by LC-HRMS/MS. Comparison of metabolomic profiles and statistical analyses are currently performed using MZMine and MetaboAnalyst with a focus on botryosulfuranol production and detection of new compounds unique to co-cultures that may correspond to molecules involved in fungal communication.

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Poster 66 - P66

How metabolomics is used to support the MetaPath solution through metabolic profiling of cheese fermentation industry ecosystem models?

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Consumer expectations and societal evolutions are at the heart of the concerns of fermentation professionals. The organoleptic properties of their products are closely correlated to the manufacturing process but also to its composition specifically the microbial ecosystems introduced to produce this fermentation. In this context, the Bpifrance MetaPath project aims to reconstruct the metabolic maps of these microbial ecosystems in order to guide the industrialist in the selection of microbial strains at the time of product design. As a partner, the MetaToul platform aims to perform metabolic profiling of ecosystems in the cheese model of industrial partner Bel. The sample preparation and analysis methods developed on the MetaToul platform on this complex matrix allow a complete and precise study of the central and energetic metabolism (~80 metabolites) of these ecosystem models. It was validated with the addition of IDMS on repeatability parameters, extraction yield and matrix effect. The samples produced for metabolomics are analyzed on the following analytical systems: ion chromatography coupled to an LTQ-Orbitrap-Velos (Thermo) for the analysis of central energetic metabolism, liquid chromatography coupled to a QExactive+ (Thermo) for the analysis of amino acids, coenzymes A and the mevalonate pathway. The metabolic profiling data produced on the different ecosystem models of the partner will be integrated with others omics data into the MetaPath solution by Abolis/ Microbiome Studio partner in order to reconstruct active metabolic maps, true identity cards of the metabolic activity of microbial ecosystems in the fermentation of cheese matrix.

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Poster 67 - P67

How metabolomics is used to support the MetaPath solution through metabolic profiling of sourdough fermentation industry ecosystem models?

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Consumer expectations and societal evolutions are at the heart of the concerns of fermentation professionals. The organoleptic properties of their products are closely correlated to its manufacturing process but also to its composition specifically the microbial ecosystems introduced to produce this fermentation. In this context, the Bpifrance MetaPath project aims to reconstruct the metabolic maps of these microbial ecosystems in order to guide the industrialist in the selection of microbial strains at the time of product design. As a partner, the MetaToul platform aims to perform metabolic profiling of ecosystems in the sourdough model of industrial partner Lesaffre. The sample preparation and analysis methods developed on the MetaToul platform on this complex matrix allow a complete and precise study of the central and energetic metabolism (~80 metabolites) of these ecosystem models. It was validated with the addition of IDMS on repeatability parameters, extraction yield and matrix effect. The samples produced for metabolomics are analyzed on the following analytical systems: ion chromatography coupled to an LTQ-Orbitrap-Velos (Thermo) for the analysis of central energetic metabolism, liquid chromatography coupled to a QExactive+ (Thermo) for the analysis of amino acids, coenzymes A and the mevalonate pathway. The metabolic profiling data produced on the different ecosystem models of the partner will be integrated with others omics data into the MetaPath solution by Abolis/ Microbiome Studio partner in order to reconstruct active metabolic maps, true identity cards of the metabolic activity of microbial ecosystems in the fermentation of sourdough matrix.

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Poster 68 - P68

Methodological study of raw MS data pretreatment for chemical forensics

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The attribution of a chemical (or a mixture) to its source (i.e. synthesis path, production batch etc.) is the aim of the developing field called "Chemical forensics". It is of special interest in order to support the attribution of a proved chemical weapons use. Data analysis workflows coming from metabolomics were shown to have a high potential to address this issue. Converting raw data from GC-MS instrument to a peak table that can be handled using statistical and data analysis methods is the first step in the data analysis workflow and a key point to achieve chemical forensic analyses.

This work consists in a methodological study in order to assess the behavior of raw MS data pretreatment tools commonly used in the metabolomics field (namely XCMS and MZmine) on several GC-MS data sets on a specific nerve agent precursor (methylphosphonic dichloride) and standard solutions. The following variability factors are studied:

- Analysis laboratory (single vs. multi-site);
- Matrix effects (standard solution vs. complex matrix);
- Injection replicates and variable selection rules based on them;
- Sample concentration.

XCMS and MZmine were compared based on both known tracers and full peaks lists. Results show similar behavior of both tools, with similar detection rates and measured area being reported. Overall, peak integration quality is very good, even though matrix complexity and sample concentration do have a significant effect on results.

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Poster 69 - P69

A Sample-centric, Sustainable and Knowledge-driven Natural Products Drug Discovery Framework

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Ultra-high performance liquid chromatography (UHPLC) high-resolution tandem mass spectrometry (HRMS/MS) profiling is the workhorse of the natural products community for extracts profiling. Thanks to modern processing and annotation strategies, large amounts of spectral and structural data can be obtained for a single extract. At the scale of a laboratory, where hundreds to thousands of extracts are profiled each year, this represents an untapped source of new discoveries. Indeed, most processing workflows imply a retention time (RT)-based feature alignment step that hinders the comparison of samples from different batches, or projects, and result in hermetic silos of data and knowledge. To enhance the potential of discovery based on data reuse, we propose a shift from a dataset-centric view (RT-aligned dataset), to a sample-centric one. For this, we implemented a Python-based workflow articulated around two cores: analytics and integration. First, LC-HRMS/MS raw data are processed to extract features with their MS1 peak area and associated fragmentation spectra. These spectra are then automatically organized - using molecular networks - and structurally annotated - using Sirius and in silico spectral database matching coupled to taxonomic reweighting - for each individual extract (analytics core). The experimental data (spectra), and their annotation (molecular structures, biological activities) are then reconciled to external sources (GNPS, ChEMBL, Wikidata) and integrated into a knowledge-graph (integration core) accessible through SPARQL queries. This framework was applied for the exploration of 1,600 plant extracts and allowed the rapid identification of new antiparasitic compounds.

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Poster 70 - P70

Hyperpolarized 13C NMR of Biofluid Samples at Natural Abundance by Dissolution Dynamic Nuclear Polarization

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NMR-based metabolomics provides important information on biological complex mixtures but mostly relies on 1D 1H experiments for sensitivity reasons. However, strong peak overlap is a limitation for the analysis of inherently complex biological mixtures. To overcome this limitation, 13C NMR benefits from a wider spectral dispersion and narrower signal linewidth but is barely used in metabolomics due to its lower sensitivity. Dissolution Dynamic Nuclear Polarization (d-DNP) offers an opportunity to improve significantly the sensitivity of 13C NMR while retaining spectral resolution advantages for metabolomics studies with natural-abundance samples. Preliminary studies demonstrated the potential of this approach for the study of plant and cell extracts. Here, we report the suitability of 13C d-DNP to provide rich information on a biofluid (urine) in conditions which are compatible with metabolomics studies. The spectral fingerprint and is extremely rich with numerous signals that would not be observable with several hours of conventional 13C acquisition at high field. Hyperpolarized 13C NMR benefits from a wider spectra dispersion (~200 ppm) and narrow singlets while 1H NMR multiplets are highly crowded even at high field. Solving such overlap issue is crucial in metabolomics, since the biologically relevant variations of key metabolites may be obscured by surrounding, less informative peaks, making biomarker discovery and quantification difficult. Furthermore, we show that NMR quantitative capabilities can be restored with these hyperpolarization experiments, enabling the precise measurement of absolute concentrations of metabolites that are challenging to quantify in 1H NMR.

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Poster 71 - P71

Untargeted NMR-based analysis of kidney allograft perfusates identifies a metabolomics signature of delayed graft function

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Kidney transplantation implies an ischemia/reperfusion (I/R) process, which impact on allograft outcomes. The use of a cold preservation solution helps to minimize I/R severity by decreasing metabolism in order to prevent delayed graft function (DGF). DGF conditions long-term graft outcomes, and the lack of linked predictive biomarkers hampers the early management of at-risk patients.

For this study, the cold preservation solution outgoing of the graft just before transplant was collected and called ”perfusate”. Through an NMR-based metabolomics approach, we aim to analyze the perfusate in way to better investigate biomarkers describing DGF profile.

A cohort of 53 perfusates was collected at the time of kidney graft, right before transplantation. Patients were categorized on the basis of (1) donor type (dead brain donors DBD/dead circulatory donor DCD) and (2) DGF occurrence. Moreover, a murine cohort of 39 perfusates was collected and classified on the basis of donor type (DBD/DCD). After sample preparation and NMR analysis, univariate and multivariate statistical analyses were performed.

Analysis on murine cohort firstly highlighted the importance to separate DBD from DCD donors since their perfusate metabolomes differ considerably, as also confirmed in the human model. Secondly, the occurrence of DGF was associated with a metabolic signature, in contrast to the one of no-DGF allografts.

In conclusion, this study shows that metabolomics could describe the DGF status through a panel of predictive biomarkers; moreover, a deeper understanding of metabolites related to donor type could improve our knowledge regarding the biochemical events linked to hypothermic storage of kidneys.

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Poster 72 - P72

NMR metabolomics multi-tissue study of the chronic low-dose exposure to a cocktail of Persistent Organic Pollutants

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Exposure to endocrine disrupting chemicals (EDCs) including persistent organic pollutants (POPs) represents one of the most critical public health threats nowadays. POPs interfere with the body's endocrine system and have been associated with a diverse array of health issues.

This innovative untargeted metabolomics study aims looking into the low-dose and chronic internal exposure to a cocktail of POPs, on multiple tissues known to accumulate these lipophilic compounds (1).

A group of donor mice was injected (2) with a cocktail of 12 POPs at different doses (0x, 5x and 15x the LOAEL*). Their adipose tissues (AT) were grafted into a group of receptor mice, from which biopsies of liver, brain, epididymal and retroperitoneal AT were obtained after different exposure times (3 and 21 days). Hydrophilic metabolites were extracted and analysed by NMR.

Interestingly, the metabolic response differs among the selected tissues in mice. In liver, we observed a dynamic effect according to the exposure time and POPs doses. In brain, the presence of POPs gave immediately a saturated effect which was independent of the dose and exposure time studied. In the opposite, for both AT nearly no effect was observed.

This metabolic profiling leads to a holistic and dynamic vision on the main metabolic pathways impacted in lipophilic tissues by a cocktail of POPs, extending our knowledge of what could be reproduced in exposed human population (3).

* LOAEL: Lowest Observed Adverse Effect Level

1. Mu¨llerov´ a, Physiol Res. (2007)
2. Joffin, Environ Int. (2018)
3. Lucas-Torres, NMR in biomedicine (submitted)

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Poster 73 - P73

Establishment of a NMR-based metabolomics protocol for salivary samples

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In contrast to the most used biofluids such as blood and urine, saliva is rarely studied in metabolomics. However, this biofluid seems to be of great interest because of its non-invasive, simple and fast collection. Moreover, it is perfectly suited for self-sampling, well-adapted to a personalized approach of medicine and is expected to be complementary with other biofluids information. This is why we aim to develop and optimize a protocol to analyse salivary samples by NMR.

Therefore, this work focused on the identification of the best suitable protocol allowing to obtain the most pertinent and huge metabolomics information. For this purpose, four methods were selected according to the literature and to in-house processes and were compared. On the basis of several analytical criteria such as the number and the concentration of identified metabolites, the repeatability and the robustness, the best method was selected. Freezedrying step followed by ultrafiltration led to the most informative and repeatable protocol. Finally, this optimized workflow was applied to a real-case study (fasting *vs* non-fasting volunteers) in order to prove the pertinence of the method and of the saliva's analysis in metabolomics. This study showed significant differences between the groups and discriminant metabolites were identified.

In conclusion, results obtained in this work are encouraging and highlight the real interest of using saliva samples in metabolomics. In the future, it will be interesting to apply the developed protocol to metabolomics studies and to combine it with the usual blood and urine's metabolomics analyses.

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Poster 74 - P74

Développement d'une méthode d'extraction et d'analyse métabolomique pour l'étude de l'impact de la mycorhization de la vigne sur son métabolisme primaire et ses réponses de défense

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La vigne doit faire face à différents stress biotiques et abiotiques entraînants, dans un contexte de réchauffement climatique, l'apparition de plus en plus fréquente de maladies de dépérissements, avec des effets grandissants allant de la baisse des rendements et de la qualité des raisins jusqu'`a la mort des ceps. L'utilisation des pesticides est progressivement remplacée par des moyens plus respectueux de l'Homme et de l'environnement tels que les microorganismes bénéfiques. Nous nous intéressons ainsi à la symbiose avec des champignons mycorhiziens à arbuscules, aidant à la nutrition et à la tolérance à divers stress, dont le stress hydrique, et nous cherchons également à montrer que la mycorhization peut être un moyen de lutte biologique face aux agresseurs.

Afin d'étudier l'effet de la mycorhization sur la vigne, une expérimentation a été menée sur des plants de Gewurztraminer de vigne préalablement mycorhizés par *Rhizophagus irregularis*. Nous avons réalisé des mesures agronomiques ainsi que des études transcriptomique et métabolomique comparatives sur des feuilles et des racines de plants non mycorhizés.

Une méthode d'extraction innovante des métabolites par extractions successives en polarité inversée a été mise en place ainsi que les méthodes analytiques non ciblées adaptées utilisant la GC-MS et la LC-MS/MS pour couvrir largement le métabolome.

Nous avons ainsi pu mettre en évidence un impact significatif de la mycorhization sur le métabolisme foliaire et racinaire de la vigne, en particulier le métabolisme primaire ainsi que sur les voies de signalisation et les réponses de défense de la vigne (Goddard et al., 2021).

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Poster 75 - P75

Quantification of bacterial lipids produced by the gut microbiota: role in visceral pain.

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Irritable bowel syndrome (IBS) is characterised by chronic abdominal pain associated with transit changes. In order to understand the role of the gut microbiota in IBS, the objective of this study was to quantify bioactive bacterial lipids that may be involved in IBS symptoms.

A wide range of quantification of microbiota metabolites was developed on feces by different chromatographic techniques. This made it possible to quantify, from the same stool homogenate, short-chain fatty acids by gas chromatography coupled to a flame ionisation detector (Trace1300, Thermo), lipoamines, GABA-lipopeptides by liquid chromatographymass spectrometer (HPLC-QqQ) (LC/MS8060, Shimadzu) and bile acids by HPLC-QqQ (G6460, Agilent) and HPLC-Q-Orbitrap (Exploris240, Thermo). These metabolites were analysed in the stools of 18 healthy volunteers and 42 IBS patients and correlated with different clinical parameters.

The stools of IBS patients showed a decrease in the concentration of alloLCA, C16LeuGABA and C12AsnGABA, and an increase in the concentration of 3beta-CA. The faecal concentrations of the other quantified lipids are not different between IBS and healthy volunteers. The fecal concentration of 3beta-CA was positively correlated and that of C16LeuGABA inversely correlated with the clinical scores of abdominal pain and severity of pathology.

This study allowed us to validate the quantification of different lipid families in human stool and to identify bacterial lipids that may be involved in the pathophysiology of IBS. To confirm these initial results, a new cohort is being recruited. These very encouraging results make it possible to envisage therapeutic avenues for these patients in the long term.

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Poster 76 - P76

Using spectral matching for evaluating the performance of LC-MS metabolomics methods

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Introduction

Untargeted metabolomics are used for biomarker discovery and hypothesis generation, but its potential is constrained by the number of detected metabolites, which is reliant on the experimental protocols (i.e. sample extraction and LC-MS methods) employed.1 Designing new experimental metabolomics protocols is challenging because these are defined according to the response of a moderate number of metabolites that may not accurately reflect the characteristics of the biological samples being examined.2

Technological and methodological innovation

A new workflow is proposed to assess the performance of metabolomics methods using a nontargeted approach based on evaluating the chromatographic peaks annotated by spectral matching3 against public mass spectral reference databases. Since this approach does not require the use of chemical standards, the bias towards these metabolites when evaluating the methods disappears. The most important steps in this workflow is the data-driven harmonization of the metabolite annotations, which facilitates comparing the performance obtained across different metabolomics methods. This workflow was validated with the reference human plasma sample SRM 1950 with more than 20 of most used LC-MS methods in the metabolomics community.

Results and impact

The chemical space of each evaluated method was characterized in terms of metabolite class coverage, reproducibility, linearity and signal suppression effects.

The workflow presented is useful for designing and optimizing high-throughput metabolomics protocols.

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Poster 77 - P77

Chemical exposure highlighted in an epidemiological study by metabolomic FT-ICR-MS fingerprinting at high throughput and high resolution and without any prior knowledge

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Cohort studies aim to assess associations between exposure to different risk factors such as chemicals and health outcomes. In our study, chemical exposure markers were detected as the main variables strongly influencing two components in a PCA exploration of the metabolomic data generated from urinary samples collected on a cohort of more than 500 individuals using by Direct Introduction coupled with a Fourier Transform Ion Cyclotron Resonance (FT-ICR) instrument. The assignment of their identity was first achieved based on their fine structures detected at very high resolution (Rp > 900,000). Their identification as dimethylbiguanide and sotalol was achieved at level 1 thanks to commercially available standards, MS/MS experiments and CCS (Collision Cross Section) measurements. Cohort metadata provided in a second step confirmed that all the subjects discriminated by PCA had declared to be prescribed these drugs for either type-II diabetes or cardiac arrhythmia. Their concentrations were also estimated by a rapid quantification using direct introduction and MS/MS fragmentation. A regression analysis showed a good correlation between these estimated concentrations and scores of individuals significantly distributed on these specific PCs. The detection of these chemical exposure markers proved the potential of our highthroughput approach without any *a priori* as a powerful emerging tool for rapid and largescale phenotyping of subjects enrolled in epidemiological studies to rapidly characterize the chemical exposome and the adherence to medicine prescription.

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Poster 78 - P78

Development of a high-throughput and high ion mobility resolution approach using automatic stitching of multiple ion mobility ranges on a TIMS-ToF instrument: application to chiral analysis

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The characterization and identification of metabolites of interest in biological matrices represent a real analytical challenge. Hyphenated methods such as liquid chromatography coupled with mass spectrometry (LC/MS) are often used to separate and identify organic compounds. However, the characterization of isomers remains difficult without adequate prior separation, even using high-resolution mass spectrometry (HRMS). Based on gas-phase mobility, ion mobility (IM) separation is not limited by solvent or stationary-phase constraints and its coupling with mass spectrometry (IM-MS) offers an additional separation dimension without lengthening the MS acquisition time. Despite its well-known ability to separate isomers, high resolution conditions are essential to distinguish very close isomeric ion mobilities.

In this work, a high-throughput and high ion mobility resolution approach was developed to allow exhaustive IM-MS measurements of as many species as possible on a trapped ion mobility spectrometry time-of-flight (TIMS-ToF) instrument. The method consists of performing single ion mobility monitoring (SIM2) at high mobility resolving power and serial acquisition at different ion mobility ranges. By stitching multiple ion mobility ranges, the IM-MS measurements can be achieved on the entire ion mobility range.

This approach was successively applied to the rapid chiral analysis of amino acids allowing characterization of almost all D- and L- amino acids using direct introduction and noncovalent CuII complexes with phenylalanine and proline as chiral references. The potential of SIM2 stitching for exhaustive ion mobility measurements at high resolving power was investigated here for the first time.

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Poster 79 – P79

New microfluidics platform at Bordeaux Metabolome facility

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Bordeaux Metabolome facility provides technological and methodological expertise for studying metabolome, lipidome and metabolic fluxes of plant or plant-derived products. The facility also participates in MetaboHUB National Infrastructure. Within the framework of MetaboHUB2.0 Workpackage 2 (Scaling down: towards single cell), our main objective is to miniaturise the sample preparation protocols in order to be able to work accurately with low volumes and use new techniques for separating or concentrating the cells.

In this view, *Bordeaux Metabolome* has invested in microfluidic devices. It is thus now possible to carry out the different steps, from creating microfluidic chips by conventional or 3D printing methods to performing microfluidic experiments. The versatility of the tools allows us to adapt to many domain of applications in plant sciences.

As a first step, two main biological questions are investigated. Firstly, we want to know how enzymes are finely regulated in order to improve the understanding of metabolic pathways. For the characterization of enzymes, biochemical measurement methods are adapted to microfluidics. Furthermore, with the encapsulation of enzymes and cofactors, it could be possible to test many conditions simultaneously. Secondly, we are questioning the metabolome of the different subcellular compartments. As such, microfluidics could help provide a solution by sorting the subcellular particles before mass spectrometry analysis, for instance. *Bordeaux Metabolome, with its new platform of microfluidic facilities dedicated to analytical biochemistry and enzymology, aims to be at the forefront of progress and thus obtain essential data for systems biology in plant sciences.*

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Poster 80 - P80

Detailed characterization of the responses and adaptation of Coelastrella sp. PCV to heavy metal-induced stress

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Pollution of terrestrial and aquatic ecosystems by heavy metals is a major and growing threat to the environment and human health. Uranium, a weakly radioactive heavy metal is very present in the environment. Its contribution to heavy metal pollution is significant. The anthropogenic origins increasing uranium concentrations in aquatic environments are diverse, and its presence constitutes a definite risk for biocenosis.

A better understanding of Uranium on terrestrial plants and microalgae is essential to develop phyto- and phycoremediation approaches. These organisms have developed sophisticated molecular mechanisms to cope with toxic elements. Deciphering these strategies is essential for understanding how plants/algae behave in scenarios of metal contamination of their environment, thus providing key information for new biotechnological applications for metal capture.

Previously, the LPCV laboratory at CEA Grenoble isolated a photosynthetic unicellular microalga, of the genus Coelastrella, that was hyper tolerant to metals in a uranium-contaminated environment. Because of its ability to grow in culture media contaminated with high concentrations of uranium, it is assumed that this alga has developed molecular mechanisms to survive in polluted environments. In this framework (ANR-21-CE34-0004 DemoniaCo), we will characterize the responses of Coelastrella to uranium-induced stresses using targeted (biochemical phenotyping, Redox profiling) and untargeted (LCMS by Orbitrap) metabolomics. Preliminary results from method development for quantifying metabolites are encouraging. Further analyses with samples treated with uranium are underway. These metabolomic data will be combined with growth rate measurements to estimate metabolic fluxes by constraintbased modelling in order to pinpoint the reactions specifically involved in uranium assimilation.

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Poster 81 - P81

Characterising the metabolomes of European soils to understand the variability in Carbonyl Sulphide (COS) fluxes

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Carbonyl Sulphide (COS) is the most abundant sulphur trace gas in the atmosphere. COS carries independent information of photosynthetic activity as it is irreversibly hydrolysed in plants cells by the Carbonic Anhydrase (CA). This enzyme also controls the reversible hydration between CO2 and HCO3-. As such, terrestrial ecosystems are commonly considered COS sinks and could be used to constrain both the COS and CO2 global mass budgets. However, some plants have been observed to emit COS and both oxic and anoxic soils can be sources of COS to the atmosphere.

This current project presents data from soils investigated as part of the ERC SOLCA project that aimed to develop mechanistic understanding and models to predict the variability of the two components of COS net fluxes from different soil types and explore the influence of the chemical and textural differences of the soil substrates.

Previously, *Kaisermann et al. (2018)* disentangled the rates of COS uptake and COS production from COS net flux measurements. They also investigated the response of soil COS fluxes to the addition of inorganic Nitrogen. Their latest results demonstrated that N addition reduced COS uptake rates and increased COS production rates leading to an overall trend of weakening the net COS sink strength of soils.

We will use LCMS-based untargeted metabolomics to characterise the events behind the consumption or production of COS through metabolic markers and identify the metabolites involved in altering the ratio of COS consumption to production in soils naturally and when exposed to N amendments.

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Poster 82 - P82

Approches métabolomiques appliquées à l'analyse génétique de la composition du bois de vigne (Vitis vinifera)

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Le bois de vigne contient de nombreux polyphénols dont certains possèdent des propriétés antimicrobiennes intéressantes. Si les déterminants génétiques de la composition des raisins ont fait l'objet de nombreuses études, peu de travaux ont analysé les bases génétiques de la composition du bois de vigne. Dans ce travail, nous avons utilisé des approches métabolomiques ciblées et non ciblées pour effectuer une analyse globale des loci de traits quantitatifs métaboliques (mQTLs) en utilisant une descendance issue d'un croisement entre deux cépages emblématiques, le Riesling (Ri) et le Gewurztraminer (Gw). L'analyse d'extraits de sarments et de ceps par chromatographie liquide couplée à la spectrométrie de masse à haute résolution a révélé une variabilité génétique dans la descendance Ri x Gw pour les teneurs en différents polyphénols. Des mQTLs significatifs pour des métabolites appartenant aux familles des stilbènes et des flavonoïdes ont été détectés grâce à des cartes génétiques à haute densité basées sur le polymorphisme nucléotidique (SNP). L'analyse bioinformatique détaillée des régions génomiques liées aux mQTLs a permis l'identification de gènes candidats dont l'impact potentiel sur la composition du bois sera discuté. La caractérisation des déterminants de la composition du bois pourra fournir les bases d'une meilleure compréhension de la sensibilité aux maladies du bois de la vigne qui frappent durement la viticulture.

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Poster 83 - P83

Breath metabolomics for the discovery of novel therapeutic biomarkers in cystic fibrosis.

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Background

Since 2020, 90% people suffering from cystic fibrosis (CF) can benefit from a new tritherapy called Kaftrio®, which drastically improves respiratory function and quality of life. Yet, side effects are poorly characterised and novel biomarkers are needed to evaluate therapeutic response in children at mild stages of the disease. Our objective was to evaluate the short-term impact of the treatment on exhaled breath using mass spectrometric (MS) profiling methods.

Methods

Ten adults and twelve children with CF were enrolled in the study and attended three clinical visits: before treatment initiation, after 1 week, after 1 month (Paris, Clinical trial IDs 2016-A00309-42 and 2021-A03119-32). Exhaled breath was either analysed in real-time by proton transfer reaction (PTR)-MS or collected on desorption tubes using a ReCIVA® device (Owlstone, Ltd) and analysed off-site by thermo-desorption and 2D-GC-MS. Compounds were tentatively identified using libraries and machine learning tools were employed to process the volatilomics breath profiles.

Results

Depending on the MS technique, hundreds to thousands of molecules were detected. Supervised longitudinal models revealed distinctive breath profiles at each visit and identified up to 50 compounds significantly modified throughout patients. Differentiating features appeared to be primarily hydrocarbons, such as branched alkanes, terpenes and phenyl compounds.

Conclusions

The metabolic origin of these volatile compounds is being investigated and could unveil novel mechanisms of action of Kaftrio®. Modifications in breath observed as soon as in the first days of treatment may serve as biomarkers for drug monitoring in patients with normal lung function such as children.

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Poster 84 - P84

Automatic search for chemical exposure markers in LC-HRMS metabolomic data

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Food is a source of chemical exposure to which humans are subjected throughout their lives. The perinatal period is of great vulnerability for child development and exposure to food chemicals at that time can have a lasting effect on health. Our work focused on the automatic search for markers of exposure to food chemicals on metabolomic datasets from a perinatal matrix of a mother-child cohort.

A list of potential contaminants was first generated from food chemicals detected in food consumed by pregnant women. We then developed an in-house bioinformatic tool to search for exposure markers to any known contaminant. It allows, from a non-exhaustive list of molecules, the prediction of their metabolites as well as their various signals that can be detected in mass spectrometry. Quality filters were also included to improve the confidence in detected signals.

The developed tool was applied to metabolomic data generated from meconium collected on the French mother-child cohort EDEN using liquid chromatography coupled to with high-resolution mass spectrometry (LC-HRMS) (Thesis of Nihel Behkti, 2021).

As a proof of concept, we successfully detected signals correlating with paracetamol (acetaminophen) exposure by the presence of the precursor molecule and its two predicted metabolites (sulfate and glucuronide conjugates). These first results show the potential of our approach for the comprehensive study of chemical exposure on LC-HRMS metabolomic data.

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Poster 85 - P85

Use of metabolomics to identify bioactive compounds from grapevine eco-extracts that can impair fungal growth and production of mycotoxins by Fusarium graminearum and to elucidate their mechanisms of action

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Fusarium Head Blight of small-grain cereals is a devastating fungal disease primarily caused by *Fusarium graminearum* in Europe that affects crops worldwide. Beyond crop losses, *F. graminearum* poses potential health risks due to the production of mycotoxins. In the context of lowering the dependency on agrochemicals, the development of environmentalfriendly strategies is of great importance to guarantee the safety of food and feed. One of these strategies lies on the promising capacity of compounds issued from natural sources to counteract phytopathogens.

In this work, the *in vitro* efficiency of 13 eco-extracts obtained from grapevine by-products was assessed against fungal growth and mycotoxin production by *F. graminearum*. To identify the compounds responsible for this bioactivity, a strategy combining untargeted metabolomics and bioguided fractionation was implemented and has led to evidence the key contribution of oligomeric stilbenes. The antifungal and antimycotoxin activity of stilbenes was then confirmed, using pure molecules (resveratrol/RES, a monomer and vitisin B/VIT B, a tetramer). The higher efficacy of VIT B compared to that of RES was demonstrated.

To deeper the knowledge on the mode of action of stilbenes, multi-omics approaches associating mRNA-seq-based transcriptomics and LC-MS/MS-based non-targeted metabolomics have been conducted.

Altogether, our data support, on the one hand, the power of metabolomics approaches to identify active molecules occurring in natural extracts and to study their mechanisms of action and, on the second hand, that grapevine by-products could be promising sustainable sources of bioactive compounds for controlling *F. graminearum* and minimizing the contamination of cereals with mycotoxins.

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Poster 86 – P86

Influence des facteurs pédoclimatiques sur la composition en métabolites du laurier du Liban

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Le laurier (*Laurus nobilis*) est un arbre présent dans tout le bassin méditerranéen. Le laurier a fait l'objet d'études approfondies sur ses composants antioxydants. Riche en 1,8- cinéole, l'huile essentielle de laurier possède de nombreuses propriétés : anti-inflammatoire, d´econgestionnante, anti-infectieuse et rééquilibrant pour le système nerveux. La plupart des lauriers existants au Liban sont marginalisés et peu investis.
Dans cette étude, nous nous sommes intéressés à l'analyse de la teneur en métabolites du laurier du Liban selon différentes conditions pédoclimatiques. La zone d'étude choisie est le sud du Liban, du fait de l'abondance de cet arbre dans cette région, la variabilité des altitudes, des sols et du climat. Les altitudes sont réparties entre 0 et 1000 m et deux types de sol caractérisent cette zone : le sol rouge (argileux) et le sol blanc (calcaire).
Nous avons travaillé sur deux types d'échantillons de cette plante : les huiles essentielles extraites des feuilles et les extraits méthanoliques des feuilles, et une étude métabolomique a été réalisée en utilisant les méthodes suivante: GC-MS, RMN et LCMS.Les analyses ont montré que les facteurs pédoclimatiques influencent la composition en métabolites du laurier.
Mots clé : Laurier, conditions pédoclimatiques, métabolites.

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Poster 87 - P87

Profilage métabolique de cultures de pomme de terre infectées par le mildiou et traitées par un agent de biocontrôle

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La pomme de terre (*Solanum tuberosum*) est l'une des cultures vivrières les plus importantes au monde ; cependant, sa production est souvent menacée par une série de maladie dont le mildiou qui est causé par *Phytophtora infestans,* un oomycete qui affecte les feuilles et les tubercules (1).
Dans un contexte de protection durable de la culture des pommes de terre, l'utilisation des substances naturelles est l'une des plus récentes stratégies utilisées comme agent de bio contrôle afin de raisonner l'utilisation des substances phytosanitaires qui sont nocives pour la santé et l'environnement (2).
La surfactine est un cyclopeptide biactif produit par différentes souches bactériennes qui a montré une résistance systémique induite dans la pomme de terre d'où son intérêt en tant qu'agent de biocontrôle (3).
Dans cette étude un profilage métabolique est effectué par RMN et LCMS pour des plantes infectées par *P.infestans* et traitées par la surfactine afin d'identifier les métabolites impactés par l'emploi de cette substance active et de comprendre le métabolisme de la résistance de la pomme de terre.
Les analyses multivariées ont mis en évidence la séparation des plantes selon le traitement par l'agent de biocontrôle et selon l'infection ; l'identification des métabolites discriminants est en cours.

Mots Clés : Pomme de terre, Mildiou, Biocontrôle, Surfactine, Métabolomique.

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Poster 88 - P88

Mise en place d'un outil analytique de Prédiction d'efficacité des alternatives aux pesticides et aux engrais minéraux en agriculture (Biostimulants et agents de Biocontrôle)

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Dans le contexte de la transition agroécologique, la recherche dans les domaines du biocontrôle et de la biostimulation sont en plein essor, afin de privilégier l'utilisation d'intrants respectueux de l'environnement pour la protection des cultures face aux stress biotiques et abiotiques. Ainsi, un certain nombre des stimulateurs de la défense des plantes (SDP) et des biostimulants, sont disponibles sur le marché pour une utilisation en agriculture. Cependant, sans essai préalable, il est impossible de pouvoir être assuré de leur efficacité, lors de leur utilisation en conditions réelles. L'Agro-K® (NUFARM) est un biostimulant, utilisé pour optimiser la floraison et la fructification des plantes et pour renforcer la résistance à la verse, chez le blé. Dans un cadre expérimental, ce produit fut également testé sur lin fibre comme biostimulant (usage non-homologué). Dans la présente étude, une analyse nonciblée des métabolites du blé et du lin, traités avec l'Agro-K®, a été réalisée en utilisant la RMN et la LC-MS. Le but était de déterminer d'éventuels biomarqueurs métaboliques, après traitement avec l'Agro-K®. Les résultats ont montré que ce traitement engendre des variations significatives, au niveau du métabolisme primaire et secondaire du blé et du lin, principalement au niveau des métabolites de la voie des phénylpropanoïdes. Ces derniers ont plusieurs fonctions dans la régulation de la différenciation cellulaire, dans la rhizogenèse et la morphogenèse des organes floraux et la maturation des fruits, mais aussi dans les réponses aux stress biotiques et abiotiques.

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Poster 89 - P89

Natural deep eutectic solvents (NaDES) : a possible link to understand seed desiccation tolerance ?

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Natural Deep eutectic solvents (NaDES) are mixtures formed between biobased hydrogen bond donor and acceptor compounds within certain molar ratios and in the presence of water. The formation of these mixtures is characterized by a very marked decrease in their melting points compared to their ingredients. These liquids mixtures are considered as an emerging class of green solvents especially for natural substances extraction. Furthermore, some DES ingredients are found in desiccation-tolerant organisms such as seeds, pollen and resurrection plants, leading to scientific curiosity about the role NaDES in water stress and desiccation resistance. Among the hypotheses suggested by scientists, NaDES could adjust the water level in cells, protect essential enzymes and even replace water in order to maintain the cellular machinery and protect certain macromolecules under osmotic stress. The objective of this work is to explore the reality of existence and functionality of eutectic mixtures in developing seeds of oilseed rape (*Brassica napus* L.) with a view to providing tangible information on their contribution to their tolerance to desiccation. We first proceeded to the analysis, through LC/GC-MS and NMR technologies, of the metabolic composition of the seed in order to identify potential ingredients of NaDES. Some new eutectic mixtures have been prepared and qualified from this natural catalog and the molecular interactions preliminarily analyzed by NMR and cold-spray ionization MS. Metabolic imaging techniques are being tested in an attempt to reveal the co-distribution of NaDES ingredients in vivo before researching the beneficial actions of these eutectic environments on cell function.

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Poster 90 - P90

Analyse rapide et quantitative du glycérolipidome des organismes photosynthétiques : présentation de la plateforme LIPANG

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Le métabolisme des lipides joue un rôle clé dans la réponse à certains stress. C'est particulièrement le cas des glycérolipides, les constituants majoritaires des membranes biologiques, composés d'une molécule de glycérol et d'acides gras. Chez les organismes photosynthétiques, on retrouve les phospholipides, présents chez tous les eucaryotes ; les glycolipides, spécifiques de tous les organismes photosynthétiques; les bétaïnes lipides, présents uniquement des algues aux bryophytes, et les lipides neutres (triacylglycérol et diacylglycérol), importants pour les réserves énergétiques des cellules. La composition en glycérolipides d'un échantillon (glycérolipidome) peut être obtenue en combinant des méthodes telles que la chromatographie en phase gazeuse couplée à la spectrométrie de masse (MS) et à un détecteur à ionisation de flamme, la trappe ionique et la chromatographie sur couche mince. L'utilisation de ces méthodes nécessite un temps d'analyse et une quantité d'échantillon importants. La chromatographie liquide (LC) couplée à la MS permet d'analyser un glycérolipidome en un temps réduit, mais les données quantitatives ne sont pas satisfaisantes du fait de la diversité chimique des espèces lipidiques et de l'absence de standards. Une méthode rapide permettant d'analyser quantitativement les glycérolipidomes de divers organismes est proposée, utilisant la LC couplée à la MS/MS. Cette méthode est utilisée au sein de la plateforme LIPANG pour un grand nombre d'organismes, en particulier des organismes photosynthétiques, afin de mieux comprendre les mécanismes régulant la composition en glycérolipides des cellules végétales, algues ou plantes, et pour des applications dans des domaines variés tels que la santé, l'alimentation, l'énergie ou la chimie verte.

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Poster 91 - P91

Uncovering Metabolic Signatures in Six Organs During a High-Fat, High-Sucrose Diet: A Cross-Sectional NMR Metabolomics Study

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Obesity is a risk factor for many diseases, such as type 2 diabetes and cardiovascular diseases. In line with the need for precision medicine, the search for biomarkers reporting the development of obesity- and diet-associated disorders is urgent. Since obesity develops progressively consequently to energy-rich diets, it also appears crucial to differentiate the biomarkers and metabolic impact of normal aging versus diet-induced obesity. We used NMR to determine the metabolic profiles of key organs (lung, liver, heart, skeletal muscles, kidneys, brain) and serum from male C57Bl/6J mice (5 weeks old) fed for 6, 10, and 14-week with a high-fat and high-sucrose diet (HFHSD) vs. standard diet (STD). This enabled us to provide tables of metabolite concentration in the organs at 3 time points and to discriminate age- and diet-related effects as well as the interaction between the two, thus highlighting the need to evaluate carefully the influence of cofounding factors over metabolic signatures.

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Poster 92 - P92

1H-NMR metabolomics for wine analysis: application to authenticity of sweet wines

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The chemical composition of wine is a key to assess in a certain way its authenticity. NMR Metabolomics provides access to the main components of wines. It is attracting increasing interest, and its use has grown consequently. This chemical fingerprint is related to biotic and abiotic factors directly linked to place and year of production, grape variety or winemaking process. 1H-NMR has already been proved to be an efficient tool to assess wine authenticity (1,2). It is a non-destructive technique, it requires a small amount of sample and a short time of analysis, yet it provides clues about several chemical families (3). The objectives of this study are: (1) to improve the methodology in a way it can be automated afterwards; and (2) to make a draft for a database which could be used in sweet wine authenticity.

The different parameters of samples' preparation and acquisition were tested and optimized. Moreover, sweet white wines from diverse French regions of production were analyzed. Performing then metabolomics analysis, data were statistically processed through supervised and unsupervised multivariate analyses.

The sample preparation has been adjusted, and automated analysis can be considered for future tests. Regarding sweet white wines, results show that they can be discriminated according to their terroir.

(1) Gougeon L. et al. (2019) *Food Chem*. 301, 125257.
(2) Le Mao I. et al. (2023) *Food Control*. 145, 109423.
(3) Le Mao I. et al. (2023) *Oeno One* 57, 15-31.

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Poster Stand

MetaboHUB (French national infrastructure in metabolomics and fluxomics for life sciences), towards next generation metabolomics and fluxomics: from population to single cells.

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Since 2013, France national infrastructure MetaboHUB (MTH) funded by the French National Research Agency (ANR) provides cutting-edge methods and tools for functional study of metabolism using metabolomics, fluxomics and bioinformatics to (i) provide high throughput quantitative analytical technologies for biochemical phenotyping of large variety of biological samples from cells to populations, (ii) identify metabolites and annotate metabolomes through implementation and maintenance of reference spectra databases, (iii) develop broadband flux measurements, (iv) provide access to high added value services to national scientific community and industrial partners, (v) attract and train a new generation of scientists and users through promotion of metabolomics in higher and continuing education.

MTH gathers 5 national leading facilities consisting of 14 NMR, 74 MS, 7 robots and more than 150 scientists with complementary skills in analytical chemistry, robotics, bioinformatics, biostatistics and biochemistry. It provides cutting edge services to academic and non-academic communities in France and worldwide. One key outcome of MTH in 2022 is the publication of PeakForest e-infrastructure, an open-source solution for FAIR reference spectra storage and sharing (Paulhe et al. 2022). A Metabolomics MOOC was freely available in 2018, and two new MOOCs on Lipidomics & Fluxomics are under construction all in collaboration with RFMF.

More globally, MTH developments revealed to be essential in order to implement multi-omics and multi-scale metabolomics approaches to move forward in our understanding of all levels of metabolism regulation.

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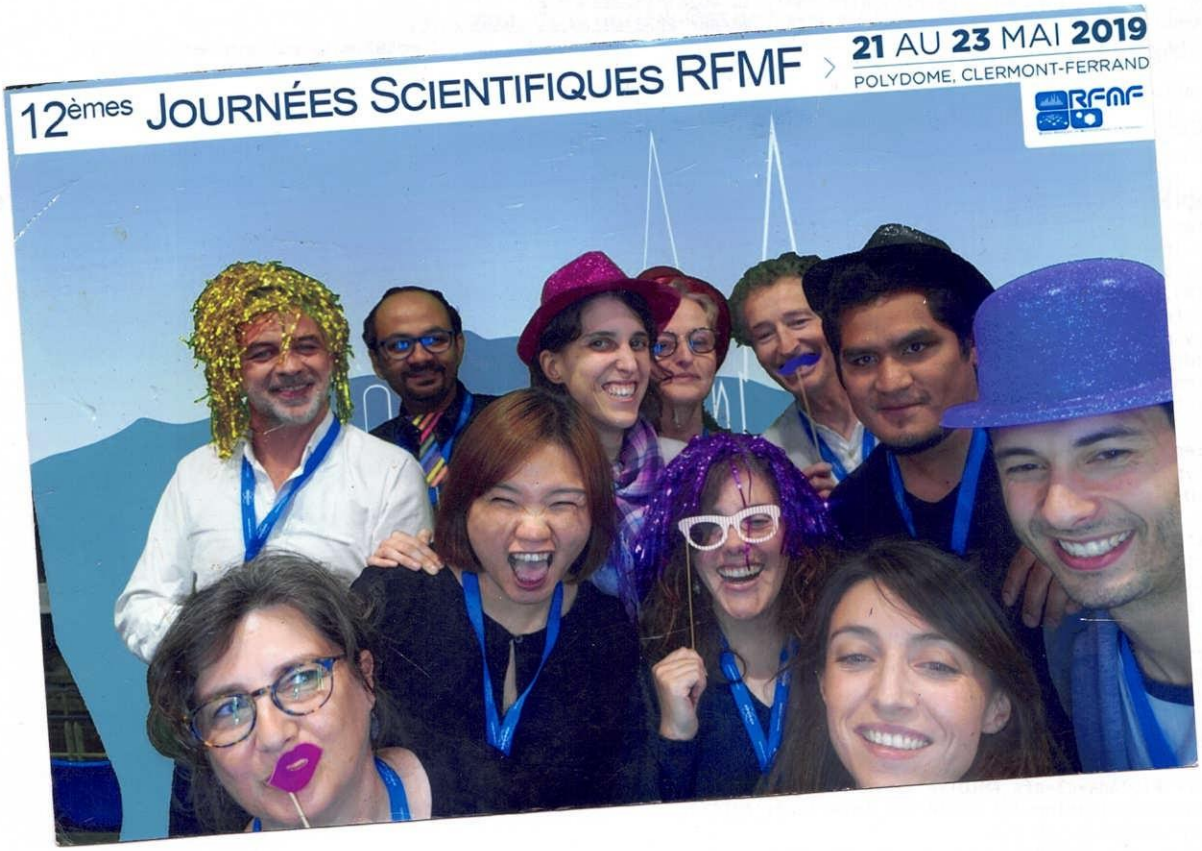
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