MS-based targeted metabolomics of eicosanoids and other oxylipins: Analytical variability and interlaboratory comparison of esterified oxylipin profile

Céline Dalle, Malwina Mainka, Jessica Dalloux-Chioccioli, Annika I Ostermann, Michael Rothe, John W. Newman, Cécile Gladine, Nils Helge Schebb

European RFMF Metabomeeting 2020

JANUARY 22 - 24, 2020

TOULOUSE, FRANCE

WITH THE PARTICIPATION OF EUROPEAN REGIONAL NETWORKS

Metabolic Profiling Forum

ORAL AND POSTER ABSTRACTS

European RFMF Metabomeeting 2020

We are very pleased to welcome you to the 2020 European RFMF-Metabomeeting conference in Toulouse. This conference gathers together two metabolomics and fluxomics networks, the RFMF (French-speaking Metabolomics and Fluxomics Network) and the MPF (Metabolic Profiling Forum) with more than 10 years of existence. MPF and RFMF share the same objectives which are to catalyze metabolomics and fluxomics research, organize conferences and promote early career scientists. Both networks also put forward networking and social aspects as strong leverage to achieve cutting edge science. For all these reasons we decided to organize this joint event.

With the growing interest in metabolomics, several regional and national networks have been created across Europe (and affiliated islands) within the last years. That is the reason why we decided to also include them in the organization of this event. They were particularly instrumental in creating a high-level scientific program and providing travel grants (a staggering 21 travel grants!).

We expect this conference to demonstrate the recent breakthroughs in metabolomics and fluxomics in a wide range of application fields. We made the choice to keep the scope of the conference as broad as possible (in terms of techniques and applications) since we think that interactions between communities is key in creating new paradigms, collaborations and scientific hypothesis.

One of our aims in this conference is to promote early career scientist participation. That is the reason why we did our best to keep registration prices as low as possible. As mentioned, networks made a strong effort to offer travel grants. Early careers were also strongly involved in the scientific organization of the conference. In fact, an early career committee was set up, led by Alison Woodward, and selected the early career presentations that you will see during the conference. They also proposed and organized workshops and social events. Finally, they will co-chair sessions with the (less early career) scientists.

Good science is generally the outcome of hard work within a friendly and supportive environment. All organisers agreed on the fact that this friendly atmosphere is of upmost importance for the organization of the conference. That is the reason why we did our best to have exciting social and networking events before and during the conference. We also aim at following RFMF mantra of “Good food Good science” with a very local and original gala dinner.

Finally, we would like to acknowledge all the academic and industrial sponsors for supporting this event and previous ones. We also thank all the people involved in the scientific and practical organization of this conference. MetaboHub-Metatoul metabolomics and fluxomics facility contribution was outstanding and our warmest thoughts go to Justine Bertrand Michel and Floriant Bellvert for their hard work and unbreakable optimism.

We wish you a fruitful scientific, human and enjoyable conference!

Jules Griffin (MPF)
Fabien Jourdan (RFMF)

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EUROPEAN REGIONAL AND NATIONAL NETWORKS AND SOCIETIES

Spanish Metabolomics Society

Committees

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Justine Bertrand Michel	RFMF	INSERM
Lorraine Brennan	Ireland	University College Dublin
Karl Burgess	Scottish Metabolomics Network	The University of Edinburgh
Marta Cascante	Spanish Metabolomics Society	University Barecelona
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EARLY CAREER SCIENTIFIC COMMITTEE

Jordi Capellades	Spanish Metabolomics Society
Sylvain Dechaumet	RFMF junior
Yoric Gagnebin	Swiss Metabolomics Society
Kate Gallagher	MPF
Natasa Giallourou	MPF
Corentine Goossens	RFMF junior
Katharina Herzog	Nordic Metabolomics Society
Justine Leenders	RFMF junior
Youzhong Liu	YoungNMC
Clément Regnault	Scottish Metabolomics Network
Sara Tortorella	Italian Metabolomics Network
Alison Woodward	MPF

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Floriant Bellvert	MetaboHub-Metatoul
Cécilia Berges	MetaboHub-Metatoul
Justine Bertrand Michel	MetaboHub-Metatoul
Maxime Chazalviel	MedDay Pharmaceuticals
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Hanna Kulyk Barbier	MetaboHub-Metatoul
Loïc Mervant	MetaboHub-Metatoul
Jean-Charles Portais	MetaboHub-Metatoul
Pablo Rodriguez Mier	INRA-Toxalim

Workshops

Workshop 1: W1

Meet the editor
(January 21st 8:30pm-10pm)

Purpose of the workshop:

Attendees will meet editors and reviewers to discuss the following points:

- Scientific inspiration versus literary perspiration: What makes a good (Metabolomics) paper?
- What you should never do in papers.
- Refereeing process: what happens once I have submitted my paper to the abyss...
- Scientific ethics: objectivity versus subjectivity

Skills acquired at the end of the workshop:

- A general understanding of the publication process.
- How to be a good reviewer and how to respond to reviewers (good and bad!)
- Some further ideas to make your paper better.

Intended audience and prerequisites:

- Public: priority to early career but can go beyond
- Prerequisites: did publish or plan to

Organisers:

Katharina Herzog, Kate Gallagher, Natasa Giallourou, Roy Goodacre and Jules Griffin.

Workshop 2: W2

Interactive tutorial, How to process LC-MS data with workflow4metabolomics.org (January 21st, 9am-12am)

Purpose of the workshop:

Metabolomics data analysis is a complex, multistep process, which is constantly evolving with the development of new analytical technologies, mathematical methods, and bioinformatics tools and databases.

The Workflow4Metabolomics(W4M) project aims at developing full LC/MS,GC/MS,FIA/MS and NMR pipelines using Galaxy framework for data analysis including preprocessing, normalization, quality control, statistical analysis and annotation steps.

https://workflow4metabolomics.org

Intended audience and prerequisites:

- Public: Researchers or students involved in metabolomics by LC-MS
- Prerequisites: basic knowledge's of the usual data processing steps for LC-MS metabolomics

Skills acquired at the end of the workshop:

- What is Galaxy?
- Comprehend the diversity of LC-MS metabolomic data analysis.
- Get familiar with the main steps constituting a metabolomic workflow for untargeted LC-MS analysis.
- Evaluate the potential of a workflow approach when dealing with LC-MS metabolomic data.

Organisers:

Franck Giacomoni, Yann Guitton and Marie Tremblay Franco

References:

https://workflow4metabolomics.org

Guitton et al (2017) Create, run, share, publish, and reference your LC–MS, FIA–MS, GC–MS, and NMR data analysis workflows with the Workflow4Metabolomics 3.0 Galaxy online

Workshop 3: W3

metaRbolomics hackathon plus
(January 21st 2020, 1pm-3:45pm.)

Purpose of the workshop:

The idea of this hackathon is to have a quiet space where active developers and advanced users can work on integrating their packages in R, adding new data to wikidata or wikipathways, mapping identifiers with BridgeDb, tailor-making MetFrag databases to answer identification questions. The hackathon will start with Jo Rainer presenting the new R packages at https://RforMassSpectrometry.org and then we will just hack away! With the new packages it will be relatively easy to implement custom "backends" to load e.g. MGF files into R, or provide access to custom, lab specific spectral libraries – lots of community contributions are possible!

Intended audience and prerequisites:

- Public: Computational metabolomics pros – active developers or advanced users

- Prerequisites: Problems to solve, questions to ask, data to integrate in open tools, ideas to discuss with active developers, desire to hack and not just talk – organisers will be happiest hidden behind laptop screens solving problems. BYO laptop(s).

Skills acquired at the end of the workshop:

Ability to integrate your R code into metaRbolomics collection, ability to add new data to wikidata/wikipathways, custom design your own high throughput non-target screening with MetFrag, map your identifiers with BridgeDb etc et

Organisers:

Johannes Rainer, Emma Schymanski, Egon Willighagen, Denise Slenter

References:

The metaRbolomics Toolbox in Bioconductor and beyond:

https://www.mdpi.com/2218-1989/9/10/200

BridgeDb tutorial: https://tess.elixir-europe.org/materials/bridgedbr-tutorial

Workshop 4: W4

How to chair sessions
(January 21st 2020, 2:45pm-4:00pm)

Purpose of the workshop:

The aim of the workshop is to provide guidelines and exchange on the role of session chairs. Robert Hall and Jennifer Kirwan will share their experience and answer questions.

Intended audience and prerequisites:

- Public: early career scientists
- Prerequisites: no specific requirements

Skills acquired at the end of the workshop:

Guidelines on how to efficiently chair a session and be part of a fruitful conference.

Organisers:

Natasa Giallourou, Robert Hall and Jennifer Kirwan

Workshop 5: W5

Advanced approaches for the analysis of metabolomics data: From experimental design to knowledge discovery
(January 21st 2020, 4pm-6pm.)

Purpose of the workshop:

The objective of this workshop is to introduce key concepts and chemometric methods (experimental strategies, data structures, multivariate models) for the analysis of multifactorial, multiblock and longitudinal metabolomic data. Several examples will be used to illustrate these different aspects.

Intended audience and prerequisites:

- Public: Researchers, engineers or students working in metabolomics and looking for information on data analysis methods and tools dedicated to complex data structures.
- Prerequisites: Basics of metabolomic data analysis.

Skills acquired at the end of the workshop:

- Overall understanding of the different experimental data structures.
- Selection of appropriate chemometric methods to extract knowledge from metabolomic data.

Organisers:

Serge Rudaz & Julien Boccard

References:

1. Harnessing the complexity of metabolomic data with chemometrics. J. Boccard, S. Rudaz. Journal of Chemometrics (2014), 28(1), 1-9.
2. Exploring Omics data from designed experiments using Analysis of Variance Multiblock Orthogonal Partial Least Squares. J. Boccard, S. Rudaz. Analytica Chimica Acta (2016), 920, 18-28.
3. Integration of metabolomic data from multiple analytical platforms: toward extensive coverage of the metabolome in Data Analysis for Omic Sciences: Methods and Applications, J. Boccard, S. Rudaz. Elsevier (2018).
4. A scoring approach for multi-platform acquisition in metabolomics. J. Pezzatti, V. González-Ruiz, S. Codesido, Y. Gagnebin, A. Joshi, D. Guillarme, J. Schappler, D. Picard, J. Boccard, S. Rudaz. Journal of Chromatography A (2019), 1592, 47-54.

Plenary and Keynote Speakers

Plenary Speaker P1:

Pr. Warwick (Rick) Dunn

Professor of Analytical and Clinical Metabolomics, School of Biosciences and Phenome Centre Birmingham, University of Birmingham, UK

Professor Warwick (Rick) Dunn is Professor of Analytical and Clinical Metabolomics, Director of Mass Spectrometry in the £8M Phenome Centre Birmingham and is Director of the Birmingham Metabolomics Training Centre at the University of Birmingham in the UK. Rick trained as an analytical chemist with a PhD focussed on placing mass spectrometers on industrial scale chemical process plants. Rick has been applying metabolomics for 20 years and now applies LC and MS platforms to the study of metabolites in mammalian biological systems though targeted and untargeted metabolomics approaches to study human health and disease across the life course. One of his research passions is increasing the efficiency and confidence of metabolite annotation in untargeted studies. His research includes the characterization of the complexity of electrospray ionization data to enhance metabolite annotation through to the optimal acquisition of MS/MS data to increase the number of metabolites annotated. His second research passion is studying how metabolites impact across time on biological processes in human health, disease and ageing in diverse areas including endocrinology, cancer, exercise/nutrition, ageing, trauma and immunological diseases.

Plenary Speaker P2:
Dr. Etienne Lefai

Université d'Auvergne, INRAE, Unité de Nutrition Humaine, Clermont-Ferrand, France.

Dr. Etienne Lefai, is a research director at the INRAE in Theix, whose work on the Scandinavian brown bear should make it possible to develop new strategies to combat muscle atrophy. Etiene Lefai studies the underlying mechanisms of brown bear hibernation which have not been understood yet. Together with a large international network of collaborator he develops new approaches combining molecular and cellular studies of bear muscle as well as human muscle cells exposed to bear serum. His recent demonstration of trans-species effects of bear serum controlling protein degradation in cultured human muscle cell holds promising potential. His exploration of winter bear serum therefore holds potential for developing new tools to fight human muscle atrophy and related metabolic disorders.

Plenary Speaker P3:
Dr. Ingela Lanekoff

Department of Chemistry - BMC, Analytical Chemistry, University of Uppsala, Sweden.

Dr. Ingela Lanekoff is a Docent and Associate Professor in Analytical Chemistry at Uppsala University, Sweden, where she heads the Division of Analytical Chemistry and her research group in surface sampling and imaging mass spectrometry. Ingela earned her PhD from Gothenburg University, Sweden, in 2011 after working with Prof. Andrew Ewing and joined the group of Prof. Julia Laskin (currently at Purdue University) at PNNL, USA, as a post doc the same year. In 2014, she started her own group at Uppsala University based on external funding from the Swedish Research Council and the Swedish Foundation for Strategic Research. Her research is focused on method and technique developments to generate 2d maps of metabolite and lipid distributions in tissue sections and detection of metabolites in single cells. Her research also includes collaborations to understand metabolic alterations in disease and dysfunction, such as diabetes and premature birth, by developing both targeted and untargeted analytical methods that enhance the signals of low abundant species and provide novel data.

Plenary Speaker P4:
Pr. Marcel Utz

Head of Magnetic Resonance, School of Chemistry, University of Southampton, UK

Marcel obtained a MSc in materials science from ETH Zürich in 1994. He then joined the research groups of Prof. Ueli Suter in Polymer Science, and of Prof. Richard R. Ernst in Physical Chemistry at ETH as a doctoral candidate. In 1998, he obtained his doctorate, with a thesis on solid-state NMR investigations of glassy polymers under plastic deformation. In 1999, he joined the group of Prof. Pablo DeBenedetti at Princeton University as a postdoc, with a stipend from the Swiss National Science Foundation. A year later, he joined the Institute of Materials Science and the Department of Physics at the University of Connecticut as an Assistant Professor. He was promoted to Associate Professor with Tenure in 2006. In the same year, he moved to the University of Virginia, where he joined the department of Mechanical and Aerospace Engineering. In 2012, Marcel moved to the UK, joining the School of Chemistry at the University of Southampton. He was promoted to a personal chair in 2014. He currently heads the section of Magnetic Resonance within the School of Chemistry. Marcel lives in Winchester, UK, with his wife Song and their son Nicolas.

Plenary Speaker P5:
Dr. Maria Fedorova

AG Bioanalytik, Institut für Bioanalytische Chemie, Fakultät für Chemie und Mineralogie, Universität Leipzig.

Maria Fedorova studied Biochemistry at Saint-Petersburg State University, Russia and obtained her PhD at Faculty of Chemistry and Mineralogy, Leipzig University, Germany. Now she is a group leader at the Institute of Bioanalytical Chemistry, Faculty of Chemistry and Mineralogy, at the University of Leipzig. Her research is focused on development and optimization of chromatography and mass spectrometry methods for analysis of lipids and their modified forms. Dr Fedorova group works on implementation of high throughput LC-MS methods in discovery lipidomics targeting in-depth identification and quantification of human lipidome in variety of tissues. By combining lipidomics data with investigation of related proteins and protein post-translational modifications via systems medicine approach, Dr Fedorova aims for a deeper understanding of pathophysiology of obesity, insulin resistance, type II diabetes and cardiovascular disorders.

Keynote Speaker K1:
Pr. Zoran Nikoloski

Max-Planck-Institut für Molekulare Pflanzenphysiologie, Wissenschaftspark Potsdam-Golm, Germany.

Pr. Zoran Nikoloski is a Chair of Bioinformatics at the University of Potsdam and leads a Cooperative Research Group at the Max Planck Institute of Molecular Plant Physiology in Potsdam, Germany. Zoran trained as a computer scientist with a PhD focused on network-based research from the University of Central Florida, Orlando, USA. The focus of his groups is on systems biology and mathematical modeling of plant metabolism and its relations to protein-protein interaction and gene regulatory networks. His research includes the development of large-scale metabolic networks of model and crop plants and their usage in conjunction with large-scale metabolomics, proteomics, and transcriptomics data to predict complex, agronomically relevant traits. Recent work includes integration of metabolomics data and large-scale metabolic models with genomics data to bridge the gap between genotype and phenotype.

Keynote Speaker K2

Pr. Marta Cascante

Marta Cascante is Full Professor at the Department of Biochemistry and Molecular Biology at University of Barcelona and leader of the Integrative Systems Biology, Metabolomics and Cancer team. She has authored over 250 publications. She is partner of European translational research projects (H2020) in the field of systems medicine and metabolomics and member of the editorial advisory boards of Metabolomics and BMC systems Biology. She has been distinguished in 2015 with Icrea Academia Prize and with the Narcis Monturiol Medal of the Catalan Government for her scientific merits.

Research interests are focused on cancer and metabolic diseases with the goal of elucidating the networks and pathways that are fundamental in their development and progression. More specifically, the Integrative Systems Biology, Metabolomics and Cancer team's mission is to devise new therapeutic strategies to forestall therapeutic resistance in cancer through integrative approaches that afford a rational identification of molecular targets, with emphasis on metabolic regulators, for effective synergistic combinations with chemotherapeutics with demonstrated efficacy and/or natural products. To tackle our mission, our team assembles high-level expertise in comprehensive metabolic analysis, bio-computational approaches, generation of cellular and pre-clinical animal models, functional genomics and drug discovery. We have generated proof-of-principle evidences that resulted in publications and a patent application of the effectiveness of this approach. We intend to generalize our approaches to inhibitors currently in use as first-in-line or emerging therapies, by unveiling and targeting the corresponding adaptive metabolic responses in order to overcome therapeutic resistance in cancer. Furthermore, in the framework of H2020 project "PheNoMenal ", coordinated by EBI, her team has been contributing to develop and deploy an e-infrastructure that makes it feasible for healthcare researchers to process analyze and mine molecular phenotyping data, to facilitate large-scale data analysis in the coming age of Precision Medicine.

Keynote Speaker K3.

Dr. Emma Schymanski

Environmental Cheminformatics and FNR ATTRACT Fellow. LCSB, University of Luxembourg.

Associate Professor Emma Schymanski is head of the Environmental Cheminformatics group at the Luxembourg Centre for Systems Biomedicine, University of Luxembourg. In 2018 she received a Luxembourg National Research Fund (FNR) ATTRACT Fellowship to establish her group in Luxembourg. She has over 50 peer-reviewed publications, book chapters and a book. Her research combines cheminformatics and computational mass spectrometry approaches to elucidate the unknowns in complex samples, primarily with non-target screening and relate these to environmental causes of disease. An advocate for open science, she is involved in and organizes several European and worldwide activities to improve the exchange of data, information and ideas between scientists to push progress in this field, detailed here:

https://wwwen.uni.lu/lcsb/research/environmental_cheminformatics

Keynote Speaker K4

Pr. Tuulia Hyötyläinen

Professor of Chemistry at Örebro University, Sweden.

Professor Tuulia Hyötyläinen is Professor of Chemistry at Örebro University, Sweden. She has a PhD in analytical chemistry from University of Helsinki, Finland. She has over 20 years of experience in analytical chemistry, mass spectrometry, environmental analysis and metabolomics. One of her main focus area is the development of robust workflows from sampling, sample preparation, analysis to data preprocessing and data mining, and quality control as well as development of methodologies for the identification of unknown metabolites. Currently, her main emphasis is to elucidate the impact of environmental factors in human health, and to identify effect-based markers of environmental exposure and the metabolic pathways behind the adverse outcomes due to the exposure . Particularly, she focuses on the impact of exposure during fetal development and early life, with focus on autoimmune diseases, particularly T1D, and most recently, also coeliac disease and irritable bowel syndrome.

Program

Tuesday January 21st 2020 **Workshop day** *University of Toulouse*			
9:00 am - 12:00 pm	**W1**: Interactive tutorial - How to process LC-MS data with workflow4metabolomics.org		
12:00 pm - 1:00 pm	LUNCH BREAK - ON YOUR OWN		
	Parallel Workshop A (room TBA)	Parallel Workshop B (room TBA)	
1:00 pm - 3:45 pm	**W2**: metaRbolomics hackathon plus	**2:45 pm - 4:00 pm**	**W5**: How to chair sessions
4:00 pm - 6:00 pm	**W4**: Advanced approaches for the analysis of metabolomics data		
6:00 pm - 6:30 pm	BREAK		
6:30 pm - 8:00 pm	**Social event** : Toulouse walking tour		
8:30 pm - 10 pm	**W6**: Meet the editor		

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Wednesday 22nd 2020 *Auditorium Marthe Condat*		
8:00 am - 9:00 am	**Registration open**	
9:00 am - 9:30 am	**Opening Ceremony**	
Methodological & Technological developments Chairs: Justine Leenders & Marta Cascante		
9:30 am - 10:15 am	**Plenary 1**: From analytical chemistry to clinical metabolomics: reflections on the experimental workflow using adrenal cancer and hypervitaminosis A case studies *Rick Dunn, UK*	
10:15 am - 10:35 am	**O1**: Compact NMR spectroscopy and advanced pulse sequences: the perfect match for the online and real-time monitoring of bioprocesses *Jonathan Farjon, France*	
10:35 am - 11:00 am	**Coffee break**	
Microbiology & Biotechnologies Chiars: Alison Woodward & Karl Burgess		
11:00 am - 11:20 am	**O2**: ScalaFlux: a scalable approach to quantify fluxes in metabolic subnetworks *Pierre Millard, France*	
11:20 am - 11:40 am	**O3:** Rational optimization of a novel synthetic pathway for methanol utilization in *E. coli* *Camille Peiro, France*	
11:40 am - 11:55 am	**F1**: Characterising the bidirectional biomolecular interactions between the intestinal microbiota and host drug metabolism *Marine Letertre, UK*	
	F2: Rapid kinetics of lipid second messengers controls secretion in Toxoplasma parasites *Nicholas Katris, France*	
	F3: Searching for differential metabolites between Mycobacterium tuberculosis and the MTBVAC vaccine using non-targeted approach metabolomics *Caridad Díaz Navarro, Spain*	
11:55 am - 12:10 pm	**S1: Sciex :** A New Tool in Metabolomics: SWATH® Acquisition Analysis for Global Profiling and Quantitation *Joerg Schlotterbec, Sciex*	
12:10 pm - 1:00 pm	**Lunch & Posters**	
1:00 pm - 1:45 pm	**Lunch & Posters**	**Career information**
1:50 pm - 2:20 pm	**Lunch & Posters**	**S2: Bruker lunch seminar. TBA**
Agriculture & Food Chairs: Loic Mervant & Steffen Neumann		
Chair:	**Plenary 2**: Physical inactivity and preservation of muscle mass: Lessons from hibernation *Etienne Lefai, France*	
3:15 pm - 3:35 pm	**O4**: Mass Spectrometry Imaging (MALDI-MSI) and LC-MS/MS reveal spatial distribution of metabolic responses to Leptosphaeria maculans infection in stems of Brassica napus *Anani Amegan Missinou, France*	
3:35 pm - 4:05 pm	**Keynote 1:** Insights from integration of metabolomics data in large-scale plant metabolic models *Zoran Nikoloski, Germany*	
4:05 pm - 4:20 pm	**F4**: Elicitor-specific reprogramming of potato's metabolome *Rafaela Martin, France*	
	F5: Biosource-guided network annotation and visualization for untargeted metabolomics *Santiago Codesido, Switzerland*	
	F6: Recommending substructures for unknown tandem mass spectra *Youzhong Liu, Belgium*	
4:20 pm - 5:00 pm	**Coffee break**	
Statistical & Computational developments Chairs: Sara Tortorella & Lorraine Brennan		
5:00 pm - 5:20 pm	**O5**: Mix it up! How to combine multiple LC-MS modes and design of experiments to understand multifactorial biological phenomena *Victor Gonzalez Ruiz, Switzerland*	
5:20 pm - 5:35 pm	**EC1**: ASICS: identification and quantification of metabolites in complex 1H NMR spectra *Gaëlle Lefort, France*	
5:35 pm - 5:55 pm	**O6:** Modelling intrahepatic metabolic rewiring during the onset of obesity using arterio-venous blood metabolomics profiles *Nathalie Poupin, France*	
5:55 pm - 6:10 pm	**S3:** *Shimadzu* . Profling experiments with DIA on the Ultra-Fast Q-TOF LCMS-9030 *Thierry Legoupil*	
6:10 pm - 8:00 pm	**Welcome drinks & Posters**	

European RFMF Metabomeeting 2020

Thursday 23rd 2020 *Auditorium Marthe Condat*		
Methodological & Technological developments Chairs: Jordi Capellades & Pietro Franceschi		
9:00 am - 9:45 am	**Plenary 3**: Spatial metabolomics in untargeted and quantitative studies *Ingela Lanekoff, Sweden*	
9:45 am - 10:05 am	**O7**: Coupling metabolomic information coming from both LC-MS and Imaging-MS to characterize experimental induction of coral larvae metamorphosis *Alain Paris, France*	
10:05 am - 10:25 am	**O8**: NMR metabolomics: a new tool in forensic sciences *Emanuela Locci, Italy*	
10:25 am - 10:40 am	**EC2**: Machine learning-based classification to improve Gas Chromatography-Mass Spectrometry data processing *Yoann Gloaguen, Germany*	
10:40 am - 11:05 am	**Coffee break**	
Human health Chairs: Corentine Goossens & Matej Oresic		
11:05 am - 11:35 am	**Keynote 2**: Unveiling the metabolic phenotypes and vulnerabilities underlying metastasis and drug resistance *Marta Cascante, Spain*	
11:35 am - 11:50 am	**EC3:** High throughput untargeted surface metabolite profiling of formalin-fixed paraffin embedded (FFPE) brain tumour tissue microarrays (TMA) using LESA-MS/MS and OrbiSIMS *Joris Meurs, UK*	
11:50 am - 12:05 pm	**EC4**: Computational modeling of p53 metabolic functions *Carlo De Blasio, France*	
12:05 pm - 12:20 pm	**F7**: Towards precision diagnostics: Untargeted metabolomics for the diagnosis of inborn errors of metabolism in individual patients *Kulkarni Purva, NL*	
	F8: Metabolic maturation in the first two years of life in resource-constrained settings and its association with postnatal growth *Natasa Giallourou, UK*	
	F9: Rapid LA-REIMS and comprehensive UHPLC-HRMS for metabolomics of biofluids in metabolic disorders *Lieven Van Meulebroek, Belgium*	
12:20 pm - 1:30 pm	**Lunch & Posters**	
1:30 pm - 2:00 pm	**Lunch & Posters**	**S4: Thermo lunch seminar** Escape the Identity Crisis with Intelligence Driven Mass Spectrometry *Amanda Souza, Marketing Manager, Life Sciences Mass Spectrometry & Anas Kamleh, Sales Support Expert - Thermo Fisher Scientific*
Environmental science & Plants Chairs: Sylvain Dechaumet & Anne-Emmanuelle Hay		
2:00 pm - 2:30 pm	**Keynote 3**: Environmental Cheminformatics: Case Study of Thirdhand Smoke in House Dust *Emma Schymanski, Luxembourg*	
2:30 pm - 2:50 pm	**O9**: Relating metabolomics and yield in a network of experiments *Sylvain Prigent* , France	
2:50 pm - 3:10 pm	**O10**: Understanding the regulation of proline content in source leaves of winter oilseed rape by using transcriptomic and 15N-isotope tracing approaches *Younes Dellèro, France*	
3:10 pm - 3:25 pm	**S5, Waters :** Multi-dimensional Metabolomics using Ion Mobility Workflows – Future Advances *Sarah Lennon, Waters*	
3:25 pm - 4:00 pm	**Coffee break**	
Methodological & Technological developments Chairs: Youzhong Liu & Patrick Giraudeau		
4:00 pm - 4:45 pm	**Plenary 4**: Micro-NMR for Metabolomic Observation of Microfluidic Culture Systems *Marcel Utz, UK*	
4:45 pm - 5:05 pm	**O11**: Coupling UPLC-MS with Microdialysis for Metabotyping Burn Injury *Elizabeth Want, UK*	
5:05 pm - 5:20 pm	**EC5**: Proton detected 31P NMR methods for metabolomics *Neil Cox, France*	
5:20 pm - 5:40 pm	**O12**: The power of LC-MS based multiomics to explore human adipogenesis *Evelyn Rampler, Austria*	
5:40 pm - 7:15 pm	**Posters Session**	
8:00 pm - 12:00 am	**Gala Dinner**	

Friday 24th 2020 *Auditorium Marthe Condat*	
Environmental science Chairs: Natasa Giallourou & Serge Rudaz	
9:00 am - 9:30 am	**Keynote 4**: Prenatal chemical exposure modulates neonatal serum phospholipids, increasing later risk of type 1 diabetes *Tuulia Hyötyläinen,* Sweden
9:30 am - 9:50 am	**O13**: TheWormJam 2019 vintage - New developments in the international research community for *C. elegans* systems biology and metabolic modelling *Horst Joachim Schirra, Australia*
9:50 am - 10:05 am	**EC6**: Child multi-omics signatures of the early life exposome *Léa Maitre, Spain*
10:05 am - 10:35 am	**Coffee break**
Human health Chairs: Kate Gallagher & Justine Bertrand Michel	
10:35 am - 11:20 am	**Plenary 5**: Lipidomics signatures of metabolic diseases *Maria Fedorova, Germany*
11:20 am - 11:40 am	**O14**: Optimisation of Biofluid and Tissue Metabolite and Lipid Extraction for Clinical Metabolic Phenotyping *Andrew Southam, UK*
11:40 am - 12:00 pm	**O15**: Integrated metabolomics and transcriptomics analysis of intra-tumour heterogeneity in paediatric brain tumours *Alison Woodward, UK*
12:00 pm - 12:20 pm	**O16**: Identifying novel activators of regulatory T-cell metabolism *Celia Berkers,The Netherlands*
12:20 pm - 12:35 pm	**Closing Ceremony & Awards**

O: Oral Presentation **- EC:** Early Career oral presentation **- F:** Flash presentation (180 seconds) **- S**: Sponsor presentation

Oral abstracts

Plenary 1: P1

From analytical chemistry to clinical metabolomics: reflections on the experimental workflow using adrenal cancer and hypervitaminosis A case studies

William NASH[1], Lukas NAJDEKR[1,2], Elliott PALMER[1], Andrew SOUTHAM[1,2], Andris JANKEVICS[1,2], Thomas LAWSON[1], Gavin LLOYD[1,2], Anthony OXLEY[3], Kieran FINNEY[3], Vasilis CHORTIS[4], Alessandro PRETE[4], Mark VIANT[1,2], Ralf WEBER[1,2], Helen COOPER[1], Georg LIETZ[3], Wiebke ARLT[4] and Warwick DUNN[1,2,4]

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Introduction

The application of untargeted and semi-targeted metabolomics tools for clinical research is increasing at an exponential rate worldwide. The metabolomics workflow [1,2] from experimental design and sample collection/preparation through data acquisition to data processing and statistical analysis is well defined though developments and validation of many of these processes are required in areas including sample collection for large human studies, standardized analytical assays [3], quality assurance and quality control [4,5] as well as metabolite identification [6]. In this presentation, I will discuss recent advances in Birmingham in analytical chemistry approaches and metabolite annotation strategies and demonstrate their application using case studies.

Technological and methodological innovation

New advances in the metabolomics workflow applying ultra-high performance liquid chromatography-mass spectrometry (UHPLC-MS) and bioinformatics are providing enhanced coverage of the metabolome, are increasing sample throughput, and are increasing annotation/identification rates. Dried blood spots are an inexpensive and simple strategy for individuals to collect blood samples; their stability, volume normalisation and analysis will be described [7]. The recent development of standardized UHPLC-MS assays to enhance metabolite annotation and enable medium-throughput data collection for hundreds of biofluid, cell and tissue samples will be discussed. Finally, new developments in the collection and use of chromatographic retention time, full scan MS1 and MS/MS data will be described with demonstrations on the quantity and quality of metabolite annotations.

Results and impact

The application of standardized untargeted UHPLC-MS metabolomics assays in clinical metabolomics will then be described. Case studies will include (1) understanding hypervitaminosis A in animal models and humans along with biomarker identification and (2) defining metabolic changes in adrenal tumours (adrenocortical carcinoma and adrenocortical adenomas). I will finish with some reflections on the next steps to strengthen metabolomics in clinical studies.

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Oral 1: O1

Compact NMR spectroscopy and advanced pulse sequences: the perfect match for the online and real-time monitoring of bioprocesses

Jonathan FARJON[1], Dylan BOUILLAUD[1,2], Benoît CHARRIER[1], Delphine DROUIN[2], Vladimir HEREDIA[2], Olivier GONCALVES[2], Patrick GIRAUDEAU[1]

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Introduction

Microalgae family host a tremendous source of chemodiversity. Moreover, thanks to their metabolic plasticity, they are able to redirect their carbon fluxes toward the biosynthesis of biomolecules of interest, i.e. lipids when grown in nitrogen limited cultures [1]. In order to monitor and therefore optimize their culture conditions, it is possible to exploit NMR spectroscopy as a suitable non-destructive approach in order to provide real time structural, kinetic and quantitative information.

Technological and methodological innovation

Emerging benchtop NMR spectrometers are low-cost, transportable and robust tools, but they suffer from a low sensitivity and a weak spectral resolution. In order to circumvent these limitations, we recently implemented advanced high-resolution NMR techniques on a 1T benchtop NMR instrument equipped with a gradient coil such as solvent suppression schemes [2]. Thanks to these developments, benchtop NMR can be applied to monitor complex bioprocesses in real time [3].

Results and impact

Our compact NMR approach was applied to the monitoring of microalgae cultures in their cultivation medium [4]. The benchtop spectrometer was coupled to a photobioreactor to monitor the total lipid production kinetics over 3 weeks under real culture conditions. Results are in perfect agreement with those obtained relying on a reference off-line GC-FID [5]. This online, non-invasive and automated approach helps making NMR accessible to the real-time monitoring and control of metabolic processes.

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Oral 2: O2

ScalaFlux: a scalable approach to quantify fluxes in metabolic subnetworks

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Introduction

^{13}C-metabolic flux analysis (^{13}C-MFA) allows metabolic fluxes to be quantified in living organisms and is a major tool in biotechnology and systems biology [1]. Current ^{13}C-MFA approaches model label propagation starting from the extracellular ^{13}C-labeled nutrient(s), which limits their applicability to the analysis of pathways close to this metabolic entry point.

Technological and methodological innovation

We have developed a new approach to quantify fluxes through any metabolic subnetwork of interest by modeling label propagation directly from the metabolic precursor(s) of this subnetwork. The flux calculations are thus purely based on information from within the subnetwork of interest, and no additional knowledge about the surrounding network (such as atom transitions in upstream reactions or the labeling of the extracellular nutrient) is required. This approach, termed ScalaFlux for SCALAble metabolic FLUX analysis [2], can be scaled up from individual reactions to pathways to sets of pathways. ScalaFlux has several benefits compared with current ^{13}C-MFA approaches: greater network coverage, lower data requirements, independence from cell physiology, robustness to gaps in data and network information, better computational efficiency, applicability to rich media, and enhanced flux identifiability.

Results and impact

We validated ScalaFlux using a theoretical network and simulated data. We also used the approach to quantify fluxes through the prenyl pyrophosphate pathway of *Saccharomyces cerevisiae* mutants engineered to produce phytoene, using a dataset for which fluxes could not be calculated using existing approaches [3]. A broad range of metabolic systems can be targeted with minimal cost and effort, making ScalaFlux a valuable tool for the analysis of metabolic fluxes.

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Oral3: O3

Rational optimization of a novel synthetic pathway for methanol utilization in E. coli

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Introduction

Methanol is an attractive feedstock due to its low price and the possibility to be produced from renewable sources. Integrating methylotrophy in *Escherichia coli* has attracted increasing attention for the production of value-added compounds. Here, we are working on a novel synthetic pathway for methanol assimilation in *E. coli* composed of a methanol dehydrogenase (Mdh) and a dihydroxyacetone synthase (Das), which ultimately produce dihydroxyacetone (DHA) and glyceraldehyde-3-phosphate (GA3P) [1].

Technological and methodological innovation

We used a high-throughput double transformation robotic platform to construct a combinatorial library of Mdh and Das orthologs resulting in 266 different combinations. To identify the most efficient one, we tested the *in vivo* activity of each combination. A high-throughput screening was developed using ^{13}C-methanol and we followed by MS the mean enrichment of a metabolite (i.e. PEP) that serves as a proxy for methanol assimilation.

Results and impact

One combination of Mdh and Das was of great interest as more than 20% of mean enrichment was reached at the PEP level [1]. We identified genetic targets [2] and demonstrated that their modification could further improve methanol assimilation. Over all, these results bring novel knowledge on synthetic methylotrophy while opening the way for developing new cell factories for methanol-based production of chemicals.

References

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Flash Poster 1: F1

Characterising the bidirectional biomolecular interactions between the intestinal microbiota and host drug metabolism

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Introduction

The gut microbiota, with trillions of microbial cells and 150 time more genes that the human genome itself, synthesises enzymes and metabolites that can influence the metabolism and toxicity of drugs in the host [1]. Conversely, drugs themselves have also been identified to exert an impact on the community structure of the gut microbiota and thus modify its overall functionality [2]. Such bidirectional interactions can have implications for host health and the outcomes of treatment strategies.

Technological and methodological innovation

The simultaneous use of targeted and untargeted LCMS and 1H NMR spectroscopy based-metabolic profiling was applied to explore (1) the potential for the gut microbiome to indirectly modulate the drug metabolism of the host[3] by feeding mice with a high-tyrosine diet and increase the microbial production of p-cresol in the presence and absence of antibiotics, and (2) the benefits of inhibiting a specific bacterial enzyme implicated in the toxicity of irinotecan to improve the outcomes of this drug[4].

Results and impact

This work demonstrates (1) the influence of microbial p-cresol on the sulfation capacity of the host liver and its effect on the phase II drug metabolism of paracetamol and (2) that selective inhibition of microbial enzymes could be an attractive target to complement the development of future drugs. The gut microbiota can explain a notable proportion of toxicity and variability in drug responses and evaluating an individual's microbiome prior to drug treatment could help to improve drug outcomes.

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Flash Poster 2: F2

Rapid kinetics of lipid second messengers controls secretion in Toxoplasma parasites.

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Introduction

Signalling of secretion in Toxoplasma parasites is regulated by lipid second messengers, phosphatidylinositol phosphates (PIPs) and diacylglycerol (DAG). Additionally, phosphatidic acid (PA)1 is implicated in vesicle fusion for release of invasion factors. However, the full network is poorly defined. We investigate the role of multiple signalling proteins including Calcium Dependent Protein Kinases (CDPKs) and an apical P-Type ATPase/Guanylyl cyclase2 (TgGC) to map the lipid signalling network.

Technological and methodological innovation

We use lipidomics approaches, notably Gas Chromatography Mass Spectrometry (GC-MS) to perform an exhaustive lipidomic analysis of the TgGC mutant. Furthermore, we used GC-MS lipid analysis to monitor lipid kinetics over time in wild type and mutant cells and observed changes in lipid second messenger turnover within a matter of seconds. Our findings are novel in the field of Toxoplasma parasite biology, where our study is the first to monitor lipid second messenger kinetics.

Results and impact

We see PA increases in WT cells less than 10 seconds after stimulus, a response which is ablated in TgGC depleted cells. We also see rapid kinetics in a TgRNG2 mutant, where a large DAG spike appears within 5 secs of stimulus, then drops to resting levels within 10 secs. We analyse proteins TgGC, TgRNG23, TgCDPK14 and TgPKG to map out the network to show that lipid signalling is crucial to control parasite secretion, which is regulated by rapid turnover of lipid second messengers.

References

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Flash Poster 3: F3

Searching for differential metabolites between Mycobacterium tuberculosis and the MTBVAC vaccine using nontargeted approach metabolomics

Caridad Díaz[1], José Pérez del Palacio[1], Patricia Mena[1], Francisca Vicente[1], Irene Pérez[2, 3], Carlos Martín[2, 3, 4], Olga Genilloud[1], Antonio Sánchez Pozo[5] and Jesús Gonzalo-Asensio[2, 3, 6]

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Introduction

MTBVAC is a live attenuated M. tuberculosis vaccine based on two genetic deletions in the *phoP* and *fadD26* virulence genes. The MTBVAC vaccine is currently in Phase 2 clinical trials with newborns and adults in South Africa[1]. Our objective is to explore *in vitro* differences in intracellular and extracellular metabolites between the vaccine candidate MTBVAC, the *phoP* mutants and their parental strains of *M. tuberculosis*.

Technological and methodological innovation

Four different modes of liquid chromatography coupled to high resolution mass spectrometry analysis were developed for a non-targeted metabolomics approach. In this study, we used a combination of reverse-phase liquid chromatography and hydrophilic interaction liquid chromatography in positive and negative ionization ion mode. For data analysis, we used a multi- and univariate statistical analysis and for metabolite identification we used several public and private database.

Results and impact

We have found significant differences in extracellular and intracellular metabolites between M. tuberculosis and theirs phoP mutants, including the MTBVAC vaccine candidate. These results expand our knowledge of the PhoP regulatory network particularly in the context of the lipid metabolism2. Differential metabolites between M. tuberculosis and MTBVAC might constitute potential biomarkers of tuberculosis infection or vaccination respectively and pave the way for future studies in vivo.

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Sponsor 1: S1 *Sciex: A New Tool in Metabolomics: SWATH® Acquisition Analysis for Global Profiling and Quantitation*

Joerg Schlotterbec, Sciex

Sponsor 2: S2 Bruker

High Speed Untargeted 4D-OMICS LC-MS/MS Workflows with Parallel Accumulation Serial Fragmentation (PASEF)

Aiko Barsch,Head of Application Development Metabolomics / Lipidomics

Endometriosis phenotypes are associated with specific metabolomic profiles on patients' blood and follicular fluids

Gildas Bertho, University Paris Descartes

Plenary 2: P2

Physical inactivity and preservation of muscle mass: Lessons from hibernation

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Muscle atrophy is one of the main deleterious consequences of ageing, diseases (e.g. cancers and AIDS), and physical inactivity. It is especially detrimental to locomotion, heat production, and metabolism thus leading to frailty, increased dependency and metabolic disorders. Apart from being a major clinical problem for older people, muscle loss is also observed during physical inactivity, which has become a major leading cause of death worldwide. Although basic knowledge regarding the underlying mechanisms of muscle atrophy is continuously growing, essentially from rodent models and clinical trials in humans, there are still no efficient therapeutic strategies for its prevention and treatment.

Hibernating bears exhibit a strong and unique ability to preserve muscle mass in conditions of muscle disuse and food deprivation, conditions during which muscle atrophy is observed in human. Bears remain inactive in winter during up to seven months without arousal episodes (without eating, drinking, urinating or defecating), with only very limited loss in muscle protein content and strength, whereas muscle and fibre cross-sectional area are preserved.

Underlying mechanisms have not been understood yet, but our approaches combine molecular and cellular studies of bear muscle as well as human muscle cells exposed to bear serum. Our recent demonstration of trans-species effects of bear serum controlling protein degradation in cultured human muscle cell holds promising potential. Hence, exploration of winter bear serum therefore holds potential for developing new tools to fight human muscle atrophy and related metabolic disorders.

Oral 4: O4

Mass Spectrometry Imaging (MALDI-MSI) and LC-MS/MS reveal spatial distribution of metabolic responses to Leptosphaeria maculans infection in stems of Brassica napus

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Introduction

Partial quantitative resistance to blackleg, caused by Leptosphaeria maculans, is expressed during the steminfection stage, thus limiting the extent of necrosis in partially resistant genotypes [Pilet-Nayel et al., 2017]. To get a better understanding of the biochemical mechanisms involved in this partial resistance at the stem stage, we developed an approach to spatially apprehend the metabolomic changes occurring in stem areas close to the fungus-infected tissues. Mass Spectrometry Imaging (MSI) [Qin et al., 2018] is a technology that enables the visualization of the spatial distribution of hundreds to thousands of metabolites in the same tissue section simultaneously.

Technological and methodological innovation

In the present work, we used high-resolution Matrix-Assisted Laser Desorption/Ionization Fourier-Transform Ion Cyclotron Resonance Mass Spectrometry Imaging (MALDI-FT-ICR MSI) to analyze the spatial distribution of metabolites in different zones of stem cross-sections after infection with L. maculans and prospect their potential role in plant response. Two genotypes, 'Bristol' (susceptible) and 'Darmor' (resistant), were analyzed at 14 days following petiole infection with L. maculans (isolate JN2).

Results and impact

Analysis of specific metabolic responses to infection highlighted that most of the accumulated discriminative features after infection were genotype-specific. In contrast, 50% of the depleted discriminative features were shared by both genotypes. Different metabolic responses were also observed in the proximal and distal tissues from the necrotic cavity in both genotypes. This difference was clearer especially in the vascular tissues of the resistant genotype, revealing that this tissue might play important metabolic functions in the reduction of necrosis extension. In addition, a metabolomic approach conducted using Liquid Chromatography separation coupled with High-Resolution Mass Spectrometry, revealed a large metabolomic shift in infected tissues of B. napus and disease-induced metabolic features (phytoalexins previously refenced [Pedras & Abdoli, 2017] and phytoalexins-like).

This work pointed out the suitability of combining mass spectrometry platforms to analyze and map metabolic responses to biotic stress in tissues of B. napus. These results, which will be consolidated by structural identification of features using direct MALDI-MSI-fragmentation and molecular networks approach, suggest some outlooks for the future understanding of the metabolic response of B. napus to pathogen infection.

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Keynote 1: K1

Insights from integration of metabolomics data in large-scale plant metabolic models

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Cellular functions are shaped by reaction networks whose dynamics are determined by the concentrations of underlying components. However, cellular mechanisms ensuring that a component's concentration resides in a given range remain elusive. I provide a systematic overview of the existing constraint-based approaches which can be used to integrate and predict metabolite concentrations in large-scale metabolic networks. The emphasis is placed on network structure conditions which can be used to arrive at simplified approaches for prediction of metabolite concentrations even in absence of data on kinetic parameters, provided there is access to metabolomics data from a reference condition. In addition, I will present a constraint-based approach that requires integration of genotype-specific metabolomics data to enable genomic prediction of plant growth. This approach is used to predict rosette growth of *Arabidopsis thaliana* accessions by employing genomic prediction of reaction rates estimated from accession-specific metabolic models. The results demonstrate that, in comparison to classical genomic selection, our approach improves the prediction accuracy of growth within and across nitrogen environments by 32.6% and 51.1%, respectively.

Flash Poster 4: F4

Elicitor-specific reprogramming of potato's metabolome

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Introduction

Potato crops are the target of major pathogens, including Phytophthora infestans, causal agent of late blight1. Control methods for this oomycete are based on the fungicides. Current regulations require a reduction of these molecules and one strategy could be to stimulate plant defenses via the PAMP-Triggered Immunity by exogenous elicitors. Unfortunately, this strategy do not always successful in the field. Our hypothesis was that the induced defence responses depend on the elicitors' origin.

Technological and methodological innovation

This hypothesis was tested on 2 potato genotypes with different levels of resistance to P. infestans treated respectively with 3 elicitors: A Concentrated Culture Filtrate-CCF, PAMPs from P. infestans2, amino acid-β-aminobutyric acid-BABA3 and an extract from Ulva spp4. The leaflet are analysed 48h after treatment by a non-target metabolomic approach using UPLCqTOF-MSe. Then a focus was carried out on secondary metabolism pathways through targeted metabolomic and transcriptomic approaches.

Results and impact

The results showed that each elicitor induce a specific reprogramming of metabolic profile independently of the genotype5. BABA and CCF preferentially regulated isoquinole alkaloids and anthocyanins and Ulva extract phenylpropanoids and sterol alkaloids. The transcript analysis confirmed these induction only for the Ulva extract. The results suggest that the antimicrobial compounds induction depends on the elicitor/genotype interaction which should be taken into account for pathogen management.

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Flash Poster 5: F5

Biosource-guided network annotation and visualization for untargeted metabolomics

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Introduction

Untargeted metabolomics has demonstrated its usefulness as a tool for biomarker exploration and sample classification. The problem remains of annotating analytical features with chemical identities, as one cannot obtain MS/MS spectra for metabolites with low concentrations, or measure LC-MS standards for all possible substances. This is noticeable in human metabolomics, where on the other hand the network of metabolites and pathways is well described [1,2], providing additional information.

Technological and methodological innovation

The use of biosources to enhance annotation recursively was used in [3] for MS/MS data. We present a Python module for network-guided annotation at several levels of reliability (such as MS/MS, LC-MS, MS), keeping track of the potentially multiple features assigned to each compound, and visualizing the relevant subnetwork. This allows to sort and select annotated features dynamically, by criteria such as their ability to separate sample groups, or their influence on a multivariate analysis.

Results and impact

We have found the resulting annotation to be highly useful to explore pathway alterations beyond the small set of metabolites identifiable via pure standards, by suggesting relevant features in their metabolic neighbourhood. Steroids are particularly benefitted, given the annotation multiplicity generated by their similar masses and retention times. Data analysis is also enhanced through the dual visualization of dysregulations both in metabolite levels and ratios between related compounds.

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Flash Poster 6: F6

Recommending substructures for unknown tandem mass spectra

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Introduction

Structural elucidation of unknown natural products, metabolites and drug derivatives from LC-MS/MS spectra remains a challenging task. However, these small molecules may share common substructures, which may result in common features in their spectra (i.e. frequently-occurring product ions and neutral losses). Through molecular networking (GNPS) and mass motif analysis (MS2LDA), an increasing number of spectral patterns have been discovered in high-quality spectral libraries. However, the lack of automated structure annotation system prevents these patterns from being useful for partial identification of unknown metabolites and further biological interpretation.

Technological and methodological innovation

We present a method that mine patterns containing both spectral features and substructures from spectral libraries. These novel patterns are represented as " diagnostic rules" that predict substructure presence/absence based on single or several observed spectral features. From GNPS spectral library, we generated 8378 rules allowing the prediction of 712 substructures. The combined use of these rules allows substructure recommendation in an automated fashion.

Results and impact

We validated GNPS rules on both expert-annotated spectral patterns (MS2LDA motifs) and independent test spectra from CASMI challenge, both showing a good agreement with the ground-truth. We further applied these rules for the characterization of impurity and degradation products of marketed drugs. From hundreds of signals (mostly unknowns) detected in a pharmaceutical product, our approach efficiently isolated signals where underlying compounds were structurally related to the parent drug (by sharing substructures). These unwanted unknown components probably arise from the manufacturing process or form during storage. The potential chemical transformations (e.g. oxidation, methylation.) were inferred using the precursor mass differences between parent compound and impurities. Combining substructure conservation and mass shifts, we proposed data-driven pathways of drug degradation and validated some of them based on literatures.

Our approach can be applied to biological systems with a goal to achieve system biochemical interpretation of MS/MS data of a complex mixture without identifying individual metabolites. The substructure recommendation system was developed into an openly-available web-tool at messar.biodatamining.be.

Oral 5: O5

Mix it up! How to combine multiple LC-MS modes and design of experiments to understand multifactorial biological phenomena

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Introduction

Fusion of data from multiple analytical techniques remains challenging, as it does the interpretation of results issued from of multi-factorial experiments in metabolomics [1]. Herein, we use multi-modal LC-MS and design of experiments to study metabolic heterogenicity in cancer cells in culture. Different cells exhibiting common or phenotypic traits were compared and the multi-modal data was merged and interpreted using the AMOPLS (ANOVA Multiblock OPLS) method [2].

Technological and methodological innovation

Metabolomic experiments were carried out to investigate differences between both cells and phenotypes simultaneously. A combination of complementary LC-MS modes (RP and two HILIC modes, with ESI+ and ESI-) [3] was implemented to broaden the explored chemical space, spanning from highly apolar lipids to polar and ionized metabolites. Thanks to AMOPLS, the contribution of each LC-MS mode, as well as individual metabolites could be evaluated with respect to the two studied experimental effects.

Results and impact

The main source of variability was the cell type (25.5%), while the phenotype accounted for 12.4%. All the three LC-MS techniques contributed equally to distinguish each experimental effect, with amide-HILIC allowing a better separation according to the phenotype. Our results show that cells exhibiting shared phenotypic traits present common metabolic patterns, independently of their genetic differences.

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EC oral 1: EC1

ASICS: identification and quantification of metabolites in complex 1H NMR spectra

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Introduction

Several high-throughput technologies allow to obtain metabolomic profiles in biological fluids: Mass Spectrometry (MS) or Nuclear Magnetic Resonance (NMR) for instance. Among them, NMR has the advantage of being less expensive and is viewed as a promising tool to detect interesting biomarkers easily. However, the interpretation of the obtained spectra is difficult since the identification and the quantification of the metabolites present in a complex mixture is not automatic.

Technological and methodological innovation

To ease and expand the use of NMR, we developed a new R package available on Bioconductor, ASICS (Automatic Statistical Identification in Complex Spectra; [1] and [2]), that proposes a complete pipeline for metabolomic spectra analysis. ASICS contains a statistical method to identify and quantify metabolites in a complex mixture by using a statistical model based on a library of pure metabolite reference spectra.

Results and impact

For some datasets, biochemical dosages of several metabolites were also available. Overall, ASICS exhibited a good sensitivity and specificity to retrieve present metabolites and a quantification that was strongly correlated to most metabolite dosages. In conclusion, ASICS allows a faster and simpler direct biological interpretation than the classical bucket approach and better results than other quantification methods such as Batman [3], Bayesil [4] or Chenomx [5].

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Oral 6: O6

Modelling intrahepatic metabolic rewiring during the onset of obesity using arteriovenous blood metabolomics profiles

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Introduction

The metabolite composition of the blood inflowing and outflowing a tissue reflects its metabolic function, with consumed (resp. released) metabolites being in higher (resp. lower) concentration in arterial inflow than venous outflow. The objective of this study was to perform a global metabolic profiling of plasma from incoming and outgoing hepatic vessels using NMR on high fat/high sugar (HFHS)-fed minipigs to explore how the hepatic metabolism is modulated during the onset of obesity [1].

Technological and methodological innovation

The originality of our approach was to translate NMR arterio-venous metabolic profiles into utilization and release fluxes that we integrated in a tuned hepatic genome-scale metabolic network model to simulate fluxes through intra-tissular metabolic reactions. By setting constraints on model exchange reactions to enforce uptake and release of metabolites fitting the experimental data and using in silico flux calculation methods we could predict associated changes in intrahepatic metabolic fluxes.

Results and impact

The interest and novelty of the presented approach is to take advantage of accessible circulating metabolites across a tissue to computationally predict rewiring in its metabolism and changes in consumed and released metabolites that could constitute biomarkers of intra-tissular metabolic modulations. Using this metabolic network modelling strategy, we predict that HFHS is associated with changes in tryptophan catabolism [2], which were further supported by biochemical and molecular analyses.

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Sponsor 3: S3

Shimadzu. Profling experiments with DIA on the Ultra-Fast Q-TOF LCMS-9030

Thierry Legoupil

Plenary 3: P3

Spatial metabolomics in untargeted and quantitative studies

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Introduction

Spatial metabolomics offers an additional analytical dimension by revealing the localization of individual metabolites in thin tissue sections. In addition, spatial metabolomics enables detection of low abundant analytes that are highly localized to distinct cellular regions, which causes them to be diluted below the limit of detection in bulk analysis. Several studies using spatial metabolomics to find metabolic signatures associated with dysfunctional biological conditions will be presented.

Technological and methodological innovation

Nanospray desorption electrospray ionization enables both untargeted and quantitative spatial metabolomics by highly localized liquid extraction followed by electrospray ionization and mass spectrometry analysis. For quantification and chemical modifications, internal standards or reagents are added into the extraction solvent and present during the entire analysis. Surface sampling capillary electrophoresis mass spectrometry offers untargeted analysis directly from the tissue surface.

Results and impact

Rodent models for premature birth, stroke, and diabetes were studied using spatial metabolomics. Both untargeted and quantitative analysis (of prostaglandins, neurotransmitters, and lipids) revealed their localization and abundance in healthy tissue and shifts in dysfunctional systems. When compared to bulk analysis using liquid chromatography ion mobility mass spectrometry, spatial metabolomics provided more detailed chemical information with higher significance to disease state.

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Oral 7: O7

Coupling metabolomic information coming from both LC-MS and Imaging-MS to characterize experimental induction of coral larvae metamorphosis

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Introduction

Benthic settlement of swimming planula larvae of corals (Cnidaria, Scleractinia) involves both physical interaction with the reef substrate (mechanosensation) and reception of chemical cues emitted by substrate biofilms (chemosensation). These interactions initiate secretion of adhesion molecules and metamorphosis of larva into polyp1. Experimentally, this sequence of events can be induced by neuropeptides2,3 and phenotyped by metabolomics using LC-MS and complementarily by MS-imaging.

Technological and methodological innovation

Planula larvae emitted by the coral species Pocillopora acuta Lamarck (1816) were collected from individual colonies in February 2019 at the Océanopolis aquarium (Brest, France) and kept in filtered natural sea water under diurnal illumination. Metamorphosis of larvae was induced by a synthetic GLW-amide neuropeptide. Metabolomic analyses were done by LC-MS fingerprinting of polar extracts, MS-imaging with MALDI-ToF-MS4,5 coupled to multivariate analyses and canonical analyses between datasets.

Results and impact

A reorganization of the larval metabolic pathways was demonstrated by metabolomic analysis of polar extracts, revealing a great residual variance explained by a "colony" effect, in addition to the "metamorphosis induction" effect. MS-imaging revealed in situ metabolomic information usable at the tissue scale. Canonical links between these two sets of data were tentatively explored, in order to deconvolute the global metabolomic information at tissue level thanks to bootstrap procedures.

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Oral 8: O8

NMR metabolomics: a new tool in forensic sciences

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Introduction

Forensic science represents the application of several aspects of the science (such as medicine, chemistry, physics, and biology) in the sphere of criminal investigation and, in a more restrict context, is mainly focused in giving evidences to be used in a legal scenario (either in the criminal or in the civil justice system) by the forensic implementation and validation of scientific methods developed for purposes far from the legal medicine ones.

NMR metabolomics is increasingly used in forensics, due to the possibility of investigating endogenous metabolic profiles and exogenous molecules that may help to describe metabolic patterns and their modifications associated to specific conditions with forensic implications.

Technological and methodological innovation

Compared to the classical analytical methods used in forensic sciences, NMR metabolomics allows to study the global profile of the low molecular weight metabolites present in the sample. It has the advantage of relying not on a single chemical feature but on a fingerprint composed by a multitude of chemical features. It is rapid, it does not need extensive sample preparation and it is non-destructive, meaning that samples are completely recovered after the analysis and available for further investigations.

Results and impact

The attention has been devoted to the identification of peculiar metabolic signatures and specific post-mortem biomarkers in different conditions of legal medicine interest, such as the identification of biological traces [1], the investigation of the metabolomic profile modifications after death for the estimation of the time since death [2, 3], the metabolomic effects of forensic relevant pathological insults such as violent mechanical asphyxia for differential diagnosis of the cause of death [4], the identification of metabolomic pathways related to acute or chronic alcohol intake. The results of these studies highlight how forensic sciences may benefit from a NMR metabolomic approach by gaining additional/complementary information that may help to unravel several forensic conundrums.

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EC oral 2: EC2

Machine learning-based classification to improve Gas Chromatography-Mass spectrometry data processing.

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Introduction

Lack of reliable peak detection impedes automated analysis of large-scale gas chromatography-mass spectrometry (GC-MS) metabolomics datasets. Performance and outcome of individual peak-picking algorithms can differ widely depending on both algorithmic approach and parameters, as well as data acquisition method. Therefore, comparing and contrasting between algorithms is difficult.

Technological and methodological innovation

We present part of the work published in [1] and implemented in our workflow for improved peak picking (WiPP), focusing on the use of machine learning-based classification to optimize and improve different steps of the common GC-MS metabolomics data processing workflow. Our approach evaluates the quality of detected peaks using a machine learning based classification scheme based on seven peak classes. The quality information returned by the classifier for each individual peak is merged with results from different peak detection algorithms to create one final high-quality peak set for immediate down-stream analysis.

Results and impact

We benchmarked our workflow to standard compound mixes and a complex biological dataset, demonstrating that peak detection is improved. Furthermore, the approach can provide an impartial performance comparison of different peak picking algorithms. We also discuss the applicability of the approach to liquid chromatography-mass spectrometry data.

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Keynote 2: K2

Unveiling the metabolic phenotypes and vulnerabilities underlying metastasis and drug resistance

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Introduction

Despite advances in therapy, progression to metastasis and acquisition of resistance to chemotherapy remain the greatest challenges in cancer. More specifically, new modalities of treatment are urgently needed for metastatic cancer and to cope with acquired drug resistance. Recent studies have highlighted metabolic reprogramming as a key player in the acquisition of metastatic potential and therapeutic resistance, which has led to propose metabolic adaptations as potential therapeutic targets.

Technological and methodological innovation

Here, we have applied a systems biology approach, including experimental data integration into genome-scale metabolic models, to unveil metabolic differences and potential vulnerabilities associated with metabolic heterogeneity of tumor cells subpopulations in metastatic prostate cancer and to cisplatin resistance. For this purpose we use a dual model clonal cell model, consisting of a CSC-subpopulation with an epithelial phenotype and a non-CSC-subpopulation with hallmarks of stable EMT.

Results and impact

We show that EMT and metastasis programmes can display distinct metabolic traits. Briefly, the major differences were observed in differential use of glucose and glutamine to fuel TCA cycle, mitochondrial respiration, one-carbon metabolism, beta-oxidation and eicosanoids metabolism. By applying similar approaches, we have identified metabolic adaptive responses associated with platinum resistance as actionable targets in combined therapeutic strategies. Finally, we have analyzed the metabolic reprogramming associated with the inhibition of the cyclin-dependent kinase CDK4/6 in colorectal cancer cells, having unveiled new mechanisms of cancer cell adaptation whose targeting averts the acquisition of pharmacological resistance to cell cycle inhibitors.

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EC Oral 3: EC3

High throughput untargeted surface metabolite profiling of formalin-fixed paraffin embedded (FFPE) brain tumour tissue microarrays (TMA) using LESA-MS/MS and OrbiSIMS

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Introduction

Understanding biochemical tumour profiles within architecturally-intact tissue specimens is important for the development of new treatments. [1] Surgically biopsied FFPE brain tumour tissue has historically only been amenable for TMA-based immunohistochemical analyses. The emergence of advanced analytical methods permits sensitive genome-wide analysis of high and low-abundant oncometabolites, maximising biochemical read-outs from limiting amounts of tissue such as from paediatric brain tumours.

Technological and methodological innovation

The potential of untargeted metabolite profiling using LESA-MS and OrbiSIMS has been shown before. [2,3] For the first time, LESA-MS/MS and OrbiSIMS have been explored for untargeted surface metabolite profiling and imaging within distinct intra-tumour regions using paediatric ependymoma TMAs as an example. OrbiSIMS and LESA-MS/MS allow acquisition of complementary data with confident identification and localization of metabolites with high mass and spatial resolution.

Results and impact

LESA-MS/MS and OrbiSIMS allowed to find complementary significant metabolites and grouping of patients based on their MS profiles, irrespective of known genetic sub-type. The ability of detecting intra-tumour metabolic difference will provide crucial information towards development of personalized treatment. Conversely, aberrant metabolic signatures consistently detected across patients may identify ubiquitous therapeutic vulnerabilities which circumvent inter-patient genetic heterogeneity.

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EC Oral 4: EC4

Computational models of p53-associated metabolic networks

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Introduction

The TP53 gene is the most commonly inactivated tumor suppressor gene in human cancers [1]. P53 is a transcription factor activated in response to multiple types of stress that regulates the expression of genes controlling cell proliferation, senescence, DNA repair and cell death. Several laboratories have also highlighted a major role of p53 in metabolism and showed that p53-associated metabolic functions contribute to its tumor suppressive activities.

Technological and methodological innovation

Our ambitious project aims at developing the first computational model of the metabolic networks regulated by the p53 pathway that we will use to underpin its tumor suppressive functions. To build these networks, we extensively profiled primary mouse embryonic fibroblasts (Mefs) harboring single or combined genetic alterations of genes encoding key components of the p53 pathway (p53, MDM2, E4F1), using different “omics” approaches (RNAseq, Proteomics, and Exometabolomics). These large datasets are integrated into Genome Scale Metabolic Networks (GSMN) that we will use to predict new biomarkers and new therapeutic strategies.

Results and impact

We performed NMR-based exometabolomic studies to determine consumption and production rates of key metabolites, as well as RNA-seq studies to determine the expression profiles of metabolic genes in all our cellular models. These analyses are pinpointing unexpected connections between these key components of the p53 pathway and pyruvate, branched-chain-aminoacids (BCAAs), or methionine metabolism. These data highlight the underestimated complexity of the metabolic networks regulated by the p53 pathway.

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Flash Poster 7: F7

Towards precision diagnostics: Untargeted metabolomics for the diagnosis of inborn errors of metabolism in individual patients

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Introduction

Inborn Errors of Metabolism are inherited conditions caused by genetic defects in enzymes or their cofactors, resulting in a specific metabolite fingerprint showing accumulation of substrate or lack of end-product in patient body fluids1. Untargeted metabolomics offers a comprehensive readout of metabolic status on an individual patient basis. This makes it a promising tool for diagnostic screening and treatment monitoring of IEM patients, especially when clinical presentations are non-specific.

Technological and methodological innovation

We have previously established Next-Generation Metabolic Screening2 as a metabolomics-based diagnostic tool for individual IEM-suspected patients. To fully exploit the clinical potential of NGMS, we have developed an automated computational pipeline to streamline analysis of complex data and make it reproducible. The pipeline features a GUI that converts raw data, detects and aligns features across samples and annotates them to identify significant deviations in patients as compared to controls.

Results and impact

Using our automated computational pipeline, we have advanced the application of metabolomics in clinical diagnostic setting to a next level. Our pipeline ensures reproducible and time-efficient metabolomics data management, processing and analysis. To validate this pipeline, we tested samples of IEM patients, including several diagnoses that were not yet measured with NGMS, for example L-2-hydroxyglutaric aciduria. Our results further expand the clinical applicability and IEM portfolio of NGMS.

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Flash Poster 8: F8

Metabolic maturation in the first two years of life in resource-constrained settings and its association with postnatal growth

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Introduction

Malnutrition continues to impact the growth and development of millions of children worldwide, and chronic undernutrition has proven to be largely refractory to interventions. Improved understanding of metabolic development in infancy and how it differs in growth-constrained children may provide insights to inform more timely, targeted and effective interventions.

Technological and methodological innovation

Untargeted ^{1}H NMR spectroscopy was used to characterize the metabolic profile of urine samples (n=2423) collected longitudinally from children from Peru, Bangladesh and Tanzania in the first two years of life. A panel of age-associated metabolites consistent across the three sites were identified and the biochemical age of the children was computed and standardized as a phenome-for-age Z score comparing a child's phenome age to that of an internal healthy reference population, providing a single continuous measure of metabolic maturity.

Results and impact

Predictive models demonstrated that growth-constrained children lag in their metabolic maturity relative to their healthier peers and that metabolic maturity can predict growth 6 months into the future providing a sensitive measure to better predict growth outcomes. The concept of a phenome age can be used to optimize interventions to the temporal metabolic demands of the developing infant and provides a metabolic framework from which nutritional programs may be more precisely constructed and evaluated.

Flash Poster 9: F9

Rapid LA-REIMS and comprehensive UHPLC-HRMS for metabolomics of biofluids in metabolic disorders

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Introduction

Metabolomics offers unique possibilities to characterize the human pathophysiological state. However, the involved LC-MS approaches are generally not suited for point-of-care testing in non-laboratory settings. In response, this study established laser-assisted rapid evaporative ionization MS (LA-REIMS) for rapid fingerprinting of biofluids (*i.e.* plasma, urine, saliva and feces). This concept was used to assess milk allergy in infants, obesity in adolescents, and type 2 diabetes in adults.

Technological and methodological innovation

The LA-REIMS platform comprised a Q-ToF mass spectrometer and Opolette™ HE2940 pump that was equipped with a Nd:YAG laser (2940 nm). A free space optics set-up was used for transmission of the laser energy to the biological material, where laser ablation (*i.e.* matrix-assisted desorption and ionization) took place. This strategy has been applied in surgical intervention (tissue analysis)[1,2] but not yet explored for metabolomics of biofluids. Methods were optimized for the various matrices.

Results and impact

LA-REIMS methods were available for each biofluid, with the acquisition time being 0.5 min/sample. Fingerprints enclosed between 1,426 (feces) and 1,818 (plasma) compound ions, and enabled rapid differentiation of samples according to pathophysiological state (OPLS-DA models, p-value <0.05 and $Q^2 > 0.5$). LC-MS[3,4,5] achieved similar discrimination and yielded new diagnostic and therapeutic biomarkers. LA-REIMS may support the translation of metabolomics into a clinical environment.

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Sponsor 4: S4

Escape the Identity Crisis with Intelligence Driven Mass Spectrometry

Amanda Souza, Marketing Manager, Life Sciences Mass Spectrometry & Anas Kamleh, Sales Support Expert - Thermo Fisher Scientific

Keynote 3: K3

Environmental Cheminformatics: Case Study of Thirdhand Smoke in House Dust

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Introduction

The environment and the chemicals to which we are exposed is incredibly complex. Household dust samples contain a summary of household exposure to various chemicals, suitable for exploration using high resolution mass spectrometry (HR-MS). Target and non-target HR-MS analysis for third hand smoke (THS) was performed on 75 dust samples (33 smoker, 42 non-smoker households) in Tarragona, Spain, focusing on tobacco-specific nitrosamines, which have been associated with higher health risk [1].

Technological and methodological innovation

Primarily open access/source metabolomics and environmental workflows for HR-MS data analysis were used to interrogate the non-target data. A list of substances in THS was compiled for suspect screening, while an XCMS-based [2] workflow was used to prioritize non-target features of interest that were significantly different between smoker and non-smoker samples. Data was extracted using RMassBank [3], features were annotated using MassBank and MetFrag [4], searching in PubChem and CompTox.

Results and impact

This case study will be used to demonstrate the potential (pros and cons) of various environmental cheminformatics approaches to interpret non-target data, and highlight challenges still facing the field, such as limited coverage of mass spectral libraries [5]. A number of other European and world-wide initiatives will be covered to demonstrate the power of HR-MS and "environmental cheminformatics" to reveal information about the exposome and move to "near real-time" analysis.

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Oral 9: O9

Relating metabolomics and yield in a network of experiments

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Introduction

Prediction of phenotypic traits based on biomarkers is promising to improve breeding efficiency and our knowledge about the links between genotypes and phenotypes. In this work, we propose a modelling method to predict maize yield from metabolomic data. With a panel of 238 maize hybrid genotypes, we were able to achieve valuable predictions of yield evaluated in 29 field experiments in Europe with metabolomic data gathered from plants grown under controlled conditions in an automated greenhouse.

Technological and methodological innovation

Generalised multilinear modelling, such as elastic nets, is a powerful method to reveal interesting links between different datasets while disregarding non-informative variables. Using this method, we were able to link metabolomic data to plant performance measured in fields under different meteorological conditions. This enabled yield predictions and the identification of metabolic biomarkers. Environmental indices were also used to improve the quality of the prediction at the field level.

Results and impact

The models presented here were able to perform good average yield predictions, with correlation between actual and predicted yield ranging around 0.6. Interestingly, the best predictions were obtained using metabolomic data gathered from stressed plants. Regarding predictions at the field level, the introduction of environmental indices in the models further improved the predictive capabilities, especially in drought conditions.

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Oral 10: O10

Understanding the regulation of proline content in source leaves of winter oilseed rape by using transcriptomic and ^{15}N-isotope tracing approaches

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Introduction

Proline metabolism is an essential component of plant adaptation to multiple environmental stress conditions that is also known to participate in specific developmental phases, particularly in reproductive organs [1,2]. Recent evidences suggested a possible role for proline catabolism in *B. napus* for nitrogen remobilization processes during sink to source transition at the vegetative stage [3,4,5].

Technological and methodological innovation

Here, we analyzed the transcriptional regulation of the *P5CS/ProDH* balance at the vegetative stage during sink to source transition with respect to net proline biosynthesis and degradation fluxes. To do so, we performed ^{15}N labelling experiment with either $^{15}NH_4$ or ^{15}N-proline during 4 hours and monitored the redistribution of ^{15}N towards amino acids by taking advantage of an UPLC-TQD analytical system.

Results and impact

We showed that the downregulation of three *P5CS1* genes during sink to source progression was accompanied by a reduced commitment of *de novo* assimilated ^{15}N towards proline biosynthesis and an overall depletion of free proline content. Inversely, the regulation of *ProDH* transcript levels during sink to source transition was not correlated with the evolution of the maximal degradation capacity for proline. Our results suggest a role for proline catabolism in *B. napus* at a late stage of senescence.

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Sponsor 5: S5

Multi-dimensional Metabolomics using Ion Mobility Workflows – Future Advances

Sarah Lennon, Waters

Plenary 4: P4

Micro-NMR for Metabolomic Observation of Microfluidic Culture Systems

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Quantitative observation of metabolism is the most direct form of studying the activity of biological systems. Our group have developed dedicated NMR probe systems and protocols that allow obtaining high-quality NMR spectra from microfluidic devices with sample volumes around 1 µl. NMR spectroscopy at such small scales must overcome significant challenges in order to provide sufficient resolution and sensitivity for meaningful metabolomic investigations.

Microfluidic lab-on-a-chip systems are increasingly used for the culture of cells, cell assemblies, tissues, and small organisms. They provide detailed environmental control, high efficiency and experimental throughput, and allow large-scale repetition of experiments under stable conditions. While traditional microfluidic assays rely on destructive end-point analysis to probe the transcriptome, proteome, or metabolome of the cultured system, NMR can provide valuable additional information non-invasively. This allows detailed observation of metabolic activity in cultured systems, complementary to the traditional end-point analysis techniques. In this talk, I will provide an overview of the opportunities and challenges associated with microlfuidic NMR, and present recent results on microfluidic perfusion culture of liver tissue slices, as well as culture of cells in adherence and in the form of cell aggregates.

Oral 11: O11

Coupling UPLC-MS with Microdialysis for Metabotyping Burn Injury

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Introduction

Burn injury is a devastating trauma affecting millions, resulting in chronic pain, often refractory to treatment. Mechanisms behind burn injury are poorly understood; studies are often destructive, prohibiting longitudinal temporal data.[1-3] Microdialysis permits in vivo collection of solutes primarily from the extracellular interstitium, ideal for studying burns. Metabolomics using mass spectrometry offers a sensitive and unbiased approach for elucidating metabolic changes - to enhance understanding of pathological processes and guide future therapeutic strategies.

Technological and methodological innovation

Subcutaneous microdialysis in a novel burn model was coupled with an optimised 12 minute ultraperformance liquid chromatography – mass spectrometry (UPLC-Q-ToF-MS) assay (ESI+/ESI mode). [4] Microdialysis was conducted for 0.5 hrs pre-burn and 3 hours post-burn, collected in 0.5 hr fractions. Data were pre-processed using XCMS software followed by multivariate analysis (PCA and PLS-DA) to elucidate discriminatory metabolite features due to burn. Model robustness was affirmed using CVANOVA. UPLC-MS/MS studies were performed for structural elucidation of key metabolites.

Results and impact

We demonstrate the application of metabolomic profiling to microdialysate collected from burns; profiling hundreds of analytes. PCA showed good acquisition stability. Clear metabolic differences were observed after burn injury; with niacinamide and uric acid potentially involved in the pathology. Further understanding metabolic changes induced by burn injury will help guide future therapeutic intervention. As this approach is applicable to other samples and conditions, it may improve our understanding of metabolic changes underlying a variety of pathological processes.

References

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EC oral 5: EC 5

Proton detected ^{31}P NMR methods for metabolomics

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Introduction

Phosphorylated metabolites are omnipresent as catabolic intermediates and anabolic building blocks of the cell components (1). NMR is an interesting approach for phosphometabolite characterization. However, despite the 100% natural abundance of the NMR active ^{31}P isotope and its large overall sensitivity, one order of magnitude stronger than ^{13}C, direct observation of ^{31}P signals suffers from line broadening at higher field and exchange broadening from interaction with cations such as Mg^{2+}(2).

Technological and methodological innovation

We have developed proton detected ^{31}P NMR experiments for the identification and quantification of phospho-metabolites. These NMR sequences can be readily implemented in metabolomics pipelines to offer (i) molecular fingerprinting of phosphometabolites, (ii) an increase in overall metabolomics data, (iii) simplification of overcrowded spectra for targeted analysis and (iv) complementarity to MS data for degradation and adducts formation control.

Results and impact

^{31}P NMR technical developments geared towards metabolomics applications will be discussed. Experimental result will be presented for phosphometabolite fingerprinting of complex media, and the complementarity with mass spectrometry will be demonstrated. The capacity to identify and follow phosphometabolites in active cell extracts allows to follow reaction networks. Additional aspects of ^{31}P NMR such as its ability to monitor in real time pH variation during a reaction will also be discussed (3).

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Oral 12: O12

The power of LC-MS based multiomics to explore human adipogenesis

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Introduction

Stem cells are the ideal source to study fat formation as they are pluripotent and their differentiation fate is directly depending on their environment. In this work, we use human mesenchymal stem cells (MSCs), received from surgery waste, and differentiate them into fat cells/adipocytes. The combination of several layers of information coming from metabolomics, lipidomics and proteomics enables in-depth analysis of the biochemical pathways in adipogenesis.

Technological and methodological innovation

We employ a multimolecular approach using an adapted two–phase extraction [1] to monitor the crosstalk between lipid metabolism and protein-based signaling in a single sample (~10^5 cells). The innovative analytical workflow includes standardization with in-house produced ^{13}C isotopically labeled metabolites/lipids, dual injection chromatography (HILIC, RP) hyphenated to high-resolution mass spectrometry for simultaneous untargeted screening and targeted quantification [2,3,4].

Results and impact

Metabolite and lipid concentrations range over four orders of magnitude and were detected down to the low fmol level. Comparing MSCs and adipocytes, we observe significant regulation of different metabolites and lipids such as triglycerides, gangliosides, carnitine and amino acids with 113 fully reprogrammed pathways [5]. The presented workflow is a proof of principle for the power of LC-MS based multiomics and is fit for purpose for applications such as adipogenic differentiation.

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Keynote 4: K4

Prenatal chemical exposure modulates neonatal serum phospholipids, increasing later risk of type 1 diabetes

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Introduction

In the last decade, the increasing incidence of type 1 diabetes (T1D) stabilized in Finland, coinciding with tighter regulation of perfluoroalkyl substances (PFAS) which are environmental chemicals that humans are continuously exposed to as they are widely-used in food packaging materials, paper and textile coatings, and fire-fighting foams (1,2). Exposure to PFAS has been linked with several adverse health outcomes, and particularly changes in lipid metabolism (2,3).

Technological and methodological innovation

In a mother-infant cohort study (n=264), we analyzed metabolite profiles of pregnant mothers and their offspring at birth with UHPLC-QTOFMS and GC-QTOFMS (260 identified lipids and metabolites) and quantified 22 PFAS with UHPLC-QqQMS. We then further examined the impact of PFAS exposure on metabolome in non-obese diabetic mouse (NOD) model, using a novel UHPLC-QqQMS method for simultaneous determination of PFAS and bile acids (BA). We also validated the findings in a second infant cohort (n=75).

Results and impact

High fetal PFAS exposure associated with reduced phospholipids in the infants and the association was exacerbated with high genetic risk of T1D (3). This lipid profile was similar to that observed in infants progressing to T1D (4,5). We verified the findings in animal models, exposed to PFAS, and also found that altered BA metabolism was associated with the changes in the T1D related lipids. Our findings suggest that fetal PFAS exposure contributes to risk and pathogenesis of T1D in children.

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Oral 13: O13

The WormJam 2019 vintage –New developments in the international research community for C. elegans systems biology and metabolic modelling

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Introduction

Caenorhabditis elegans is an important biological model organism. In recent years, metabolism has been increasingly recognised as being of pivotal importance for ageing, development, or disease, as well as the basis for resistance to toxic insults or adaptation to different environments. In this context, the combination of metabolomics with systems biology techniques enables a more detailed characterisation of this organism and its physiological processes [1-2].

Technological and methodological innovation

Recent advances in this area are driven by metabolic reconstructions which allow to model metabolism *in silico*. The *WormJam* genome-scale model (GSM) is a consensus metabolic reconstruction of *C. elegans*, created by the *WormJam* consortium [3-4], and represents the most comprehensive knowledgebase for *C. elegans* metabolism. In 2019 we further improved the metabolic reconstruction especially in lipid metabolism, and created an innovative technological framework for continuous community curation.

Results and impact

We will cover *WormJam*'s aims, 2019 progress, and planned developments. The *WormJam* GSM and NMR-based metabolomics were also applied to characterise *C. elegans* resistance to the toxic gas phosphine, which is used world-wide as agricultural fumigant. The metabolic changes behind phosphine resistance hint at conserved mechanisms for global metabolic regulation [1-2]. A highly curated community-driven consensus *C. elegans* GSM will bring this organism to the forefront of metabolism research.

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EC Oral 6: EC6

Child multi-omics signatures of the early life exposome

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Introduction

It is widely recognised that early life environmental factors play a critical role in influencing life health outcomes, yet the global molecular impact of the whole exposome in utero and in childhood is poorly characterised. The Lifecycle project offers a unique opportunity to explore the interplay between the early-life exposome and the molecular responses in childhood in multiple European birth cohorts (Maitre et al. 2018).

Technological and methodological innovation

Here we present a large population-based study of the exposome in 1,300 children at age 6-11 years incorporating climate, outdoor urban environment, lifestyle, social factors & chemicals. The blood methylome, transcriptome, plasma proteins, serum and urinary metabolites were measured in childhood (Lau et al. 2018) and an exposome-wide association study (ExWAS) performed.

To gain insight into the complex interconnected nature of the exposome and its potential biological signature patterns, we applied network analytic and visualisation methods.

Results and impact

We identified 1170 significant associations, which were organized in 3 pregnancy and 11 postnatal network clusters. We found that the child methylome best captured the influence of pregnancy exposures, such as tobacco smoking and supply of essential minerals (Mo, K, Zn). The omics signatures associated with postnatal exposures, revealed the major influence of adiposity and lifestyle factors in mediating the interaction between exposure and phenotype. Clusters of association could also distinguish routes of exposure through diet to chemical pollutants such as heavy metals (As, Hg) and pesticides (organophosphates) known to have significant impact on health later in life.

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Oral 14: O14

Optimisation of Biofluid and Tissue Metabolite and Lipid Extraction for Clinical Metabolic Phenotyping

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Introduction

Clinical metabolic phenotyping aims to detect and measure 1000s of metabolites and lipids within clinical samples (e.g. biofluids and tissues) to identify changes between metabolic phenotypes (e.g. disease status) and to understand biochemical mechanisms driving the phenotype. Sample extraction is a critical step in clinical metabolic phenotyping: it must be reproducible and give a high extraction yield of metabolites and lipids.

Technological and methodological innovation

We tested multiple monophasic and biphasic metabolite/lipid extraction methods for biofluids (urine/plasma) and tissue (heart/kidney/liver). We also tested solvent-biofluid incubation time/temperature. Extracts were analysed by UHPLC-MS assays: HILIC (urine, plasma, tissue polar extracts); C_{18} aqueous reversed phase [RP] (urine polar extracts); C_{18} reversed phase (plasma & tissue lipid extracts). Each method was assessed for yield, reproducibility and class of extracted metabolites.

Results and impact

Based on yield and reproducibility the best methods were: plasma/urine HILIC– monophasic 50:50 methanol (MeOH):acetonitrile (ACN); urine RP – any tested monophasic method except 100% ACN; plasma lipids – monophasic 100% isopropanol (IPA). Altering solvent-biofluid incubation time/temperature had little effect on yield. For tissue, MeOH/CHCl3/H2O was the best all-round method; however, for some specific compounds other methods performed better, e.g. cardiolipins were better extracted by 100% IPA.

Oral 15: O15

Integrated metabolomics and transcriptomics analysis of intra-tumour heterogeneity in paediatric brain tumours

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Introduction

Despite standard-of-care treatment, up to 50% of childhood ependymomas relapse; the prognosis is then abysmal. One likely explanation for the failure of adjuvant therapy, is the under appreciation of intra-tumour heterogeneity. Integrated metabolomics and transcriptomics offers an opportunity to elucidate aberrant metabolic pathways and reveal new therapeutic avenues in ependymoma tissue neurosurgically resected from different spatial regions of individual tumours.

Technological and methodological innovation

Here we present broad metabolome coverage using liquid chromatography-tandem mass spectrometry (LC-MS/MS) to separately analyse ependymoma metabolites and lipids, and broad transcriptome coverage by RNA sequencing. We homogenised surgically resected ependymoma tissue and extracted polar metabolites and lipids using methanol/water/chloroform (1:1:3), leaving RNA in the solid interphase, ensuring integrated omics analysis will be conducted on the same population of tumour tissue.

Results and impact

First, we compared two subgroups from distinct neuro-anatomical compartments, PF-A (n=10) and ST-RELA (n=5). Integration of metabolomics with transcriptomics revealed the metabolic network centred on GMP was important in STRELA. Next, intra-tumour regions within each of 8 PF-A patients were compared, revealing an abundance of metabolites in the arginine and proline metabolism pathways. The identified intra-tumour heterogeneity exposes new drug targets representing metabolic vulnerabilities

Oral 16: O16

Identifying novel activators of regulatory T-cell metabolism

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Introduction

In a healthy immune response there is a balance between the opposing activities of two T-cell types: conventional T cells (Tconv) and regulatory T cells (Treg). In contrast, immune related diseases are often characterized by a disbalance between these cell types. Upon activation, T cells place unique demands on their metabolism. However, whereas proliferation of Tconv is fully depending on the mTORC1 pathway, Treg proliferation is not, potentially allowing for selective drug targeting.

Technological and methodological innovation

We hypothesized that Tconv and Treg differ in their metabolic features. We used a novel procedure to sort and expand human Treg cells without the use of the metabolic inhibitor rapamycin. Subsequently, we combined metabolomics, fluxomics and proteomics analyses to map metabolic differences between resting and activated Tconv and Treg.

Results and impact

We here show that unlike Tconv, human Treg cells do not activate a glycolytic program upon CD3 activation and CD28 costimulation. Moreover, we identify a novel co-stimulatory signal that does enable Treg to undergo a metabolic switch and activate glycolysis. Finally, using tracer studies, we identify unique aspects in the Treg glycolytic program. Further exploration of these differences could identify metabolic targets that can be exploited to selectively modulate T cell activity.

Poster abstracts

Poster 001: P001
Revealing age-related sex differences in human lipidome: Metabolic phenotyping of Lausanne population

Poster 002: P002
Untargeted metabolomic analysis for the discovery of treatment response biomarkers in breast cancer patients through Hotelling T2 multivariate profiling

Poster 003: P003
Metabolomic investigation of immune cell activation by Liquid Chromatography coupled with High Resolution Mass Spectrometry (LC-HRMS)

Poster 004: P004
GH-OMICs: applying metabolomics to the fight against hormonal doping

Poster 005: P005
NMR metabolomics for understanding the role of alkaline phosphatase in neurotransmission and inflammation processes

Poster 006: P006
A multiplatform approach: HPLC, GC and NMR for urinary metabolomic analysis of muscle-invasive bladder cancer

Poster 007: P007
NMR metabolic profiling can help discriminate between normal primary hepatocytes and diverging hepatic cancer cell lines

Poster 008: P008
Quantitative metabolomics analysis reveals altered lipid profile in women with endometriosis: a pilot case-control study

Poster 009: P009
Steroid profile in human seminal fluid: is it linked to sperm quality?

Poster 010: P010
Combination of two semi-targeted approaches to detect toxicological biomarkers as a tool to understand the relation between red meat consumption and colorectal cancer promotion

Poster 011: P011
Mre11 nuclease activity removes the replication blocking nucleoside analogue Gemcitabine from the nascent strand during DNA replication

Poster 012: P012
Metabolic characterization of Leishmania mexicana-infected macrophages

Poster 013: P013
A comprehensive analysis of metabolome and lipidome in different sleep deprivation models using a multiplatform technique of chromatography-mass spectrometry

Poster 014: P014
Study on Alterations of Metabolome by Blood Transfusion using LC-Q-TOF and GC-MS

Poster 015: P015
Dried Blood Spots for the Determination of Reduced to Oxidized Glutathione Status

Poster 016: P016
Preliminary investiogations of alkaloid composition from Thalictrum isopyroides plant of Azerbaijan Flora

Poster 017: P017
Serum targeted and untargeted metabolomics-based analyses in infants with Ureteropelvic Junction Obstruction

Poster 018: P018
Investigating urinary and faecal metabolic differences among native Africans, African Americans and Alaskans using mass spectrometry

Poster 019: P019

Alterations in plasmatic levels of metabolites involved in purine, urea cycle and tryptophan metabolic pathways during right ventricular remodeling in chronic thromboembolic pulmonary hypertension

Poster 020: P020
Molecular networking-based mass spectrometry to decipher tumor cells metabolism

Poster 021: P021
"-Omics- sciences and therapeutic"

Poster 022: P022
Metabolomic study of commercial mistletoe extracts used in alternative anticancer therapy

Poster 023: P023
Optimization of the untargeted metabolomics workflow for studying the impact of dysbiosis on the host

Poster 024: P024
Rapid extraction and High-Resolution Tandem Mass Spectrometry analysis of the acylCoA metabolome

Poster 025: P025
Serum metabolic profiling following traumatic brain injury in rats using 1H Nuclear Magnetic Resonance spectroscopy

Poster 026: P026
Surface analysis of lipids in rat mesenteric fat tissue sections by MALDI-MS imaging to understand the impact of obesity

Poster 027: P027
Combined transcriptomics–metabolomics profiling of the heat shock response in the hyperthermophilic archaeon Pyrococcus furiosus

Poster 028: P028
Alternative carbon sources for the central metabolism of *Trypanosoma brucei* procyclic form

Poster 029: P029
Characterization of bioherbicidal compounds stemming from micro-algae diversity by differential metabolomic and spectral similarity network assisted dereplication (ALL DREAM)

Poster 030: P030
Threonine metabolism in Leishmania

Poster 031: P031
Fluorinating Putida: Annotating Novel and Undesired Metabolites

Poster 032: P032
Analysis of metabolome time-variations of an endophytic fungal strain of Botryosphaeria mamane through untargeted LC-MS profiling

Poster 033: P033
Study of the production of (phyto)hormones by mycorrhizal fungi

Poster 034: P034
Metabolites characterization of nine cyanobacteria isolated from thermal mud.

Poster 035: P035
Metabolomics in plant-microbe interactions: "MetaboHUB-MetaToul-plant metabolites" platform facilities

Poster 036: P036
Investigating the effects of future climate scenarios in soft fruit: application of HPLC-PDA-MS metabolite profiling in blackcurrant cultivated under increased temperature and altered light regimes

Poster 037: P037
The role of food polyphenols in energy metabolism

Poster 038: P038
Study of dietary polyphenols metabolites by a top-down metabolomics approach

Poster 039: P039
NSP19 an original eco-extract from Dioscorea villosa with neuroprotective effects in in vitro Alzheimer's disease models

Poster 040: P040
Digging out the terroir influence on bioactive polyphenols from grape stems: A correlation_network_ driven approach to spatialize metabolomics data.

Poster 041: P041
Development of a UHPLC-MS/MS targeted method in various matrices for the quantification of toxicologically relevant aldehydes produced from lipid peroxidation

Poster 042: P042
LC-MS based untargeted metabolite profiling of wholegrain spelt (Triticum spelta) cultivated in Switzerland

Poster 043: P043
Isotope Labelling and Molecular Networking to Identify New Fungal Secondary Metabolites

Poster 044: P044
Specific derivatization with isotopic labeling introduction and mass spectrometry to monitor lipid peroxidation carbonyl compounds in the intestinal lumen by both targeted and non-targeted approaches.

Poster 045: P045
Characterization of A. thaliana responses to plant defence elicitors using untargeted metabolomics

Poster 046: P046
Metabolomics implemented to the Equine Biologic Passport (EBP) for doping control.

Poster 047: P047
Unravelling the impact of the antidepressant venlafaxine on the metabolome of meagre fish

Poster 048: P048 Comparison of effects produced by the exposure of bisphenol A and estradiol in zebrafish embryos (Danio rerio) using an untargeted metabolomics approach

Poster 049: P049
Let's talk about POÆMS !! Photosynthetic Organisms from Atmospheric Ecosystem in a Multiscale Study

Poster 050: P050
Developing a multi-omics approach for chemical grouping

Poster 051: P051
Microbiome and metabolome analyses of the medaka fish gut during a chronic exposure to toxic cyanobacterial blooms

Poster 052: P052
High-Throughput Targeted Lipidomics Analysis of Dihydroceramide Desaturase-1 (DES1) Knockout Mice

Poster 053: P053
In Vivo Microdialysis of Endogenous and 13C-labeled TCA Metabolites in Rat Brain:Reversible and Persistent Effects of Mitochondrial Inhibition and Transient Cerebral Ischemia

Poster 054: P054
Ion source optimization in liquid chromatography time-of-flight mass spectrometry for metabolomics studies using the design of experiments

Poster 055: P055
A novel multi-targeted quantitative approach for nutrimetabolomics research

Poster 056: P056
FOBI: An ontology to represent food intake data and associate it with metabolomic data

Poster 057: P057
Comparison of different methods for lipidic extraction

Poster 058: P058
Development of an untargeted metabolomics workflow for investigating drug-induced cardiotoxicity using cardiac microtissues.

Poster 059: P059
A metabolomics-like approach for chemical forensic: preliminary study and perspectives

Poster 060: P060
Simultaneous measurement of absolute metabolite concentration and isotope incorporation by mass spectrometry

Poster 061: P061
Evaluation of pure shift NMR method in metabolomics

Poster 062: P062
A high throughput fluxomic workflow for exploration of metabolic phenotypes

Poster 063: P063
Metabolomic fingerprint of aging on red blood cells

Poster 064: P064
Intelligent Acquisiton for Comprehensive Metabolome Coverage in Plants, Mammals, and Bacteria

Poster 065: P065
Novel MALDI imaging solution empowered by a dual-source Q-TOF and a dedicated bioinformatics pipeline for identification of peaks from tissue

Poster 066: P066
Establishing a Spectral Library and Accurate Mass Retention Time (AMRT) Database for Pediatric Metabolomics Analysis

Poster 067: P067
Investigation of mevalonate pathway by quantitative metabolomics and isotopoloque profiling

Poster 068: P068
High-throughput metabolite profiling of cell media for improved antibody production utilizing a dual separation/mass spectrometry system

Poster 069: P069
The development of selective sorbent for isolation of catecholamines and their metabolites from biological fluids

Poster 070: P070
Data processing workflow to study the exposome by suspect screening

Poster 071: P071
Development of an untargeted approach based on isotope profiling of metabolic networks by high resolution mass spectrometry

Poster 072: P072
Improving lipid annotation coverage using intelligent precursor selection software on an Orbitrap-based mass spectrometer

Poster 073: P073
Comparison of normalization techniques for untargeted metabolomics of urine samples.

Poster 074: P074
Comparison of two sampling and extraction protocols to perform metabolomic studies on human adherent cells

Poster 075: P075
Development of a System Suitability QC Sample for Nano-Electrospray Direct Infusion Mass Spectrometry Metabolomics

Poster 076: P076

Evaluation of U-13C Spirulina (Arthrospira platensis) for stable isotope assisted untargeted metabolomics and liquid chromatography-isotope dilution mass spectrometry (LC-IDMS)

Poster 077: P077
Increased throughput and coverage for the annotation of Saponins using a structurebased MSn approach on a Tribrid Orbitrap mass spectrometer

Poster 078: P078
Application of 13C flux analysis in diagnosis of metabolic disorders

Poster 079: P079
Study on Intracellular Tracking of 13C isotope - labeled Volatile Cancer Markers

Poster 080: P080
Metabolomics profiling urine by Benchtop NMR

Poster 081: P081
Multi-platform and multi-matrix analytical development

Poster 082: P082
A comparison between a targeted and an untargeted approach in the frame of a pilot biomonitoring study

Poster 083: P083
Development and validation of a RPLC-MS/MS method for the quantification of ceramides in human serum of patients with CAD

Poster 084: P084
AlpsNMR: an R package for fully untargeted NMR-based metabolomics workflow

Poster 085: P085
Costs and benefits of switching from vendor-based to open source pipelines for untargeted LC-MS metabolomics

Poster 086: P086
Volatile Biomarker discovery in flatus by GC-MS and GC-IMS to diagnose digestive disorders and diseases.

Poster 087: P087
MetExplore: Omics data analysis in the context of metabolic networks

Poster 088: P088
BARSA: BidimensionAl nmR Spectra Annotation

Poster 089: P089
Assessing the impact of physicochemical parameters on the predictive capabilities of thermodynamics-based stoichiometric approaches under mesophilic and thermophilic conditions

Poster 090: P090
Uniting metabolomics data processing and highly confident annotation across six MS instrumental set ups: MetaboScape 5.0

Poster 091: P091
Integrated workflow with quality control for large cohort and clinical metabolomics research using robust hardware and signal correction

Poster 092: P092
Automatic annotation in untargeted metabolomics: a proof of concept protocol based on recurrent experiments

Poster 093: P093
Metabolomics results interpretation using recommender system embedded in metabolic network visualization

Poster 094: P094
Technological developments and opportunities with Workflow4Metabolomics

Poster 095: P095

Taxonomically informed metabolite annotation enhances confidence in specialized metabolomes annotation

Poster 096: P096
Improving prediction of essential genes using context-specific metabolic network ensembles

Poster 097: P097
Showcasing Longitudinal Metabolomic Datasets: Accessible, Fast and Simple

Poster 098: P098
Developing methods for targeted metablomics by LC-MS

Poster 099: P099
The SMRT dataset for machine learning-based metabolite retention time prediction.

Poster 100: P100
LC-MS/MS data analysis with xcms

Poster 101: P101
Targeting MDM2-dependent serine metabolism as a new therapeutic strategy for liposarcoma

Poster 102: P102
Biofluid Applications in Benchtop Low-frequency 60 MHz NMR: A Metabolomics Investigation

Poster 103: P103
Analysis of Penillium sclerotiorum metabolome by molecular networking

Poster 104: P104
FoodomicsGR National research infrastructure for the comprehensive characterisation of Foods

Poster 105: P105
Bioavailability of dietary long chain-PUFAs in the rat retina: mapping by MALDI imaging mass spectrometry

Poster 106: P106
Colorectal Cancer: Biomarkers and Effect Size

Poster 107: P107
Inflammation signaling by cardiac stromal exosomes

Poster 108: P108
Assessment of Preterm Infant's Nutrition by Untargeted Human Milk Lipidomics

Poster 109: P109
Exploring the use of GC-CI-MS for stable isotope labeling in metabolomics

Poster 110: P110
Development of a targeted urinary metabolic profiling technique for inflammatory bowel disease using Liquid Chromatography Mass Spectrometry with Electrospray Ionization Quantification.

Poster 111: P111 CEU MASS MEDIATOR: THE TOOL TO ANNOTATE COMPOUNDS IN CE-MS FOR METABOLOMICS

Poster 112: P112
Improved Metabolite Identification in a Single Injection with SWATH® Acquisition for Untargeted Metabolomics Workflow

Poster 113: P113
High-throughtput single-step sample preparation coupled to targeted LC-MS/MS approach for extended coverage of human plasma metabolome and lipidome

Poster 114: P114
In vivo isotopic tracing experiments reveal lactate as an oxidative substrate for brown/beige adipose tissues

Poster 115: P115

LipostarMSI: Comprehensive and Vendor-Neutral Data Analysis Platform for Mass Spectrometry Imaging

Poster 116: P116
SWATH® Acquisition Allows a Deeper Level of Comprehensive Metabolite Quantitation

Poster 117: P117
Community-based urine sampling methodology and biomarker technology for assessment of dietary exposure

Poster 118: P118
Simultaneous quantification of urine metabolites to allow comprehensive assessment of dietary exposure

Poster 119: P119
Utilizing the semantic web for kinetic modeling of metabolic disease pathways

Poster 120: P120
Untargeted Lipidomic analysis of bioactive lipids in epicardial adipose tissue and the effect of coronary artery disease status

Poster 121: P121
Using metabolomics to investigate the role of the gut microbiota in mediating the effects of diet on appetite in humans

Poster 122: P122
Seeking new biomarkers of atherogenesis in an atherogenic-prone down-sized pig model and their relevance in a cardiometabolic risk human cohort using LC-MS/MS lipidomics

Poster 123: P123
MS-based targeted metabolomics of eicosanoids and other oxylipins: analytical variability and interlaboratory comparison

Poster 124: P124
Migrating from PLS to Artificial Neural Networks – Adapting Interpretation Strategies

Poster 125: P125
Identification of biomarkers in Attention Deficit with Hyperactivity Disorder (ADHD)

Poster 126: P126
Metabolic signatures of urinary schistosomiasis and pharmacometabolomics of praziquantel treatment efficacy in children from rural Côte d'Ivoire

Poster 127: P127
Metabolomics at the top: Characterizing hypoxic responses and chronic mountain sickness in the highest city of the world

Poster 128: P128
Input of deep phenotyping in the metabolic syndrome stratification

Poster 129: P129
The circulating metabolites in the progression to islet autoimmunity and type 1 diabetes

Poster 130: P130
Milk molecular species of triacylglycerols characterized by lipidomic approach in cows and goats fed diets supplemented with various lipid sources

Poster 131: P131
Genome-scale metabolic modeling of human CD4+ T cells reveals ceramides as metabolic signature of Th17 and regulatory T-cell differentiation

Poster 132: P132
How metabolomic data support an innovative read across approach for safety assessment in cosmetics

Poster 133: P133
LC-MS-based semi-targeted lipidomics method: Application to the discovery of lipid biomarkers in diabetes

Poster 134: P134

Optimization of fecal NMR-based metabolomics to study the developing infant gut

Poster 135: P135
Biomarkers involved in glioma development in Drosophila melanogaster model

Poster 136: P136
Differential Mobility Separation Enhances the Quantification of Lysophosphatidic Acid in Plasma

Poster 137: P137 The Ichem'Algae ANR project: Untargeted chemotyping of algae bank by GC-MS and non invasive vibrational spectroscopy

Poster 138: P138 Prolonged Exposure to Milk Casein Results in Depressive Behaviour, Impaired Brian Development and Altered Metabolism in Wistar Rats

Poster 139: P139
Assessment of the Substrate Biodegradability in Anaerobic Co-digestion using a Metabolomic Approach

Poster 140: P40
Comparative metabolomics in Mamiellales

Poster 141: P41
Deciphering B .methanolicus metabolism for a one carbon-based production platform

Poster 142: P142
A Community-led Initiative to Develop and Promote Quality Assurance and Quality Control in Untargeted Metabolomics Research: the metabolomics Quality Assurance and quality Control Consortium (mQACC)

Poster 143: P43
Analysis of Endophytic Colletotrichum sp. strains by MALDI-TOF mass spectrometry and t-SNE Molecular Networking

Poster 144: P44
Fungal endophyte exo-metabolites alter the morphology and metabolome of the plant pathogen Fusarium graminearum: an LC-MS based metabolomics approach to unravel the biocontrol effect

Poster 145: P145
Evaluation of micro Pillar Array Columns (µPAC™) Combined with High Resolution Mass Spectrometry for Lipidomics

Poster 146: P146
Chemical mappings for the flowers of Abeliophyllum distichum using metabolomics tools

Poster 147: P147
METABOLOMIC INSIGHT IN THE RESPONSES OF STREAM BIOFILMS TO THE HERBICIDE DIURON AND ITS MODULATION BY ENVIRONMENTAL FACTORS

Poster 148: P148
The Power of MS/MSALL Acquisition for High-Throughput Metabolomics Studies

Poster 149: P149
Exploring a volatomic-based strategy to study Lavendula semiochemicals

Poster 150: P150
Plasma metabolomics to identify biomarkers of foie gras quality in mule duck

Poster 151: P151
Effect of organic fertiliser on metabolic profile of hydroponically cultivated tomato

Poster 152: P152
Tackking the antioxidant metabolomie of white wine

Poster 153: P153
Metabolomics approach reveals disruption of metabolic pathways in the marine bivalve Mytilus galloprovincialis exposed to WWTP effluent

Poster 154: P154
Molecular networking as a novel approach to unravel chemical diversity of Dinophysis spp. from French coastal waters

Poster 155: P155
Fast quantitative 2D NMR for targeted and untargeted lipidomics and metabolomics.

Poster 156: P156
4D-Lipidomics investigation of in C. elegans daf-2 mutants related to ageing and longevity

Poster 157: P157
Lipid profiling to identify changes in lipid metabolism in Caenorhabditis elegans upon starvation

Poster 158: P158
High speed untargeted 4D-Lipidomics LC-MS/MS workflows with Parallel Accumulation Serial Fragmentation (PASEF)

Poster 159: P159
Electrospray Ionization and samples complexity in Meta-metabolomics: a biomarker or a suppressed ion?

Poster 160: P160
Experimental strategy to discover new bioactive lipopeptides produced by gut microbiota: unknownknown approach by LC-HRMS.

Poster 161: P161
Novel UHPLC-MS method for detailed analysis of lipid species using a scheduled MS/MS acquisition approach for improved metabolite annotation

Poster 162: P162
Ammonium fluoride as suitable additive for HILIC-based LC-HRMS metabolomics

Poster 163: P163 Metabolomics profiles of Annona species cultivated in Egypt by using bioassay-guided fractionation process as antiproliferative agent.

Poster 164: P164
Targeting Esterified Oxylipins – Optimized Sample Preparation for LC-MS Analysis

Poster 165: P165
Stability of total oxylipins – influence of plasma generation and long-term storage

Poster 166: P166
Understand the metabolic crosstalk in photosymbiosis

Poster 167: P167
Molecular networks as a metabolomic tool to link traditional uses to biological activities and potential valuation of Polynesian plants

Poster 168: P168
A New Tool in Metabolomics: SWATH® Acquisition Analysis for Global Profiling and Quantitation

Poster 169: P169
How to automate boring lipidomic extraction?

Poster 170: P170
Development of lipidomic profiling by SFC-HRMS

Poster 171: P171
Parsimonious 13C Metabolic Flux Analysis

Poster 172: P172
The development of microbore UHPLC-MS assays to enhance sensitivity of untargeted metabolomic analysis of mammalian biofluids.

Poster 173: P173
MeTaQuaC: Quality Control Measures for Targeted Metabolomics Studies

Flash Poster 1: F1
Characterising the bidirectional biomolecular interactions between the intestinal microbiota and host drug metabolism

Flash Poster 2: F2
Rapid kinetics of lipid second messengers controls secretion in Toxoplasma parasites

Flash Poster 3: F3
Searching for differential metabolites between Mycobacterium tuberculosis and the MTBVAC vaccine using non-targeted approach metabolomics

Flash Poster 4: F4
Elicitor-specific reprogramming of potato's metabolome

Flash Poster 5: F5
Biosource-guided network annotation and visualization for untargeted metabolomics

Flash Poster 6: F6
Recommending substructures for unknown tandem mass spectra

Flash Poster 7: F7
Towards precision diagnostics: Untargeted metabolomics for the diagnosis of inborn errors of metabolism in individual patients

Flash Poster 8: F8
Metabolic maturation in the first two years of life in resource-constrained settings and its association with postnatal growth

Flash Poster 9: F9
Rapid LA-REIMS and comprehensive UHPLC-HRMS for metabolomics of biofluids in metabolic disorders

Poster 001: P001 *Revealing age-related sex differences in human lipidome: Metabolic phenotyping of Lausanne population*

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Introduction

Men and women have different predisposition to acquire metabolic disorders with age. However, beyond sex hormones, the sex-specific lipidome variation with age, in health and in disease, remains unexplored. Lipids have a far-reaching role in the regulation of metabolic processes and given the alterations in lipid metabolism with ageing, the sex-related framework of lipid variation needs to be defined along lifespan in a 'healthy' population, prior to relating altered pathways with a disease.

Technological and methodological innovation

We will comprehensively quantify complex lipid species and steroids in human plasma of the apparently healthy sex-matched subjects from 40 to 80 years old. To obtain the complex lipid profile, we have developed a high-throughput single-step extraction with BUME (butanol-methanol 1:1 v/v %) coupled to HILIC-ESI-MS/MS analysis. With our method we can measure approximately 600 lipid species including glycerolipids, cholesterol esters, sphingolipids, glycerophospholipids and free fatty acids.

Results and impact

Quantitative data on these structural, fuel and signaling lipids, will allow us to draw a comprehensive picture of plasma lipid profile. The correlation with the steroid profile will allow us to characterize its variation in the context of female and male reproductive cycle and hormonal status. The advanced data analysis will comprise the exploration of sex-differences within specific biochemical pathways in addition to associations with other clinical metadata.

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Poster 002: P002 *Untargeted metabolomic analysis for the discovery of treatment response biomarkers in breast cancer patients through Hotelling T2 multivariate profiling*

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Introduction

Breast cancer is the leading cause of women cancer-related deaths worldwide. Thus, new technologies with enhanced sensitivity and specificity for diagnosis and monitoring are in critical demand. The aim of our study was to identify those metabolites that suffered the greatest variations over time between patients who responded and did not respond to treatment in the four-breast cancer molecular subtypes: Luminal B, Luminal A, Triple Negative and Human Epidermal growth factor Receptor 2 positive.

Technological and methodological innovation

Plasma samples of 104 breast cancer patients were collected before any type of chemotherapy, prior to the surgery and before the surgery. They were classified into responders or non-responders based on Miller-Payne response. Samples were analyzed following an untargeted approach using liquid chromatography coupled to high-resolution mass spectrometry in positive mode. Multivariate Empirical Bayes Analysis detected differences in temporal profiles in the different biological conditions.

Results and impact

Hotelling´s T2 multivariate profiling allowed us to detect differential metabolites whose expression varied over time between responders and non-responders. It could be used to determine the existence of residual disease after neoadjuvant treatment and thereby contribute to the identification of patients who will absolutely benefit from additional treatment. LC-HRMS plasma metabolomics is a non-invasive, faster and less costly technique than the current screening methods for breast cancer.

Poster 003: P003 *Metabolomic investigation of immune cell activation by Liquid Chromatography coupled with High Resolution Mass Spectrometry (LC-HRMS)*

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Introduction

In recent years, it has been shown that some specific metabolites could be involved in immune cell polarization during infection, thus shaping the immune response [1]. Within the adaptive immune system, dendritic cells (DCs) play a central role, orienting the immune response according to the perceived danger signal [2]. While metabolism seems to significantly impact DC activation, the underlying mechanisms are still poorly described and understood.

Technological and methodological innovation

We aimed to develop a comprehensive method to get deeper insight into DC metabolic reprogramming. Monocyte-derived DCs were *in vitro* activated either with lipopolysaccharide (LPS, bacterial signal) or poly (I:C) (viral signal) and their corresponding metabolic responses studied by an untargeted LC/HRMS-based metabolomics approach [3]. Statistical data analyses were used for thoroughly assessing specific metabolic differences.

Results and impact

We described the first complete metabolic map of DCs (150 metabolites robustly monitored) and identified central metabolic changes following activation by LPS or poly(I:C). Two distinct signatures were obtained with alterations of four main metabolic pathways such as Krebs cycle. Our approach represents a powerful and simple way to robustly monitor the metabolic activation of immune cells that would facilitate the discovery of biomarkers that specifically sign a bacterial or viral infection.

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Poster 004: P004 *GH-OMICs: applying metabolomics to the fight against hormonal doping*

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Introduction

Growth hormone (GH), an endogenous protein regulating anabolism and lipolysis in humans, is known to be abused by the athletes to improve their performances. GH detectability is very limited due to its quick turnover and its variability across humans [1]. Given the several effects of GH on metabolism [2], the aim of this project is to i) develop a metabolomics strategy to identify GH users, ii) validate it and iii) determine its performance to propose its application as official anti-doping method [3].

Technological and methodological innovation

The project GH-OMICs uses metabolomics to study metabolic changes induced by GH, identifying "markers of effect" to track GH administration in athletes. Given the several and long-lasting effects of GH on metabolism, we expect to find markers exhibiting disrupted patterns longer than the GH turnover. In the method development step, urine and plasma samples of two groups of athletes treated either with EPO or EPO + GH [4] have been collected and analyzed using metabolomics and lipidomics approaches.

Results and impact

The results of the method development show that a longitudinal metabolomics screening of the athletes is able to identify correctly the GH users in spite of the confusing presence of EPO. Several biomarkers have been identified in both metabolomics and lipidomics [5]. Further validation with new experimental and real anti-doping samples is required to confirm the biomarkers, complete the biological interpretation and implement the official method.

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Poster 005: P005 *NMR metabolomics for understanding the role of alkaline phosphatase in neurotransmission and inflammation processes*

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Introduction

Tissue Non-specific Alkaline Phosphatase (TNAP) is a ubiquitous ectoenzyme located in different body tissues. Mutations of the TNAP gene are responsible for hypophosphatasia, a rare disease characterized mainly by defective bone mineralization caused by pyrophosphate accumulation in bone tissue and accompanied by neurological symptoms (pain, seizure…) [1]. We aimed in identifying metabolic pathways associated with TNAP activity in brain and in exploring its role in the inflammatory process.

Technological and methodological innovation

Untargeted ^{1}H NMR-based metabolomics was used on tissue extracts from two original models: (i) Akp2 mice, which is the rodent model of hypophosphatasia by invalidation of the TNAP gene and (ii) a model of postprandial inflammation based on food intake [2].

Results and impact

Eight metabolites differentiated TNAP-deficient mouse brain (in 7 day-old animals), among which GABA, adenosine and cystathionine were the most discriminant [3]. Metabolic changes were also observed in both liver and brain in the postprandial inflammation model. These metabolic changes suggest that TNAP is (i) involved in the production of antiinflammatory agents (adenosine) or their precursor (cystathionine, precursor of H_2S), (ii) regulated by pro-inflammatory cytokines.

References

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Poster 006: P006 *A multiplatform approach: HPLC, GC and NMR for urinary metabolomic analysis of muscle-invasive bladder cancer*

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Introduction

Bladder cancer (BCa) constitutes one of the most common cancers and the second most common malignancy of the urinary tract in the human population worldwide [1]. The main known risk factors of BCa include tobacco smoking, age, obesity, lack of physical activity, gender, genetic and occupational exposure [2]. This study aimed to perform and analyze the urinary metabolomic signatures of high-grade muscle-invasive BCa patients and healthy volunteers thanks to a multiplatform analytical approach.

Technological and methodological innovation

Three complementary analytical techniques were used: (i) high performance liquid chromatography (reversed phase and hydrophilic interaction) coupled with mass spectrometry (MS) detection in two ionization modes, (ii) gas chromatography with MS detection in full scan mode and (iii) proton nuclear magnetic resonance spectroscopy. Datasets were submitted to multivariate statistical analyses and discriminating compounds between patients and healthy urinary samples were identified.

Results and impact

The multiplatform analysis allowed to identify 17 urinary metabolites that differentiate bladder cancer patients and healthy volunteers at a statistically significant level. For the two metabolites hippuric acid and lactate, concentration changes were observed with the different analytical techniques. The present research showed that a combination of several techniques is better to maximize metabolite coverage and validate their variation, particularly for a biological fluid such as urine.

References

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Poster 007: P007 *NMR metabolic profiling can help discriminate between normal primary hepatocytes and diverging hepatic cancer cell lines*

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Introduction

Metabolic alterations are a hallmark of cancer cells. Recent studies suggest that the metabolic reprogramming of cancer cells is essential to support their growth in nutrient-restricted environments. We previously showed that Dt81Hepa1-6 cells, a highly tumorigenic derivative of Hepa1-6 murine cells, display increased metabolic plasticity *in vitro*. Recently, novel omics technologies have been shown to perform multi-parametric quantification of metabolic responses in normal and pathological cells. To characterize the metabolic pathways active in primary hepatocytes and these two diverging cancer cell lines under proliferative conditions, we examined production and consumption rates of metabolites *in vitro* using a nuclear magnetic resonance (NMR) metabolomic approach.

Technological and methodological innovation

Primary hepatocytes were isolated using the two-step collagenase perfusion method from C57BL/6 mice. Dt81hepa1-6 cells have been isolated after an in vivo passage of parental Hepa1-6 cells. Cells were cultured in vitro in high glucose DMEM (25mM) with 10% FBS. After 48 hours, medium was collected and cells were snap-frozen. The extraction of intracellular metabolites was performed using an ice-cold MeOH/CHCl3/H2O solution. Cell extracts and culture medium samples were analyzed using a cryoprobe Bruker 700 MHz AVANCE NMR spectrometer for both structure elucidation and quantification.

Results and impact

More than 30 metabolites were identified and quantified in both cellular extracts and extracellular culture medium. Metabolomic analysis revealed distinct metabolic profiles between primary hepatocytes, Hepa1-6 and Dt81-hepa1-6 cell lines. We identified several metabolites that were differentially regulated between normal and cancer cells (that both displayed increased glycolytic activity) as well as metabolites that were specifically associated with the highly tumorigenic Dt81Hepa1-6 cell line. Hence, a significant decrease in glucose, glutamine as well as a specific increase in lactate, alanine, acetate were found in the extracellular medium of both cancer cell lines. Interestingly, ketoacids by-products originating from the valine, leucine and isoleucine degradation pathway were found in the Dt81-hepa1-6 medium. Lactate, alanine, creatine and ATP were also increased in cancer cell compared to primary cells extracts.

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Poster 008: P008 *Quantitative metabolomics analysis reveals altered lipid profile in women with endometriosis: a pilot case-control study*

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Introduction

Endometriosis is a gynaecological disease characterised by the presence of endometrial tissues outside of the uterus. Presently, the gold standard for diagnosis is laparoscopy with an average delay of 8-10 years between symptom onset and surgical confirmation[1]. Thus, exploring early biomarkers of endometriosis is a clinical priority to shorten diagnostic delays. Most biomarker research to date has focused on proteomics of inflammation with few studies exploring the application of metabolomics.

Technological and methodological innovation

A novel quantitative mass spectrometry (MS) metabolomics approach was piloted in serum from 66 women with and without endometriosis from a case-control study. Serum samples were processed with the quantitative metabolomics kit MxP®Quant 500 and the extracts analysed by liquid chromatography (LC-MS/MS) and flow injection analysis (FIA-MS/MS), for small molecules and lipids, respectively. Multiple reaction monitoring in combination with internal standards served quantification of metabolites.

Results and Impact

The tested kit was able to quantify 102 small metabolites and 454 lipids in the serum samples. Statistical analyses revealed significant differences in the lipid profiles between women with vs without endometriosis (94% classification accuracy) but not in the profiles of small metabolites. 11 lipids in particular were as significant biomarkers through multivariate regression analysis. Quantitative metabolomics profiling offers a promising contribution to aetiological research on endometriosis.

References

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Poster 009: P009 *Steroid profile in human seminal fluid: is it linked to sperm quality?*

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Introduction

Steroids are essential hormones that play a crucial role in homeostasis of many biological processes including sexual development, spermatogenesis, sperm physiology and fertility [1]. Although steroids have been largely studied in common biological matrices such as plasma or urine, there is a lack of knowledge about the presence of steroids in the seminal fluid, as well as of their potential as indicators of sperm quality. In this study, we aimed to firstly develop a robust LC-HRMS (liquid chromatography coupled with high resolution mass spectrometry) strategy to map the steroidome in seminal fluid and study the relationship between the steroid profile and the seminal quality.

Technological and methodological innovation

In this study, a novel LC-HRMS strategy has been developed for obtaining the extended steroid profile in human seminal fluid. An optimized supported solid-phase extraction (SPE) with an automatic annotation (by DynaStI, a publicly available retention time prediction tool developed in house) were carried out for the steroidome method analysis. Then, the relationship between the steroid profile and the sperm quality was evaluated on samples from the FABER Swiss nationwide crosssectional epidemiological study [2] by choosing 200 seminal fluid samples [50 with high total sperm count (TS) and good morphology (M), 50 with low TS and good M, 50 with high TS and poor M and 50 with low TS and poor M].

Results and impact

Preliminary results showed different patterns of steroids altered. We observed that patterns of androgens were increased in subjects with high total sperm counts while the production of cortisol appears to be higher in subjects with lower sperm counts. The variations on the steroid profile provided insights for further understanding the biochemistry behind male infertility diseases.

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Poster 010: P010 *Combination of two semi-targeted approaches to detect toxicological biomarkers as a tool to understand the relation between red meat consumption and colorectal cancer promotion*

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Introduction

Among existing hypotheses to explain the link between red meat consumption and colorectal cancer promotion, heme iron is highly suspected as the key element of this mechanism. It catalyses reactive oxygen species production that subsequently attack dietary poly-unsaturated fatty acids giving rise to lipid peroxidation products (LPPs) that are known to be highly reactive towards biomolecules. Thus, monitoring our exposure to these compounds could be highly informative in a toxicological context.

Technological and methodological innovation

LPPs present in free form in the colon lumen can be detoxified via glutathione conjugation and elimination in urine as mercapturate conjugates. Two LC-MS strategies were set up to analyse these compounds. Urinary mercapturate conjugates were screened without a priori by neutral loss monitoring using DIA HRMS. In addition, free reactive aldehydes were analysed using a selective derivatization introducing a bromine atom enabling their detection via the specific isotopic pattern of bromine.

Results and impact

Analyses of urine and feces samples obtained from rats fed various diets supplemented or not with heme iron showed specific modulation in LPPs formation. Interestingly statistical analyses performed only on features associated to LPPs showed a much better segregation. These two approaches allowed highlighting the diversity of LPPs formed from various fatty acid precursors and evidencing new compounds linked to lipid peroxidation which exhibited cytotoxic properties as other known LPPs.

Poster 011: P011 *Mre11 nuclease activity removes the replication blocking nucleoside analogue Gemcitabine from the nascent strand during DNA replication*

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Introduction

Nucleoside analogues are clinically important anticancer drugs[1,2]. They exert their cytotoxicity by inhibiting nucleotide synthesis and through incorporation into nascent DNA during replication, causing chain termination[2,3]. A candidate for the excision of nucleoside analogues from DNA is Mre11. Using eukaryotic model organisms, we demonstrate that Mre11 nuclease activity is required for the removal of Gemcitabine from the nascent DNA strand during replication.

Technological and methodological innovation

MRE11-dependent removal of Gemcitabine from newly synthesized DNA during replication was assessed by LC-MS/MS based measurement of Gemcitabine content in wild-type and mutant cells and in cells treated with a nuclease inhibitor.

Results and impact

Our data show that the *Schizosaccharomyces pombe* nuclease mutants are sensitive to the nucleoside analogue Gemcitabine and show a higher amount of Gemcitabine in genomic DNA. Chicken lymphoblast DT40 cells treated with an MRE11 3'-5' exonuclease inhibitor also showed increased genomic Gemcitabine content. This suggests that Mre11 nuclease activity contributes to the removal of chain-terminating Gemcitabine from the nascent DNA strand in these cells.

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Poster 012: P012 *Metabolic characterization of Leishmania mexicana-infected macrophages*

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Introduction

Leishmaniasis is a Neglected Tropical Disease representing a major burden across the world, with an estimated incidence of 12 million cases and 350 million people at risk of developing the cutaneous or visceral form of the disease. In humans, Leishmania parasites invade macrophages and replicate within those cells as infection progresses. Thus, characterizing the macrophage-leishmania interaction at the metabolic level could lead to the identification of new drug targets to treat leishmaniasis.

Technological and methodological innovation

We employed untargeted and stable isotope labelling metabolomics approaches using [U-^{13}C]-D-glucose to study the effect of infection with *L. mexicana* on the metabolism of murine bone marrow-derived macrophages.

Results and impact

TCA cycle intermediates in infected macrophages incorporated higher levels of $^{13}C_3$, $^{13}C_4$ and $^{13}C_5$ labelling from D-glucose indicating higher TCA cycle activity, which has been associated with an anti-inflammatory phenotype (Mills & O'Neill, 2016). Our results also point to the existence of previously unreported metabolic routes in *Leishmania* parasites, including a novel degradation route for the immunomodulatory metabolite itaconate as well as a propionate detoxification pathway.

References

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Poster 013: P013 *A comprehensive analysis of metabolome and lipidome in different sleep deprivation models using a multiplatform technique of chromatography-mass spectrometry*

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Introduction

Sleep deprivation (SD) has been investigated for its association with chronic diseases and metabolic disorders. Until now, several metabolic changes were reported in various SD models. However, comprehensive investigation on the systemic and sleep-related regional effect were more to be explored.

Technological and methodological innovation

The multiple platform technique was used to induce SD and two different circadian-phase intervention was adopted for acute and chronic SD models. A multiplatform of chromatography-mass spectrometry, including untargeted and semi-targeted metabolomics and untargeted lipidomics, was conducted to exclusively profile and assess the biomolecules in serum, hypothalamus, and hippocampus for biochemical investigation from acute and chronic SD models.

Results and impact

From the annotated features in metabolomics and lipidomics results in acute SD model, 37 metabolites and 28 lipids were selected based on VIP score (≥1) from multivariate statistical analysis for validation of chronic SD model. Systemical metabolic alteration showed similar aspect between two SD models, whereas lipid alteration was model-dependent. Local metabolic perturbation was also observed in hypothalamus. As a result, our finding may extend the understanding biochemical perturbation in SD.

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Poster 014: P014 *Study on Alterations of Metabolome by Blood Transfusion using LC-Q-TOF and GC-MS*

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Introduction

Blood transfusions improve somatic state of patient for a while but it has effects on immune and non-immune systems. Allogenic blood transfusions during surgery are related with various dangers like allergic reactions, incompatibility and coagulopathy. Although, there were studies about side effect of blood transfusions, the global metabolic changes were almost unknown.

Technological and methodological innovation

In our study, metabolite changes produced by allogenic blood transfusions were researched applying our metabolomics technology in Lewis rats and 14SD rats. We operated liquid chromatography-quadrupole time-of-flight mass spectrometry (LC-Q-TOF) and gas chromatography-mass spectrometry (GC-MS) for profiling metabolome after allogenic and autologous blood transfusions.

Results and impact

Allogenic blood transfusions accelerate microenvironment change connected with relatively high expression of GLUT1/GLUT4. To support this event, there was abnormal expression of IL-6 and IRS1 which is glucose metabolism related enzyme. Also, in ABT groups, LysoPC and PLA2 were meaningfully changed compared to autologous groups. Synthetically, difference between autologous and allogenic blood transfusions was demonstrated. And connection with cancer associated metabolic changes was proved.

References

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Poster 015: P015 *Dried Blood Spots for the Determination of Reduced to Oxidized Glutathione Status*

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Introduction

Glutathione (GSH) to glutathione disulfide (GSSG) ratio is considered a clear indicator of intracellular redox status. GSH/GSSG determination in whole blood can be accomplished by liquid chromatography-mass spectrometry (LC-MS) after derivatization of GSH with N-ethylmaleimide (NEM). We propose an alternative collection procedure based on dried blood spot (DBS) testing in order to postpone laboratory processing providing accurate and reproducible results for both, GSH and GSSG.

Technological and methodological innovation

An experimental procedure was developed for the determination of GSH/GSSG in DBS samples after *in-situ* derivatization with NEM. In this protocol, filter paper was treated with NEM solution prior to blood collection, thus initiating GSH derivatization immediately after sample withdrawal. Using only 10 µL of blood and after up to 24 h of storage at RT, extraction of target species (GSH, GSSG) was carried out and further analyzed by means of LC-MS.

Results and impact

Levels of GSH and GSSG measured in DBS samples were comparable to those obtained by a reference method [1]. Filter paper impregnated with NEM could be stored at least for one week and the studied biomarkers were stable during at least 24 h at RT. DBS testing reduces the immediate manipulation of samples by clinical staff. We expect that this method will prevent an overestimation of GSSG and in turn, provide a means for an accurate determination of GSH/GSSG values reported in clinical trials.

References

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Poster 016: P016 *Preliminary investiogations of alkaloid composition from Thalictrum isopyroides plant of Azerbaijan Flora*

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Introduction

Alkaloids are high biological active compounds and secondary metabolites with a high pharmacological sphere. One of the plant species compositions with alkaloids of the Azerbaijan flora is Thalictrum isopyroides C.A. Mey. This plant is widely spread around the Greater Caucasus Mountains. It has been used as a folk medicine. It shows pharmacological activity such as anti-inflammatory, diuresis and anticancer effects [1] [3]. That is why it is beneficial to identify alkaloids of this plant.

Technological and methodological innovation

The air-dried roots, leaves and seeds of *T. isopyroides* were collected, powdered and extracted with 95% ethanol three times and after removal of ethanol in vacuum the extract was moistened with a 15% ammonia solution and extracted with dichloroethane. Extracts of different parts were collected using reference [2]. Thin layer chromatography (TLC) was carried with chromatography solvent system: chloroform-methanol-25% solution of ammonia (8:2:0.1), plate: Merck 254, and detector: Dragondorff's reagent.

Results and impact

In the work consequence of this division of T. isopyroides with the application, TLC has resulted and the retention factor (Rf) parameters are defined. From leaves, stalks of herb alkaloid extracts of T. isopyroides allocated and divided. Experimental researches by TLC, we have been identified from the roots (Ext. 1) - 6 spots (Rf - 0,19; 0,29; 0,36; 0,45; 0,51 və 0,74), from the upper ground parts (Ext. 2) - 3 spots (Rf - 0,12; 0,18 və 0,27). T. isopyroides plant research continues in this direction.

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Poster 017: P017 *Serum targeted and untargeted metabolomics-based analyses in infants with Ureteropelvic Junction Obstruction*

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Introduction

Ureteropelvic junction obstruction (UPJO) constitutes the predominant cause of obstructive hydronephrosis/neuropathy in both neonates and infants. Fundamental questions regarding the mechanisms of the diseases in the specific cohort remain still unanswered. The aim of this study was to elucidate potential differences through serum metabolic profiling of surgical cases of infants with UPJO compared to both non-surgical cases and healthy matched controls.

Technological and methodological innovation

Serum samples were collected from 19 patients preoperatively, 19 patients with mild stenosis treated conservatively, and 17 healthy matched controls and subjected to both targeted and untargeted metabolomics analyses by HILIC-MS/MS and RPHRMS, respectively. The combination of the two analytical platforms provided a holistic approach of serum metabolic profile of neonates and infants with UPJO, revealing a larger panel of biochemical pathways.

Results and impact

Both univariate and multivariate statistical analysis managed to highlight significant differences in the metabolic profile of the studied groups, distinguishing patients who required surgery from those followed by systematic monitoring as well as from healthy controls. A panel of metabolites, including creatinine, tryptophan, choline, and aspartate, showed high specificity of UPJO and could be used as early diagnosis biomarkers complementary to clinical practice.

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Poster 018: P018 *Investigating urinary and faecal metabolic differences among native Africans, African Americans and Alaskans using mass spectrometry*

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Introduction

Colorectal cancer (CRC) is the 3rd most commonly diagnosed cancer worldwide however, there is marked geographical variation in incidence rates [1]. Differences in diet may contribute to this variation, specifically the consumption of a 'Western', high-fat and low-fibre diet. This diet-associated risk is, in part, mediated by the colonic microbiota, specifically through the metabolism of undigested dietary residues, resulting in the production of either pro- or anti-carcinogenic metabolites [2].

Methods

To investigate the impact of these metabolites in mediating CRC risk, semi-targeted bile acid profiling and untargeted global profiling methods based on liquid chromatography-mass spectrometry (LC-MS) were used to elucidate the faecal and urinary metabolic differences between high- and low-risk CRC populations, with a view to identifying metabolites that contribute to/ help prevent CRC development.

Results and impact

Analyses of global profiling data revealed significant differences between the metabolic profiles of high- and low-risk groups, partly driven by increased faecal butenylcarnitine in high-risk groups. Semi-targeted bile acid analyses showed a general increase in carcinogenic secondary bile acids in high-risk groups, together with increases in lithocholic acid derivative, 3ketocholanic acid in those consuming a high-fibre, low-fat diet, suggestive of potential microbiota mediated detoxification.

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Poster 019: P019 *Alterations in plasmatic levels of metabolites involved in purine, urea cycle and tryptophan metabolic pathways during right ventricular remodeling in chronic thromboembolic pulmonary hypertension*

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Introduction

Chronic thromboembolic pulmonary hypertension (CTEPH) is a complication of acute pulmonary embolism, leading to right ventricular (RV) failure and premature mortality if left untreated [1]. In this context, metabolomic analyses were performed on plasma samples of CETPH patients in order to help for patient stratification according to the severity of the disease before surgery. In addition, histological characteristics were conducted on RV biopsies to confirm fibrosis and capillary depletion.

Technological and methodological innovations

Among 62 screened CTEPH patients, 10 were rigorously selected according to their clinical characteristics and compared with 10 controls. Untargeted metabolomic and lipidomic approaches were performed on plasma samples by LC-HRMS using a C18 and a ZIC-pHILIC Column coupled to an Exactive mass spectrometer for metabolomics [2] and a C8 column coupled to a tribrid Orbitrap Fusion mass spectrometer for lipidomics [3]. Data processing was achieved using the Workflow4Metabolomics platform [4].

Results and impact

Metabolomic results highlighted a dysregulation of the urea cycle, tryptophan and purine metabolisms in addition to significant histological alterations of RV in CTEPH patients such as macrophagic infiltration suggesting inflammatory processes compare to controls without RV dysfunctions. These preliminary results will serve as a basis of a longitudinal study of CETPH to determine prognostic biomarkers of right ventricle reshaping after surgery based on pulmonary thromboendarterectomy.

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Poster 020: P020 *Molecular networking-based mass spectrometry to decipher tumor cells metabolism*

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Introduction

The understanding of mechanisms that regulate the formation and evolution of tumor cells requires a careful study of its endogenous cellular metabolism. Recent advances in mass-spectrometry and related bioinformatics tools are opening new doors to the study of tumor cells metabolism.
This work focuses on mass spectrometry-based metabolomics coupled to molecular networking to highlight the metabolic adaptive mechanisms and expand the metabolic repertoire of tumor cells.

Technological and methodological innovation

Mouse primary Eμ-Myc lymphomas cells were cultured [1] and metabolites were extracted using methanol:acetonitrile:water (40:40:20, v/v/v). We developed an ion-pair ultra-high-performance-liquid chromatography coupled to high-resolution mass-spectrometry (IP-UHLPC-HRMS) method for fingerprinting the specific metabolism of Bcell lymphoma. Molecular networking tools (GNPS; [2]) will be used to reinforce the DLBCL metabolic repertoire and metabolic pathways.

Results and impact

A database of 34 chosen metabolites from 6 distinct metabolic pathways was set up to develop an IP-UHPLC-HRMS method in a SMART manner (Specific, Measurable, Attainable, Realistic, Timely). Detection of those metabolites in B-cell lymphoma extracts using single ion monitoring followed by data dependant MS2 serves as a proof of concept for further targeting a larger (100-150) metabolic cohort.
Molecular networking analyses will then be applied to detect all the metabolites within the same metabolic pathway.

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Poster 021: P021 *"-Omics- sciences and therapeutic"*

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Introduction

The aim of our poster is to present the capabilities of the new MetaboUCA platform of University Côte d'azur (UCA) in the field of "omics sciences". To illustrate this, we present 3 research projects.

Technological and methodological innovation

MetaboUCA is a platform of the Cote d'Azur University (UCA). It aims to coordinate scientific experts and heavy equipment (Nuclear magnetic resonance (NMR), Mass Spectrometry (MS)) around "omics" sciences. PFTC is an actor of MetaboUCA and is located in the Institute of Chemistry, Nice (ICN). The available techniques are NMR (500MHz, 400MHz and 200MHz), LC/MS/MS (2 systems), GC/MSn and molecular modeling.

Results and impact

In the first one, we investigated the metabolic composition of Anemonia viridis and its photosynthetic dinoflagellate endosymbionts in order to find biomarker of thermal stress. In the second one, we investigated the metabolic finger print of marine sponges to find relevant chemical biomarker in order to be able to discriminate Ircinia species. In the third one, we investigated the abilities a new series of C-nucleoside structure to bind The A site RNA.

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Poster 022: P022 *Metabolomic study of commercial mistletoe extracts used in alternative anticancer therapy*

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Introduction

Mistletoe is a parasitic plant reported to have anticancer activity and used in Europe as a complementary therapy. However, its effectiveness in treating cancer is not understood. Various companies sell extracts of mistletoe for injection by the subcutaneous route or directly intra-tumoral. Therefore, we undertook a metabolome study of these commercial extracts sold as food supplements to explore their composition considering the totum interest.

Technological and methodological innovation

An untargeted LC-HRMS metabolomics analysis was performed on 18 extracts of mistletoe commercial products from 3 producers at different concentrations.
A classical workflow was conducted on the online and freely available Workflow4Metabolomics (W4M) platform. For a better interpretation of the chemical space, molecular networking has been undertaken, using the user-friendly Metgem software. This approach aids in the recognition of structurally related chemical derivatives.

Results and impact

Multivariate exploration highlights metabolomic profiles significantly different from a company to another. Differences between extracts from a host plant to another are also observed. Moreover, our results clearly show that the composition of these extracts is very rich. Molecular networks allowed to separate and identify many classes of metabolites, such as amino acids, carbohydrates, carotenoids, diterpene alkaloids. Thus, their status of food supplements should really be questioned.

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Poster 023: P023 *Optimization of the untargeted metabolomics workflow for studying the impact of dysbiosis on the host*

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Introduction

The gut microbiota is known for its metabolic and nutritional functions, and moreover influences host immunity (1,2,3). This by direct interactions at the level of the intestinal mucosa, but also via soluble molecules, and in particular the bacterial metabolites. In order to study the impact of bacterial metabolites on immunity, mice were fed either with antibiotics or prebiotics to modulate their microbiota composition. Then, we developed a workflow of non-targeted metabolomics for this study.

Technological and methodological innovation

Mice (n= 18 mice/group) were fed with water, antibiotics or prebiotics. 10uL of plasma were deproteinized and analyzed by LC-MS/MS in positive mode on a QExactive plus connected to a Vanquish UHPLC System (ThermoFisher Scientific). The column used for the chromatography separation was an accucore RP column. Raw data were converted in mzXML with Proteowizard, then LC-MS feature detection and alignment was done with MZmine2.0 and statistical analysis with MetaboAnalyst 3.0 software.

Results and impact

We demonstrated that we got a rich metabolomics fingerprint from only 10 µL of mouse plasma. We obtained more than 700 features after data curation. Each group was well distinguished with a predictivity of 96.3% in an OPLS-DA model. We annotated 118 metabolites with GNPS and highlighted VIP metabolites (Amino acids metabolism, Tryptophan catabolites, bile acids …). Our workflow is suitable to understand modifications of the metabolomic activity caused by a modulation of gut microbiota.

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Poster 024: P024 *Rapid extraction and High-Resolution Tandem Mass Spectrometry analysis of the acylCoA metabolome*

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Introduction

Thioesters of Coenzyme A (CoA) carrying different acyl chains (Acyl-CoAs) may play a critical role in epigenetics and cell signalling. Their steady state concentrations and fluxes seem to reflect the metabolic state of a cell, determined by the genetic background and the environment, in particular fuel supply. One difficulty is the large concentration differences between acyl-CoA species and their varying chemical properties, ranging from very hydrophobic to rather hydrophilic chains. Here we attempt to obtain the acyl-metabolome of the lymphoblast cell line Reh, derived from acute lymphocytic leukemia, and to compare clones differing in their metabolism due to the knockdown of a mitochondrial gene.

Technological and methodological innovation

We first established a rapid extraction and metabolic quenching method to preserve endogenous acyl-CoA levels, followed by a novel high-resolution LC-MSMS method. The characterization of CoA and related substances was performed on a Vanquish UPLC system coupled to a Q Exactive plus Orbitrap mass spectrometer. The LC-MSMS method is based on a 24 min total run and using a reverse phase-chromatographic gradient. The dry cell pellets were dissolved into 30 µL of 30 mM ammonium acetate pH 6.5 containing 20% of methanol. Samples were centrifuged at 14000xg at 4°C during 3 min and the supernatant was transferred directly into LC vials. The injection volume was 10µL.

Results and impact

In the present work, we aimed at establishing an useful LC-MS/MS method for the profiling of a set of different acyl-CoAs in the lymphoblast cell line Reh that differ in their mitochondrial metabolism. Moreover, the use of Parallel Reaction Monitoring (PRM) technique allowed us to identify with high resolution and high precision a panel from short to long chain of acyl-CoAs.

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Poster 025: P025 *Serum metabolic profiling following traumatic brain injury in rats using 1H Nuclear Magnetic Resonance spectroscopy*

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Introduction:

Traumatic brain injury (TBI) is a complex disorder associated with short- and long-term neurological dysfunction. Subtle symptomology is a challenge in the management of mild TBI, that is commonly misdiagnosed, hence the need for biomarkers of injury severity. Circulating blood metabolites may be markers of pathological pathways altered in response to TBI. Here we use a 1H Nuclear Magnetic Resonance (NMR) spectroscopy biomarker approach to assess the metabolome of varying TBI injuries in rats.

Technological and methodological innovation:

Sprague-Dawley rats (n=28) were randomly assigned to naïve (n=4) or to TBI groups with different Controlled Cortical Impact (CCI) injury parameters (speed (m/s), depth (mm), tip shape): 6m/s, 2mm, flat (n=8); 8m/s, 1mm, flat (n=8); 8m/s, 2mm, flat (n=4); 8m/s, 2mm, round (n=4). Brains and serum samples were collected at 15min (n=12) or 24h (n=12) postCCI for histological and 1H NMR analysis respectively. Multivariate statistics was used to assess serum metabolic changes with injury severity.

Results and impact:

Histological evaluation showed that different CCI injury parameter combinations result in different lesion sizes at 15min and 24h with differential injury development. Multivariate modelling of 1H NMR serum data showed that the metabolic signature of the injury groups correlates with lesion volume measurements as early as 15min post-injury. This data suggests a predictive ability of the metabolome for lesion size and development, relevant for the clinical diagnosis of TBI injury severities.

Poster 026: P026 *Surface analysis of lipids in rat mesenteric fat tissue sections by MALDI-MS imaging to understand the impact of obesity*

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Introduction

In the obese state, excessive fat accumulation affects adipose tissue homeostasis, modifies the secretion of adipokines and cytokines by adipocytes, and favors the recruitment of immune cells [1]. The current interest in lipoinflammation has led to interest in understanding the heterogeneity and molecular remodeling of adipose tissue microenvironments. However, the spatial localization of lipids is lost when using traditional mass spectrometry as lipids are homogenized and extracted from tissue.

Technological and methodological innovation

MALDI LTQ Orbitrap XL MS (Thermo Fisher Scientific) was used to characterize the distribution of lipids in mesenteric fat of Sprague-Dawley rats under PPAR-pan activator (0-1000mg/kg/d) treatment [2]. Tissue sections (15µm) were sprayed with matrix solution of 2,5-dihydroxybenzoic acid (DHB), norharmane, α-cyano-4-hydroxycinnamic acid (CHCA) and 2,4,6trihydroxyacetophenone (THAP). Spectra between 200-1000 m/z were collected at 50µm step increments and 60,000 resolution.

Results and impact

MALDI-MS imaging allowed the visualization and mapping of 40 to 90 lipids in positive mode, including phosphatidylcholines, phosphatidylethanolamines, sphingomyelins, lyso-phosphocholine, phosphatidic acids, diacylglycerides and triacylglycerols (TG). Adipocytes presented a characteristic distribution of lipids and were identified as a TG-rich region. The data will be displayed and discussed to derive knowledge of the lipid species and distribution within mesenteric fat tissue.

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Poster 027: P027 *Combined transcriptomics–metabolomics profiling of the heat shock response in the hyperthermophilic archaeon Pyrococcus furiosus*

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Introduction

Microorganisms are frequently challenged by various environmental stresses that can result in structural damage and ultimately in disruption of vital cellular functions [1]. To survive, cells must be able to sense, respond, and adapt rapidly once sudden and adverse conditions arise. The archaeon Pyrococcus furiosus, which grows at 99°C, is regarded as a useful hyperthermophilic model. To better understand the adaptation strategies of this organism to thrive at such high temperatures, we used state-of-the-art omics approaches to investigate the metabolic response of P. furiosus to heat stress.

Technological and methodological innovation

To gain insight into how this model hyperthermophile copes with heat stress, we compared transcriptomic and metabolomic data of cells subjected to a temperature shift from 90 °C to 97 °C. In this study, we used RNA-sequencing to characterize the global variation in gene expression levels, while nuclear magnetic resonance (NMR) and targeted ion exchange liquid chromatography–mass spectrometry (LC–MS) were used to determine changes in metabolite levels.

Results and impact

At the transcriptomic level, 257 were upregulated and 295 were downregulated. At the metabolite level, 37 compounds were quantified. The level of di-myo-inositol phosphate, a canonical heat stress solute among marine hyperthermophiles [2], increased considerably (5.4-fold) at elevated temperature. Also, the levels of activated sugars were enhanced and it was concurrent with transcriptomics data. This work provides the first metabolomic analysis of the heat shock response of a hyperthermophile.

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Poster 028: P028 *Alternative carbon sources for the central metabolism of Trypanosoma brucei procyclic form*

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Introduction

The parasite Trypanosoma brucei adapts to the conditions encountered during the life cycle by remodeling its metabolism. In the glucose-free midgut of the insect vector, the procyclic form develops an elaborated energy metabolism with proline as a major carbon and energy source [1]. Intermediates from the TCA cycle like **α**-ketoglutarate (**α** -KG), succinate and to a lesser extent malate, can stimulate the oxygen uptake [2]. Here we explored in detail the metabolism of these three carbon sources.

Technological and methodological innovation

In this work, we used a dozen of T. brucei mutants impaired for enzymes of the TCA cycle, proline degradation and acetate production pathways in combination with isotopic profiling of the exometabolome by proton (1H) NMR analysis [3]. The assay allows distinguishing 13C-enriched from 12C-non enriched molecules. We used combinations of D-[U-13C]-glucose, L[U-13C]-proline, succinate, malate and **α**-ketoglutarate to quantify the compounds consumed and the final products excreted by the parasites.

Results and impact

We found that T. brucei re-metabolize succinate excreted from glucose degradation. In addition, malate and **α**-KG are metabolized and stimulate growth. Strikingly, **α**-KG is consumed 10 times faster than glucose, probably due to maintenance of the mitochondrial redox balance at the substrate level. By testing the three metabolites using 1H NMR analysis with mutants of enzymes from the TCA cycle, we show for the first time evidence supporting the functionality of a complete TCA cycle in trypanosomes.

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Poster 029: P029 *Characterization of bioherbicidal compounds stemming from micro-algae diversity by differential metabolomic and spectral similarity network assisted dereplication* ***(****ALL DREAM****)***

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Responding to public concerns about the impact of pesticides on health and the environment, European legislation has led to the development of new strategies to assess and limit the potential impact of different agricultural practices. Thus, the development of new "green" practices such as the use of biocontrol products are booming. In this context, biomass from the production of photosynthetic microorganisms can be an alternative to highlight. Indeed, micro-algae constitute, within the marine environment, a source of bioactive molecules. Micro-algae metabolites are active on a wide range of biological targets and can be recycled in various ways: bio-pesticide, cosmetic, pharmaceutical, etc.

"ALL DREAM" project is aimed to develop an innovative tool adapted to the analysis of the chemical diversity from the microalgae and evaluation of their potential as a bio-control product. This project proposes to combine, on the one hand, differential metabolic fingerprints and, on the other hand, analysis of the molecular diversity maps drawn by the study of "molecular networks. The "molecular network" is an innovative bio-informatics approach that allows characterizing classes of metabolites based on their spectral data and studying their degrees of similarity.

Through the use of molecular networks, data and complex mixtures can be compared in silico and annotated. This approach facilitates the study of the metabolome of living organisms and the characterization by dereplication of the studied active metabolites.

For this project, we selected 150 extracts of microalgae from various families that were subjected to a microplate photosynthesis inhibition as well as antioxidant assay. Their chemical fingerprints were analysed by a meabolomic approach and spectral similarity networking to facilitate the annotation and characterization of bioactive compounds. The data was first processed via W4M platform and R software, then subjected to a comparative analysis between the MetGem and GNPS platforms and finally processed under Cytoscape for their annotation and VIP search. Second, active extracts were subjected to bio-guided fractionation via an LC-PDA-ELSD coupled with a fraction collector and LC-HRMS to confirm the characterization of active metabolites. The active extracts are then tested on young seedlings to analyse their herbicide effect.

This poster presents all the results highlighting the potential of this "blue" resource that are microalgae as well as the "green" alternative they represent for the agriculture of the future.

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Poster 030: P030 *Threonine metabolism in Leishmania*

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The parasitic protozoan Leishmania is responsible for various clinical manifestations in humans. Deletion of the glucose transporter genes in the Leishmania □GT1/3 mutant1 induced a possible shift to amino acid metabolism, and seemed to point to threonine as a new carbon source. Indeed, serine hydroxymethyltransferase (SHMT) and aldehyde dehydrogenase (ALDH) are two

possible enzymes of threonine catabolism found overexpressed2 in the □GT1/3 mutant.

By combining different approaches, i.e. overexpression of SHMT and ALDH, gene inactivation using CRISPR-Cas9 technique and NMR analysis of metabolites excreted by the parasite using enriched carbons sources (threonine), we were able to study the involvement of these enzymes in the energy metabolism of the parasite.

We have demonstrated that ALDH is indeed involved in the catabolism of threonine by catalyzing the transformation of acetaldehyde into acetate, while SHMT does not seem to be involved in this metabolic pathway. However, we identified a third enzyme, threonine aldolase (TA), which converts threonine into acetaldehyde, thus allowing the reconstitution of threonine degradation pathway in Leishmania. Future studies will focus on the requirements of these enzymes over the parasite's life cycle.

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Poster 031: P031 *Fluorinating Putida: Annotating Novel and Undesired Metabolites*

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Introduction

SinFonia, an EU H2020 project, aims to integrate the nonnative element fluorine into the metabolism of Pseudomonas putida to produce novel fluorinated biopolymers [1]. Metabolic rewiring may result in an accumulation of non-canonical metabolites that induce toxicity or cause poor yield [2,3]. To identify these byproducts, non-targeted mass spectrometry will be used to screen for fluorinated and undesirable metabolites in engineered bacteria.

Technological and methodological innovation

Engineered systems may lack sufficient metabolite repair capacity, which we aim to counteract by screening for metabolite damages in our cell factories and envisaging strategies to repair them. Metabolite repair may be particularly important in SinFonia due to the load of adding heterologous pathways and a nonnative element to create new-to-nature products. If successful, SinFonia will provide a less hazardous and more sustainable solution to synthesizing fluorochemicals.

Results and impact

Untargeted LC-MS and GC-MS was used to measure metabolic differences in engineered P. putida strains induced or not to produce polyhydroxyalkanoates, the chemical backbone in which we aim to incorporate fluorine. Prioritize features were annotated using internal and external libraries, metabolic models, and MetFrag [4]. These methods will be used to identify undesirable (fluoro)metabolites and construct metabolite repair modules to suppress toxicity and maximize polymer accumulation.

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Poster 032: P032 *Analysis of metabolome time-variations of an endophytic fungal strain of Botryosphaeria mamane through untargeted LC-MS profiling*

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Introduction

The metabolome of all organisms change overtime as a result of all the dynamic biochemical processes involved in metabolism [1]. Nevertheless, fungi metabolome has not been extensively studied in terms of time-variations, which is an important factor to consider in gaining access to a greater chemical diversity [2]. This work aimed at studying the metabolome variations of an endophytic strain of Botryosphaeria mamane (BM) with a focus on 3 bioactive thiodiketopiperazines already isolated [3].

Technological and methodological innovation

Botryosphaeria mamane was cultured during 28 days at 27ºC under natural light in 6-well plates containing malt extract agar. Each fungal culture (from day 1 to 28) was extracted with DCM-MeOH-H2O 64:36:8 (v/v/v) and crude extracts were then analyzed by UHPLC-DAD-LTQ Orbitrap XL. MZmine 2.51 and Metaboanalyst 4.0 software were used for data treatment and data analysis, respectively.

Results and impact

Chemical profiles highlighted global variations in the BM metabolome due to the production and metabolization of secondary metabolites. Incubation time is an important parameter to consider in the search for new compounds such as the maximum observed detection of the TKP's, between day 5 and 7. Moreover, the TKP's appeareance dynamic could give us insights to understand their pathways in order to optimize the production of these bioactive metabolites.

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Poster 033: P033 *Study of the production of (phyto)hormones by mycorrhizal fungi*

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Introduction

Arbuscular mycorrhizal fungi (AMF) live symbiotically with 72% of land plant species for which they have played and still play a major nutritional role.

Phytohormones are involved in the establishment of this symbiosis, but their role has been mainly examined from the plant side. Since other fungi were reported to produce phytohormones [1], and putative receptors of cytokinines and ethylene were identified in AMF [2], we wondered if AMF produce and/or perceive phytohormones.

Technological and methodological innovation

A protocol of global extraction and enrichment of phytohormones was adapted to different fungal samples, and their detection and identification by LC-MS was optimized. Isotopic labelling assays were performed to establish their fungal or plant biosynthetic origin. Ethylene production and biosynthesis pathways were investigated by GC-FID. Cytokinin and ethylene effects were assessed by bioassays and RNAseq.

Results and impact

We identified for the first time an AMF production of ethylene, and an AMF exudation of several phytohormones. Identification of the metabolic origin(s) of these hormones and their role in AMF biology and in AM symbiosis still require further investigation.

This work is the first step toward the study of phytohormones as inter-kingdom mediators and genuine fungal hormones.

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Poster 034: P034 *Metabolites characterization of nine cyanobacteria isolated from thermal mud.*

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Introduction

Cyanobacteria are photosynthetic microorganisms that colonize diverse environments worldwide, ranging from ocean to freshwaters, soils, and extreme environments. Their adaptation capacities and the diversity of natural products that they synthesize support cyanobacterial success in the colonization of their respective ecological niches. Although cyanobacteria are well-known for their toxin production and their relative deleterious consequences, they also produce a large variety of molecules that exhibit beneficial properties with high potential in various fields [1].

Technological and methodological innovation

As part of my thesis project (CIFRE project in partnership with the thermal institute of Balaruc-Les-Bains, France), we decide to study the diversity of metabolites produced by 9 cyanobacteria isolated from thermal mud. We used two approaches: metabolites prediction by metagenomics analysis and identification of metabolites produced by LC-MS/MS. We decided to check 3 extraction methods (water, acetone and methanol) according to the large chemical diversity of cyanobacterial metabolites [2] [3].

Results and impact

We showed that the genomic potential for secondary metabolite is important for the 9 cyanobacteria. Surprisingly, there is a lot of unknown cluster into our metagenomes, despite the growing number of cluster describes until now [4]. As expected, we showed that there is a huge difference in metabolites extracted between the 3 methods used. Surprisingly, acetone extraction (often used in plant extraction) is not the most effective one, with a weak number of metabolites extracted. We also confirm the absence of cyanotoxins (through the use of standards to identify them) which is important for further development and exploitation of the cyanobacteria.

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Max 5

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Poster 035: P035 *Metabolomics in plant-microbe interactions: "MetaboHUB-MetaToul-plant metabolites" platform facilities*

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The MetaboHUB-MetaToul-Plant Metabolites (MV) facilities hosted at the LRSV belong to the Metabolomics and Fluxomics platform of Toulouse and is part of the French resource core MetaboHUB. The MV platform provides research and development services dedicated to the characterization of metabolomes from plants, fungi or bacteria in the field areas of agrobiosciences and phytochemistry.

Targeted quantitative approaches are routinely available such as phytohormonomics which allows the detection and quantification of more than thirty phytohormones from plant (roots, aerial samples) or microbes in a single experiment. In addition, other classes of plant secondary metabolites are reachable or under development (signaling molecules, elicitors derived of amino acids, blumenol…), or specific fungal and bacterial metabolites (chitinic molecules).

A dedicated workflow for untargeted metabolomics has been developed in the platform to provide a global metabolite mapping from complex extracts and detect compounds of particular interest with the use of specific multivariate data analysis. This approach is endorsed to our in-house databases of more than 1000 standards and an in silico database of 500 000 unique metabolites to improve annotation rates.

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www.lrsv.ups-tlse.fr/metatoul/
www.metatoul.fr
www.metabohub.fr

Poster 036: P036 *Investigating the effects of future climate scenarios in soft fruit: application of HPLC-PDA-MS metabolite profiling in blackcurrant cultivated under increased temperature and altered light regimes*

Introduction J. William Allwood[1]; Tomasz L. Woznicki[2, 3]; Yun Xu[4, 5]; Alexandre Foito[1]; Kjersti Aaby[6]; Julie Sungurtas[1]; Sabine Freitag[1]; Royston Goodacre[4, 5]; Derek Stewart[1, 7]; Siv F. Remberg[3]; Ola M. Heide[8]; Anita Sønsteby[2]

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Introduction

Blackcurrant (Ribes nigrum L.) is an excellent example of a super fruit with potential health benefits. Both genotype and cultivation environment (temperature, solar radiation and precipitation) are known to affect the chemical composition of blackcurrant, especially ascorbic acid and various phenolic compounds (e.g. anthocyanins amongst other quality traits). The objective was to deepen understanding of the effects of temperature and day length, on fruit quality and chemical composition. The studies relevance is accentuated by predicted and ongoing changes in global climate.

Technological and methodological innovation

A phytotron experiment with cultivation of single-stemmed potted plants of blackcurrant cv. Narve Viking was conducted at 12, 18 or 24 °C, and three different photoperiods (short day, short day with night interruption, natural summer conditions). Plants were also grown outdoors. Ripe berries were analyzed via HPLC–PDA–LTQOrbitrap XL MS to detect metabolites affected by the climatic factors. By applying online DDA- MS2 and MS3, 53 compounds were identified at MSI level 1, 103 and 221 at MSI levels 2 and 3, respectively.

Results and impact

Temperature had significant effect on a total of 365 metabolites. Tryptophan was upregulated as temperature increased, in turn impacting upon the regulation of Indole Acetic Acid and its control of fruit ripening. Phenylalanine, the major flavonoid precursor, also increased with temperature, as did its downstream metabolites including catechins, anthocyanins, anthocyanidins, flavones and flavanols. The levels of delphinidin- and cyanidin- 3-O-rutinosides (60% of total anthocyanins), were elevated between 12 - 18 °C, before being strongly reduced at 24 °C, suggesting that global warming will have large negative impacts on anthocyanin content. Interestingly, 34 annotated flavonoids were more abundant under outdoor conditions, illustrating the impact that UVA has on their synthesis. Based upon the most abundant anthocyanins, a comparison between targeted and untargeted analyses, revealed a close convergence of the methods, illustrating the precision of the metabolomics approach.

Poster 037: P037 *The role of food polyphenols in energy metabolism*

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Introduction

Polyphenols are secondary metabolites naturally synthetized in plants and have demonstrated biological properties against disorders related to oxidative stress or inflammation, among others. Lippia citriodora, which is used as food seasoning or infusion, is rich in verbascoside, a potent anti-inflammatory phenylpropanoid. Theobroma cacao is worldwide consumed and common ingredient of many food products. Cocoa, containing proanthocyanidins, is a valuable source of antioxidant compounds.

Technological and methodological innovation

The antioxidant and anti-inflammatory properties of these extracts were tested in 2 mouse embryonic fibroblast (MEF) cell lines: a double knock-out mouse of paraoxonase-1, an important endogenous antioxidant enzyme, and a double knock-in mouse for monocyte chemoattractant protein 1, a pro-inflammatory cytokine. MEF wild-type were used as lean samples. Targeted metabolomics of energy metabolism was performed on gas chromatography coupled to a QTOF mass spectrometer and an electron impact source [1].

Results and impact

Energy metabolism changes drastically when MEF are incubated with LPS. *L. citriodora* and *T. cacao* have an important effect on energy metabolism in MEF PON-1 KO and MCP-1 KI incubated with LPS, leading metabolite values to these found in MEF wild-type. Special attention merits a-ketoglutarate since both plant extracts decrease strongly its levels. These results could be an approximation of how polyphenols act in oxidation or inflammation models.

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Poster 038: P038 *Study of dietary polyphenols metabolites by a top-down metabolomics approach*

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Introduction

Phenolic compounds from plant sources have been reported to have beneficial properties for the treatment of several human chronic diseases [1]. Traditionally, the study of these compounds has been carried out using a bottom-up approach (from *in vitro* to *in vivo* models). Therefore, our goal is to propose a top-down approach, starting from human *in vivo* assays, with the purpose of detecting metabolites derived from these compounds present in plants as well as knowing their mechanisms of action.

Technological and methodological innovation

The first stage corresponds to nutritional intervention assay in humans. In this trial, volunteers ingest supplements rich in phenolic compounds from *Lippia citriodora*, *Hibiscus sabdariffa*, *Olea europaea*, *Silybum marianum* and *Theobroma cacao*. After that, biological samples are collected at different times after intake and the identification of circulating metabolites is carried out using analytical techniques such as liquid chromatography coupled to mass spectrometry (HPLC-ESI-qTOF-MS).

Results and impact

The expected results of the use of this new approach will allow us to know which metabolites are really responsible for the bioactivity properties of different plant sources rich in phenolic compounds. In addition, a great advance of knowledge in relation to metabolic transformations and mechanisms of action of phenolic compounds will be achieved.

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Poster 039: P039 *NSP19 an original eco-extract from Dioscorea villosa with neuroprotective effects in in vitro Alzheimer's disease models*

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Introduction

Increasing evidence suggests that a loss in estrogen can increase the women's risk of developing Alzheimer's disease (AD)[1]. AD is a neurodegenerative disease characterized by a progressive loss of neurons associated with memory deterioration. Postmenopausal women receiving estrogen replacement therapy would exhibit a decreased risk of developing AD. Diosgenin, one of the most important steroidal saponins of *D. villosa,* has been shown to protect neurons via estrogen receptor pathway.

Technological and methodological innovation

Here, we have prepared an optimized ecofriendly extract from *D. villosa* rhizomes (NSP19) using microwave technology and green solvents, water and ethanol. NSP19 was tested on primary neuronal culture injured with beta Amyloid peptide (Aβ) or glutamate, cellular models of AD [2]. A deep analysis of the extract was performed using UHPLC-DAD-ELSD technology in order to evaluate Diosgenin content and associated actives.

Results and impact

NSP19 showed a significant neuroprotective effect. We proved that a) a synergistic effect between Diosgenin and phenolic compounds was involved in this neuroprotective action, b) a specific ratio of these compounds was needed to get the optimal neuroprotective efficacy of NSP19. Altogether, these results support the usage of NSP19, a steroidal natural product, as a food supplement that may prevent cognitive dysfunction and therefore reduce the risk of Alzheimer's disease in postmenopausal women.

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Poster 040: P040 *Digging out the terroir influence on bioactive polyphenols from grape stems: A correlation_network_ driven approach to spatialize metabolomics data.*

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Introduction

Grape stems are low-cost viticultural by-products rich in bioactive polyphenols. While their phytochemical composition highly depends on the genotype [1-3], the terroir influence is still unknown. In oenology, terroir defines all the interactions between genotype, environment and viticultural practices that give wine typicity [4]. Here, we hypothesized that change in environmental conditions, particularly in soil properties, may influence polyphenol composition.

Technological and methodological innovation

A vineyard parcel planted with a unique grape clone covering four different geological layers was selected. Grape stems were harvested at the same GPS positions over 3 consecutive years and analysed by UPLC-DAD-MS-based metabolomics targeted on 43 polyphenols. Correlation-based networks were employed to select co-varying metabolites conserved over the three years and Geographic System Information (GIS) was used to study the spatialization of clustered metabolites.

Results and impact

Beyond vintage effect, principal component analyses on intra-vintage data were reproducible and showed metabolites covariation by polyphenol subclasses. Flavonoids were spatialized on sandy soils when stilbenoid DP4 over-accumulated on loamy-silty soils. The unprecedented combination of metabolomics correlation-based networks and GIS mapping represents a powerful workflow to spatialize field omics data, a key challenge in precision agriculture.

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Poster 041: P041 *Development of a UHPLC-MS/MS targeted method in various matrices for the quantification of toxicologically relevant aldehydes produced from lipid peroxidation*

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Introduction

Lipid peroxidation catalyzed by dietary heme iron leading to reactive aldehydes such as hydroxyl-alkenals (e.g. HHE and HNE) is a major hypothesis for linking red and processed meat-rich diets consumption with an elevated risk of colorectal cancer (CRC) [1]. The quantification of these representative hydroxyl-alkenals in different matrices is therefore of central importance to assess their occurrence and correlate it with other biological and biochemical markers of CRC promotion.

Technological and methodological innovation

UHPLC-ESI-MS/MS method has been developed and validated for HNE and HHE quantification in fecal water [2]. Samples were derivatized in-situ using a brominated reagent (BBHA) in presence of deuterated internal standards (HNE-D_{11}/HHE-D_5), extracted by solid phase extraction, and then analyzed by LC-positive ESI-MS/MS in MRM mode. This method was then extended to be used in other matrices, such as plasma by adding a protein precipitation step to the sample pretreatment.

Results and impact

BBHA allowed efficient stabilization of the reactive aldehydes [3] which detection is based on characteristic transitions monitored from bromine isotopic peaks and selective MRM mode. The method was validated according to the EMEA guidelines [4], for selectivity, sensitivity, linearity, carry-over effect, recovery, matrix effect, repeatability, trueness and intermediate precision. The method is compatible with the detection of physiological HNE / HHE levels (ca. 100 nM) in the studied matrices.

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Poster 042: P042 *LC-MS based untargeted metabolite profiling of wholegrain spelt (Triticum spelta) cultivated in Switzerland*

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Introduction

Spelt is an ancient cereal related to common wheat, now being rediscovered due to their eco-friendly agronomical and valuable nutritional characteristics[1]. Wholegrain spelt is rich in dietary fiber and bioactive phytochemicals, which may reduce the risk of some chronic diseases[2, 3]. We developed a novel robust high-throughput method based on untargeted metabolomics to evaluate phytochemical profiles in various spelt varieties from a wide range of breeder diversities.

Technological and methodological innovation

A panel of 124 spelt varieties were cultivated under uniform conditions in the field in 2016. Phytochemicals of their grains were extracted with an automatic sample preparation device, and further analysed with a UPLC-ESI-Q-TOF-MS based untargeted metabolite profiling approach. The MS data from each variety was converted into markers for comparison. Multivariate statistical analysis was applied to find the characteristic markers, enabling us to find the discrimination among the varieties.

Results and impact

Our research indicated that metabolite profiles were significantly different among spelt varieties. Characteristic markers were tentatively identified by in-house and public databases. Abundance of some compounds of interest can be compared efficiently among varieties with semiquantitative metabolomics data. The results of this study will provide insights for better selection of spelt varieties in agriculture with a high nutrient content and improvement of spelt food products in the future.

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Poster 043: P043 *Isotope Labelling and Molecular Networking to Identify New Fungal Secondary Metabolites*

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Introduction

Characterization of fungal secondary metabolomes has become a great challenge in the last decades due to both the emergence of fungal threats leading to the destruction of food, and the industrial interest of many natural products. Fungal toxicity and the spreading of fungi into stock feed and food are caused by secondary metabolites. Therefore, it is crucial to characterize the metabolites produced by fungi and investigate the food contamination by potentially not yet known mycotoxins.

Technological and methodological innovation

A strategy based on double isotope labelling and untargeted metabolomic high resolution MS based analysis was adopted [1]. This strategy enabled the specific detection of metabolites naturally produced by a fungus, the unambiguous determination of their chemical formulas, and the generation of curated molecular networks using GNPS [2] and MetGem [3]. The combination of these 2 networks, curated using isotopes, allowed at the end the identification of unknown metabolites of Penicillium nordicum.

Results and impact

By this way, 92 metabolites were detected following infection of a natural substrate by P. nordicum, among which 69 were completely unknown. Two curated molecular networks of MS/MS data, specifically generated on the features of interest, highlighted 13 fungisporin-related metabolites previously unidentified in this fungus and 8 never observed in any fungus. The combination of the two molecular networks also allowed to identify some unknown fungisporins [4].

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Poster 044: P044 *Specific derivatization with isotopic labeling introduction and mass spectrometry to monitor lipid peroxidation carbonyl compounds in the intestinal lumen by both targeted and non-targeted approaches.*

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Introduction

Diets rich in red meat are associated with an elevated risk of colorectal cancer. A major hypothesis involves dietary heme iron which induces the formation of lipid oxidation products such as reactive aldehydes[1]. A strategy based on the simultaneous derivatization and stable isotope labeling of these carbonyl compounds is proposed, enabling both targeted monitoring of representative aldehydes and non-targeted carbonyl profiling to get an image of the lipid peroxidation in the intestinal lumen.

Technological and methodological innovation

Fecal water samples from rats fed various pro-oxidant diets were derivatized in situ using 1-[(aminooxy) methyl]-2bromobenzene hydrochloride (BBHA) [2] and analyzed by LC-ESI-MS after SPE extraction. High resolution MS (LTQOrbitrap) is used to filter the signals of the brominated compounds and derivatized aldehydes are identified by MS/MS by comparison with standards. LC-MS/MS in the MRM mode (TSQ) is used for the targeted quantification of two aldehydes (HHE and HNE) in internal calibration.

Results and impact

Derivatization by BBHA makes it possible to stabilize the reactive aldehydes and to use the characteristic isotopic signature of the bromine atom for specific detection by both non-targeted and targeted approaches. On the one hand, LC-HRMS allows specific signal filtering to obtain a qualitative image of lipid peroxidation in colon lumen without a priori [3]. On the other hand, targeted LC-MS/MS made it possible to quantify HNE and HHE using a method validated according to EMEA guidelines [4-5].

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Poster 045: P045*Characterization of A. thaliana responses to plant defence elicitors using untargeted metabolomics*

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…

Introduction

The green revolution of the past century has led to an explosion in agricultural yields, supported by the intensive use of pesticides and chemical fertilizers. The limits of this model are gradually being revealed, leading to a change in the mindset from producing "more" to producing "better". Today the objective is to develop innovative solutions based on natural & biocontrol concepts. Among the proposed solutions, the elicitation of plant defense responses is a strategy for the control of diseases and pests. The concept of induced resistance is based on the application of a natural (plant extract, microorganisms, and mineral) or synthetic (hormone analogues) product, which triggers natural defence mechanisms and thus enables the plant to be in a state of resistance to a pathogen during an attack1. Signaling pathways leading to the induction of plant defenses involved phytohormones and notably salicylic acid. Arabidopsis thaliana is a model organism for the study of physiological, cellular and molecular factors in plants. In this sense, it is also a good model to study the mechanisms involved in plant resistance and to better understand the mechanisms underlying elicitor protection. In our study 3 elicitors were tested: a commercialized synthetic product (Bion - Syngenta) containing a functional analog of the phytohormone Salicylic acid, an essential oil for Gaultheria procumbens (GEO) containing more than 95% of methylsalicylate2 and an extract of seeds of Fenugreek (Fen) known to activate A. thaliana defense through the salicylic acid signaling pathway3. The objective of our study was to characterize the plant response to these three compounds using an untargeted metabolomics approach.

Technological and methodological innovation

Obtaining precise information on the chemical composition of complex natural extracts is a challenging task that requires sophisticated methods.4 In this project we have developed, with the MetaboHUBMetaToul-Plant Metabolites platform, a method using liquid chromatography high resolution tandem mass spectrometry (LC-HRMS/MS) to compare the effect of plant defense elicitors on the metabolomic profile of Arabidopsis thaliana. Plant leaves were treated with solutions containing each of the three products and tissues were harvested 48 hours after the treatment. To analyse the results, a workflow including MS-Dial and MS-Finder softwares have been used to annotate and identify peaks with databases (PlantCyc, HMDB…)5. The identification of compounds whose accumulation was correlated with the treatments has been investigated by a multivariate and univariate statistical analysis combination.

Results and impact

This study showed common and specific plant metabolomic responses to the three treatment, Even if their activity relies to the salicylic acid signaling pathway. Globally, GEO treatment increases phenolic glycosides compounds whereas Bion treatment increases alkythiols and Fen treatment increase both flavonoids and fatty acids. This result highlights the significant role of downstream metabolic response regulation activated thought common signaling salicylic acid pathway.

Poster 046: P046 *Metabolomics implemented to the Equine Biologic Passport (EBP) for doping control.*

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Introduction

In the horseracing industry drug of abuse is an important concern and the doping control laboratories continuously develop new strategies to detect misuses of doping substances. In this context, the LCH began in 2009 the development of the Equine Biologic Passport (EBP) based on omics and biomarkers monitoring. The EBP is applied to the follow up of the best French trotters and combined different levels of monitoring including implementations of metabolomic approaches.

Technological and methodological innovation

LC-HRMS and GC-MS/MS metabolomics was conducted on horse urine samples collected after administration with growth promoters, erythropoiesis stimulating agents, immune castration. OPLS-DA models were developed allowing discrimination between treated and control animals[1-4]. These models are employed in the EBP and allow to routinely control French trotters at training. An analytical strategy based on quality controls enables to normalize data, manage analytical drifts and correct batch effects.

Results and impact

The results are routinely processed and the OPLS-DA models constitute new potential methods in the fight against doping. These models highlight metabolic disturbances due to the misuse of doping agents and provide a convenient method for result interpretation. Models are continuously improved with additional population studies. Moreover, annotation and structural identification of relevant features are in progress and could possibly find out new candidate biomarkers for doping control.

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Poster 047: P047*Unravelling the impact of the antidepressant venlafaxine on the metabolome of meagre fish*

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Introduction

There is a growing interest on the application of metabolomics in environmental science to study how the exposure to xenobiotics affects the metabolism of aquatic organisms. The present work aimed to evaluate the changes in the metabolome of the juvenile fish Argyrosomus regius when exposed to the antidepressant venlafaxine (VLF). VLF was selected due to its ability of inducing toxic effects [1] and behavioral changes [2] in fish, as well as bioaccumulation in their tissues [3].

Technological and methodological innovation

Fish were exposed to 20 µg/L of VLF via water for 28 days followed by an elimination phase of 7 days. Metabolomics was applied to assess tissue-dependent changes in the profile of endogenous metabolites in different tissues (liver, brain and plasma) at 28 and 35 days. Analysis was performed using a non-target screening approach based on liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS). Data was processed using Compound Discoverer 3.0.

Results and impact

Tissue-dependent variations on endogenous metabolites of fish exposed to VLF were observed, showing that metabolomics allow to detect changes at an early stage of exposure. Several endogenous metabolites, altered after contaminant exposure, were tentatively identified and can be proposed as markers of contamination impact. In general, the metabolic pathways more significantly affected were phenylalanine, tyrosine and tryptophan metabolism; purine metabolism; and signaling pathway.

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Acknowledgments

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Poster 048: P048 *Comparison of effects produced by the exposure of bisphenol A and estradiol in zebrafish embryos (Danio rerio) using an untargeted metabolomics approach*

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Introduction

Bisphenol A (BPA) is an exogenous endocrine disruptor, well known due to its estrogenicity [1], but it is also related to alterations in adipogenesis and obesity [2, 3]. Previous studies show disruption of lipid-related genes and in the metabolome of zebrafish embryos (Danio rerio) [4], but further characterization is desirable. Therefore, we aim to determine changes in the metabolomic profiles of zebrafish embryos exposed to BPA, and a natural hormone, estradiol (E2) during early development.

Technological and methodological innovation

A HILIC-HRMS/MS workflow has been employed to assess the effects caused by the exposure of zebrafish embryos to BPA and E2. Zebrafish embryos were exposed at two concentration levels (sub-lethal doses), from 48 to 120 hours postfertilization (hpf) and collected at 120hpf. A TSK Amide 80 column and a QTOF mass spectrometer in ESI negative mode were used for the analysis. W4m online platform, PLS Toolbox and HMDB database were employed for feature detection, multivariate analysis and annotation.

Results and impact

There is a need in deepening the knowledge of the consequences of BPA in the metabolism of exposed organisms. This study aims to achieve a better understanding of the metabolic consequences of its exposure, beyond its estrogenic effect, when compared to the effect produced by a natural hormone, as E2.

Preliminary results show that the main markers associated with the exposure correspond to different metabolomic pathways, including amino acid and sugar metabolisms, as previously reported [4].

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Poster 049: P049 *Let's talk about POÆMS !!*

Photosynthetic Organisms from Atmospheric Ecosystem in a Multiscale Study

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Introduction

Atmosphere is a poorly understood compartment, ecologically speaking. Preliminary studies try to show impact of bioaerosols on climate changes and few of them try to understand physiological adaptations upon such harsh conditions [1, 2]. Among microorganisms from this compartment, several are photosynthetically active, from prokaryotic (e.g. cyanobacteria [3]) & eukaryotic phyla [4]. As the others, they come from – and fall to – all over the world, possibly influencing ecosystems of destination.

Technological and methodological innovation

Meta-metabolomics [5] aim to focus on environmental aspects and try to harvest evidences about how physiological plasticity leads to evolution among consortia or holobiontic contexts. However, the assumptions need to be reinforced / supported with other sources of information. Then, dealing with data from meteorological, ecological and omics is a challenge to reveal capabilities of bioaerosols to influence terrestrial reservoirs.

Results and impact

First results show on one way that atmospheric diversity is linked to several unpredictable parameters. On the other way, microbial lifespan and metabolome seem to be modified according to the recruited consortia. This project is at its alder and needs to mature methodological issues, collect more data and validate hypothesis with much more well-documented observations. Such meta-metabolomics breakthroughs will permit to open ecology field to much more accurate phenotypic assessments.

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Poster 050: P050 *Developing a multi-omics approach for chemical grouping*

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Introduction

With the globally growing use of chemicals there is an ever-increasing demand for improved safety assessment. However, toxicity data is often lacking for substances[1]and their impacts on human and environmental health are poorly understood. One of the approaches frequently applied in regulatory toxicology for data gap filling is forming groups of substances and 'reading across' toxicity[2]. It is traditionally based on structural similarity and often does not fully consider toxicity mechanisms.

Technological and methodological innovation

Here, we evaluated the capabilities of high-throughput multi-omics approaches to substantiate or disprove the structure-based grouping of seven azo dyes, where the grouping was based on the molecular toxicity responses in Daphnia magna. This utilised direct infusion mass spectrometry (DIMS) non-targeted metabolomics[3] and the TempO-SeqTM platform for transcriptomics. The multi-omics data streams were integrated and analysed using hierarchical cluster analysis to derive mechanism-driven grouping.

Results and impact

The multi-omics investigation discovered sufficient metabolic and transcriptional responses to facilitate grouping of the azo dyes based on their toxicological fingerprints, demonstrating the potential of the approach. In addition, Ingenuity Pathway Analysis (IPA) of the gene expression data provided an independent mechanistic rationale for the multi-omics grouping. The groupings based on the two approaches of structural similarity vs. multi-omics responses were similar but not identical.

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Poster 051: P051 *Microbiome and metabolome analyses of the medaka fish gut during a chronic exposure to toxic cyanobacterial blooms*

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Introduction

Cyanobacterial blooms are freshwater natural events in which Microcystis is one of the most encountered genus. Those microorganisms are able to produce a large set of bioactive secondary metabolites [1], some of which are known to have antimicrobial activities [2]. Although metabolites effects on fish physiology have been well investigated, effects on microbiota are still lacking. Here, we assess potential interactions between the M. aeruginosa species and the gut microbiota of a model fish.

Technological and methodological innovation

Medaka fishes (Oryzias latipes) have been exposed during 28 days in microcosms to environmentally relevant and increasing concentrations of M. aeruginosa blooms. Both microbiome (16S rDNA) and metabolome have been analysed from gut samples to study respectively the composition of bacterial gut communities associated and the secondary metabolites diversity. Furthermore, multi-omics analyses allowed to determine variations and correlations between amplicon sequence variants and metabolic compounds.

Results and impact

Preliminary results for the microbiome revealed a shift in the composition of bacterial communities for fishes exposed to the highest concentration of M. aeruginosa, suggesting the existence of a tipping point. Additionnally, the change in metabolic profiles with the increase in concentration is following a common continuous trajectory between samples and replicates. This suggests a possible progressive change in metabolite compositions.

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Poster 052: P052 *High-Throughput Targeted Lipidomics Analysis of Dihydroceramide Desaturase-1 (DES1) Knockout Mice*

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Introduction

Lipidomics is a fast-growing area of science, aiming to quantitatively define functions of lipid classes at the molecular species levels. There are about 150,000 different lipid molecular species present across the biological spectrum. This diversity reflects the different roles lipids play at the cellular, tissue, and organismal levels. Traditional approaches to lipid analysis in biological samples have relied on 'Shotgun' lipidomics, a high throughput infusion-based approach for screening studies, or IDA-based (i.e., data-dependent) discovery experiments. The challenge to both approaches is the need for extensive data analysis to identify molecules, and the quantitative results are limited to sum composition (e.g., PC 34:2) rather than molecular species composition (e.g., PC 16:0/18:2). The ability to identify and quantitate lipids at the molecular species level is essential to correlate lipidomics to metabolism and hence biology.

For studies requiring more sensitivity and specificity, a targeted lipidomics approach using liquid chromatography (LC) coupled with triple quadrupole based instrumentation in the multiple reaction monitoring (MRM) mode has advantages. Amide column separation is an attractive chromatographic strategy that effectively resolves both polar and non-polar lipids into classes and subclasses. This is an essential aspect of a column-based strategy due to the extensive isobaric overlap among different lipid classes that confounds identification and quantification. To validate this method, we selected DES1 knockout mice to measure lipid changes in liver and adipose tissues. The method was then adjusted to optimize window width and dwell weight to enhance sensitivity and coverage.

The QTRAP® 6500+ System offers unparalleled sensitivity even at high scan speed (2-5 msec transition time) with rapid positive/negative switching (< 5msec). Using qualified MRM transitions and commercially available lipid internal standard mixtures, this method provides quantitative measurement of over 1150 different lipid molecular species in a rapid, highly reproducible manner. The target list is customizable and expandable to include new lipid classes, or the list can be shortened for a class-specific study.

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CONCLUSIONS

Liver and epidymal white adipose tissue (eWAT) tissues were harvested from dihydroceramide desaturase-1 (DES1) knockout and control mice. DES1 is the enzyme responsible for inserting the 4,5-trans-double bond into the sphingolipid backbone causing dihydroceramides conversion to ceramides. While both of these classes of lipids are lower in abundance in the chosen tissues, this method enable the researcher to quantify them and show significant changes in these lipid classes in this study, however lipids from another 17 classes were not changing.

In this study we present an optimized method to target a broad array of different lipids at the molecular species level. An amide column-based chromatographic method was developed to minimize isobaric interference, which allows for a relatively rapid and specific lipid screening technique. The target list of lipids is comprehensive, covering most major lipid classes and categories, and MRMs were selected to cover lipids containing fatty acids with 14 to 22 carbons and 0 to 6 double bonds. The method is customizable, so new lipid categories, classes and molecular species can be added. In simple terms, this method is a specific, quantitative and comprehensive workflow to rapidly screen the lipid profile of any type of matrix and serves as an excellent starting point for further method development and optimization.

Poster 053: P053 *In Vivo Microdialysis of Endogenous and 13C-labeled TCA Metabolites in Rat Brain:Reversible and Persistent Effects of Mitochondrial Inhibition and Transient Cerebral Ischemia*

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Introduction

Cerebral microdialysis is routinely used, in neuro-intensive care patients after an ischemic insult, to monitor interstitial levels of glucose, lactate and pyruvate and thus mitochondrial function. Understanding the underlying mechanisms of disturbed energy metabolism are essential to improve treatments. Thus, we applied mitochondrial dysfunction rat models and measured tricarboxylic acid cycle (TCA)-centered metabolites and their incorporation of 13C upon perfusion with 13C-labelled succinate [1].

Technological and methodological innovation

Two models of mitochondrial dysfunction were applied: a malonate-perfusion and endothelin-1-induced transient cerebral ischemia. The cerebral microdialysis fractions with a temporal resolution of 30 min were analyzed using a LC-MS to identify and relatively quantify TCA-centered metabolites. Moreover, as the rat brains were perfused with 13C-labeled succinate, compounds with 13C incorporated from 13C-succinate were also measured [1].

Results and impact

Use of the malonate model confirmed the functionality of the approach. In the ET-1 model, increases in non-labeled TCA metabolites (reflecting release of intracellular compounds) and decreases in 13C-labeled TCA metabolites (reflecting inhibition of de novo synthesis) were observed. The analysis of 13C incorporation provides further layers of information to identify metabolic disturbances in experimental models and neuro-intensive care patients [1].

References

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Poster 054: P054 *Ion source optimization in liquid chromatography time-of-flight mass spectrometry for metabolomics studies using the design of experiments*

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Introduction

Metabolomics studies in the context of microbial cell factories are essential to assess influences on product yield and quality[1,2]. Consequently, analytical techniques for determining intra-cellular metabolites with high coverage and accuracy are required. The analysis using liquid chromatography mass spectrometry (LC-MS) represents a technical challenge due to the miss-annotation of in-source fragmentation[3] and requires careful optimization of the electrospray ionization (ESI) process.

Technological and methodological innovation

ESI parameters, including temperature and flow rate of drying gas and sheath gas, fragmentor, nozzle and capillary voltage, as well as nebulizer pressure were systematically investigated by experimental designs. The signal of several metabolites was determined using hydrophilic interaction liquid chromatography combined with time-of-flight mass spectrometry. PlackettBurman and central composite experimental designs were employed for screening and optimizing ESI parameters, respectively.

Results and impact

The experimental design approach was a suitable method for evaluating the influence and interaction of ESI parameters for MS measurements. Our findings contribute significantly not only to explain ESI parameters on ion source suppression and fragmentation, but also to expand metabolome coverage and correct annotation in the intra-cellular metabolomics studies. First results addressing the non-targeted analysis of yeast cell extracts will be discussed.

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Poster 055: P055 *A novel multi-targeted quantitative approach for nutrimetabolomics research*

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Introduction

Nowadays, metabolomics plays an essential role in nutritional research for accurate dietary assessment by means of the monitoring of food intake biomarkers, as well as for investigating the effect of dietary habits on health. To address the metabolome complexity, in this work we have developed a multi-targeted quantitative approach for comprehensive and rapid metabolomic fingerprinting of biological samples.

Technological and methodological innovation

A solid phase extraction method was optimized for the simultaneous extraction and pre-concentration of around 350 foodderived metabolites. Complementarily, simple extraction methods based on dilution and protein precipitation were also validated for the analysis of more than 500 endogenous metabolites from urine and plasma samples, respectively. Metabolomic fingerprinting is then conducted by reversed-phase UHPLC-MS/MS.

Results and impact

A novel multi-targeted approach was developed for the simultaneous quantitation of about 900 exogenous and endogenous metabolites in very short run times and using low volumes of biological sample, which facilitates its application to epidemiological studies. This metabolomic approach thus represents one-step further towards precision nutrition and dietary assessment, as well as for the investigation of the role of diet and nutrition in health status.

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Poster 056: P056 *FOBI: An ontology to represent food intake data and associate it with metabolomic data*

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Introduction

Nutrition research can be conducted by using two complementary approaches: 1) traditional self-reporting methods or 2) via metabolomics techniques to analyze food intake biomarkers in biofluids. However, the complexity and heterogeneity of these two very different types of data often hinder their analysis and integration. To manage this challenge, we have developed a novel ontology that describes food and their associated metabolite entities in a hierarchical way.

Technological and methodological innovation

The ontology presented is called FOBI (Food-Biomarker Ontology) and it is composed of two interconnected sub-ontologies. One is a ”Food Ontology” consisting of raw foods and prepared foods while the second is a ”Biomarker Ontology” containing food intake biomarkers classified by their chemical classes. These two sub-ontologies are conceptually independent but interconnected by different properties.

Results and impact

This ontology uses a formal naming system, category definitions, properties and relations between both types of data. Potential applications of this ontology include the annotation of foods and biomarkers using a well-defined and consistent nomenclature, the standardized reporting of metabolomics workflows (e.g. metabolite identification, experimental design), or the application of different enrichment analysis approaches to analyze nutrimetabolomic data.

Poster 057: P057 *Comparison of different methods for lipidic extraction*

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Introduction

On the MetaToul-Lipidomic facility, we usually use Bligh and Dyer (modified) method for lipidic extraction. During the automation (Tecan® robot), the results of experimentations revealed that lipids extractions yields were very low. Then to improve this step, we decided to compare different methods of liquid-liquid extraction (LLE) for the extraction of neutral lipids and phospholipids. In this study, the different methods were tested on liver, plasma and appropriate internal standards.

Technological and methodological innovation

The LLEs tested were a modified Bligh and Dyer method1, a Folch method2, a three phase liquid extraction (TPLE)3, the BUME method4 and a lipid extraction by methyl-tert-butyl ether (MTBE)5. Neutral lipids were analyzed on a GC-FID (Trace 1300, Thermo®) and phospholipids on a LC-MS/MS (1290 Infinity/6460 Triple quad, Agilent Technologies®).

Results and impact

The poster presents the results of the different extraction methods. Overall, they are similar, except for the TPLE, which has lower yields. We thus focused on the modified Bligh and Dyer, the BUME method, the MTBE method and the Folch method was replaced by a microBligh and Dyer method (using less solvent)6.

There are large variations in extraction yields from one method to another and from one lipid family to another. The prospects of this study would be to optimize sample preparation for each studied matrix and lipid family of interested.

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Poster 058: P058 *Development of an untargeted metabolomics workflow for investigating drug-induced cardiotoxicity using cardiac microtissues.*

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Introduction

Cardiac microtissues are a promising in vitro model system for exploring drug-induced cardiotoxicity, a major contributor to attrition during drug development[1]. Cardiac microtissues are 3D co-cultures of cardiomyocytes, fibroblasts and endothelial cells, consisting of ~500 cells[2]. Untargeted metabolomics offers an opportunity to gain molecular-level insight into druginduced cardiotoxicity. The aim of this research is to develop an untargeted metabolomics workflow for cardiac microtissues.

Technological and methodological innovation

Sampling of cardiac microtissues for untargeted metabolomics must be rapid, effectively remove extracellular contaminants, and limit cell lysis and/or leakage. Presented here is a novel approach that uses cell strainers to meet these requirements. Additionally, a highly sensitive direct infusion mass spectrometry (DIMS) metabolomics method[3] has been applied to assess the minimum biomass required for sensitive and reproducible measurement of the cardiac microtissue metabolome.

Results and impact

Approximately 2000 and 3500 polar and non-polar features, respectively, were robustly detected (biological and technical median RSDs <30% and <20%, respectively) for 28 pooled microtissues using a rapid sampling approach and highly sensitive DIMS metabolomics method. The optimized workflow will be applied to investigate drug-induced cardiotoxicity in a physiologically relevant in vitro system, enabling greater mechanistic understanding of underlying molecular perturbations and toxicity pathways.

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Poster 059: P059 *A metabolomics-like approach for chemical forensic: preliminary study and perspectives*

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Introduction

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 forensic" [1]. It is of special interest in order to support the attribution of a chemical weapons use. Data analysis workflows coming from metabolomics were shown to have a high potential to address this issue [2]. However, more studies are needed to identify technological and methodological blocking points in the workflow.

Technological and methodological innovation

Two production batches of a nerve-agent precursor (methylphosphonic dichloride, DC) were analyzed by GC-MS after hydrolysis and extraction (both done in two separate laboratories for reproducibility assessment). An instrument-independent data treatment workflow based on R (peak-picking by XCMS) and Matlab (data analysis) was set up to differentiate samples according their impurity profiles and to detect potential technical bias (e.g. influence of operator or laboratory consumables).

Results and impact

A chemical profile was established for each sample. Interestingly, no major technical bias can be noticed regarding sample preparation. The following blocking points were identified in the chemical forensic workflow: (i) lack of complete coverage of MS data by peak-picking algorithms (ii) lack of flexible and validated variable selection strategies (iii) lack of harmonized analytical and data analysis strategy for inter-laboratory studies.

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Poster 060: P060 *Simultaneous measurement of absolute metabolite concentration and isotope incorporation by mass spectrometry*

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Introduction

Studies of the topology, functioning and regulation of metabolic systems are based on two main types of information which can be measured by mass spectrometry: the absolute concentration of metabolites and their isotope incorporation in 13Clabeling experiments. These data are currently obtained from two independent experiments because the 13C-labeled internal standard (IS) used to determine the absolute concentration of a given metabolite overlaps the 13C-mass fractions from which its 13C-isotopologue distribution (CID) is quantified.

Technological and methodological innovation

Here, we developed a generic method with a dedicated processing workflow to obtain these two information simultaneously in a unique sample collected from a single cultivation, thereby reducing by a factor of two both the number of cultivations to perform and the number of samples to collect, prepare and analyze. The proposed approach is based on an IS labeled with other isotope(s) which can be resolved from the 13C-mass fractions of interest.

Results and impact

As proof-of-principle, we analyzed amino acids using a doubly labeled 15N13C-cell extract as IS. Extensive evaluation of the proposed approach shows a similar accuracy and precision compared to state-of-the-art approaches. We demonstrate the value of this approach by investigating the dynamic response of amino acids metabolism in mammalian cells upon activation of the PERK kinase, a key component of the unfolded protein response. Integration of metabolite concentrations and isotopic profiles reveals a reduced de novo biosynthesis of amino-acids upon PERK activation. The proposed approach is generic and can be applied to other (micro)organisms, analytical platforms, isotopic tracers, or classes of metabolites.

Poster 061: P061 *Evaluation of pure shift NMR method in metabolomics*

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Introduction

Although 1H NMR spectroscopy is commonly used in metabolic profiling, the analysis of the spectrum is complex because of extensive signals overlap. To overcome this limitation, pure shift NMR, method through which multiplicities are collapsed into single lines, has shown to be a convenient tool to address crowded spectra [1,2] but has been rarely used for metabolomics [3]. In this study pure shift NMR was evaluated to explore hepatic metabolic disruptions of pigs exposed to a food contaminant.

Technological and methodological innovation

Aqueous liver extracts of pigs fed two different diets and exposed or not to Fumonisin B1 (FB1) were analyzed using standard 1D proton sequence with suppression of water signal (noesypr1d) and pure shift NMR sequence (SAPPHIRE-PSYCHE [4]) on a 800 MHz NMR spectrometer. After spectra bucketing, multivariate statistical analyses (Partial Least Squares Discriminant Analysis – PLS-DA) were performed on the two datasets. Discriminant metabolites were identified for each sequence and compared.

Results and impact

1H NMR spectra of aqueous liver spectra were simplified using the pure shift sequence, thereby allowing easier metabolite identification but at the expense of lower sensitivity than the standard proton NMR sequence. PLS-DA analysis showed a separation between the two diets, and between control and FB1 exposed animals for the first diet. In addition to the standard proton sequence, pure shift sequence enables the identification of additional discriminant metabolites such as betaine and lactate.

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Poster 062: P062 *A high throughput fluxomic workflow for exploration of metabolic phenotypes*

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Introduction

High-throughput (HT) omics approaches enable the large-scale analysis of biological molecules and try to get a comprehensive understanding of living organism and their regulation. Among those, metabolic flux analysis aims at measuring the actual rates of biochemical reactions in metabolic networks. It is a powerful and growing approach to get comprehensive understanding of cellular metabolism in many fields of investigation, ranging from biotechnology to medical sciences.

Technological and methodological innovation

However, a 13C fluxomic experiment requires several complex experimental and computational steps as weel as large input of manual steps which may lower robustness of flux analyses. These makes classical fluxomics a low throughput approach, which limits the number conditions which can be investigated at once. To increase fluxomics throughput and robustness while reducing experimental costs and human efforts, we designed a complete workflow in order to automatize, parallelize, integrate and optimize each step of fluxomics experiments.

Results and impact

Thanks to this development, we are able to quantify flux responses to environmental or genetic perturbations in complement to other omics approaches. It opens the way to very large-scale fluxomics screening experiment. Thanks to our HT-Fluxomics workflow, 240 flux maps were generated in 3 months; this workflow can now be applied to large-scale studies in many fields: biotechnology, biomarker, drug development, medical science, toxicology or pharmacology.

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Poster 063: P063 *Metabolomic fingerprint of aging on red blood cells*

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Introduction

Increases in the lifespan due to better life conditions, together with the drop in the birth rates are leading to a more aged society1. The aging process leads to many changes in the human organism and has been associated with the development of many conditions2,3. Since these changes are reflected in the human metabolome, the evaluation of the metabolomic alterations occurring in the human body during aging is an attractive strategy to address diseases.

Technological and methodological innovation

Metabolomics is a relatively novel and personalized strategy4 that has been widely used in human blood samples. However, Red Blood Cells (RBCs) have been poorly assessed5. RBCs are abundant, readily available and they contain many metabolites, making them a potential tool to obtain information about the health condition of each person. Therefore, the goal of this work was to perform an NMR metabolic study of RBCs to assess aging alterations and the effect of lifestyle factors, such as BMI.

Results and impact

Aging showed a clear fingerprint on RBCs with alterations on aminoacid, carbohydrate, lipid and energy metabolism. BMI also presented metabolite patterns in RBCs and altered the age-related metabolic changes, whereas gender had a less significant impact on its metabolomic profile. Metabolomic profiling of RBCs by NMR proved to be a fast and robust method to detect the fingerprint of age and determine relationships between the biological processes that take place in aging and obesity.

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Poster 064: P064 *Intelligent Acquisiton for Comprehensive Metabolome Coverage in Plants, Mammals, and Bacteria*

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Introduction (500/500 characters spaces included)

Unknown identification remains a challenge in LC/MS based untargeted metabolomics where thousands of features from a single sample are routinely detected. However, not all features represent metabolites of biological origin resulting in fragmentation spectra of background ions and degenerate signals. Thermo Scientific™ AcquireX intelligent data acquisition was applied to plant, mammal and bacterial samples to deliver an efficient workflow for deeper metabolome coverage and increased annotations.

Technological and methodological innovation (490/500 characters spaces included)

Data-dependent acquisition often provides information for the most abundant ions. Data-independent acquisition can get fragmentation data for all features, but results in convoluted MS/MS spectra complicating identification. The AcquireX acquisition software determines on-the-fly features corresponding to background contaminants and compound degeneracy, like isotopes, adducts, and dimers, enabling more efficient precursor selection to target unique metabolites of biological relevance.

Results and impact (496/500 characters spaces included)

Samples of varying complexity were used to demonstrate the utility of AcquireX acquisition. By excluding background and degenerate signals, the total number of fragmentation targets was reduced without losing metabolite coverage. Over 10,000 features were detected in E. coli extracts, but after data reduction on-the-fly, only 1,040 features corresponded to unique compounds. A similar 10-fold reduction in precursors to target was seen in extracts of yeast, tea, human urine, and human plasma.

Poster 065: P065 *Novel MALDI imaging solution empowered by a dual-source Q-TOF and a dedicated bioinformatics pipeline for identification of peaks from tissue*

Sabine Jourdain[5], Janina Oetjen[1],Arne Fuetterer[1], Juergen Suetering[1], Niels Goedecke[1], Stephanie Kaspar-Schoenefeld[1], Scarlet Koch[1], Shannon Cornett[2], Christian Marsching[3], Carsten Hopf[3], Nikolas Kessler[1], Wiebke Timm[1]; Aiko Barsch[1]; Heiko Neuweger[1], Jan H. Kobarg[4], Dennis Trede[4], Sören-Oliver Deininger[1], Alice Ly[1], Jens Fuchser[1], Lucy Woods[1], Oliver Raether[1], Jens Höhndorf[1]

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Introduction

MALDI Mass Spectrometry Imaging (MALDI-MSI) has emerged as a technique with a broad range of applications in Omics research. We present initial results from the timsTOF fleX system; a timsTOF Pro QTOF mounted with a high-throughput, high spatial resolution MALDI source and stage. The instrument allows for the robust, high speed acquisition of both MALDI and LC-MS/MS data. When combined with a software processing pipeline for automatically annotating measured ions, this enabled generation of annotated images from MALDI-imaging data.

Technological and methodological innovation

ESI mode performance on the timsTOF fleX was evaluated by analyzing a commercially available HeLa digest (Pierce) using DDA PASEF. For MALDI-MSI experiments, tissue sections were mounted on conductive glass slides, and coated with matrix using standard protocols or a specialised sublimation device. Ion mobility imaging experiments were acquired at 150 1/K0 mobility resolution. SCiLS Lab was used for visualising and statistical analysis of MALDI-MSI data. MetaboScape was used for feature extraction, de-isotoping, ion deconvolution and automatic annotations.

Results and impact

MALDI Imaging data was acquired at a rate of up to 20 pixels/second in positive and negative mode . Rat testis sections measured at 10µm zoom-mode showed different seminiferous tubule structures. In experiments designed to stress the system, 20 hours of image acquisition showed no decline in imaging dataset quality and a mass deviation of RMS 2.06 without lock mass. Trapped ion mobility imaging measurements removed isobaric interferences in lipid imaging.

MetaboScape was used for lipid annotation using untargeted (raw formula generation) or targeted (e.g. HMDB, LipidMaps) methods.

Poster 066: P066 *Establishing a Spectral Library and Accurate Mass Retention Time (AMRT) Database for Pediatric Metabolomics Analysis*

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Introduction

Metabolomics is an established discovery tool for biomarker discovery, disease diagnosis and novel mechanistic insights of pathophysiological processes. Liquid-chromatography mass spectrometry (LCMS) is currently the method of choice for metabolome analysis, however, the wide variety of chromatographic columns and conditions is presenting a challenge in sharing and interpretation of metabolite identities.

Technological and methodological innovation

We have established a spectral library of compounds in the (IROA) kit for metabolite identification. Data was acquired using flow injection analysis (FIA) which allowed the fast acquisition of spectra at a wide range of collision energies (0-200, normalized collision energy NCE). Reference standards were injected on an Vanquish™-Q-Exactive™ LCMS system. Two libraries were established, one spanning all NCEs and one restricted to a carefully selected NCE for each compound.

Results and impact

The libraries were used as a training set to select generalized NCE values for unknowns. We provide a scheme to produce the optimal number of mixtures for IROA metabolites or similar reference libraries. The mixtures were used to establish an accurate mass/retention time (AMRT) databases on the optimized chromatographic conditions (reversed-phase and HILIC). Metabolite identification in matrices (plasma, urine and sweat) were further validated using the established AMRT databases and libraries.

Poster 067: P067 *Investigation of mevalonate pathway by quantitative metabolomics and isotopoloque profiling*

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Mevalonate pathway is an important metabolic pathway which plays a key role in multiple cellular processes. In recent years the mevalonate pathway has become a challenging and, in the meantime, fascinating topic, when a large number of experimental and clinical studies suggested that inhibition of nonsterol isoprenoids might have valuable interest in human pathology. These molecules that are essential for cell growth and differentiation appear to be potentially interesting therapeutic targets for many areas of ongoing research: oncology, autoimmune disorders, atherosclerosis, and Alzheimer disease. Thus, functional understanding of their biosynthesis is of high importance. However, available methods do not yet allow accurate quantification and tracing of stable isotopes incorporation for all the isoprenoids precursors (mevalonate intermediates and prenyl pyrophosphates). We present a complete methodology for functional analysis of isoprenoids biosynthesis, with a novel quantification method based on liquid chromatography coupled to high-resolution mass spectrometry. This workflow covers all the experimental and computational steps from sample collection and preparation to data acquisition and processing. It ensures accurate, absolute quantification (RSD < 20 %) of all mevalonate and prenyl pyrophosphates intermediates with a high sensitivity over a large linear range (from 0.1 to 50 pmol). Determination of their isotopologue distributions in isotopic labeling experiments was performed, opening the way for 13C-metabolic flux analysis of isoprenoids biosynthesis.

In conclusion, the described methodology fills one of the last technical gaps for functional studies of isoprenoids biosynthesis and should be applicable to other eukaryotic and prokaryotic (micro)organisms after adaptation of some organism-dependent steps.

Poster 068: P068 *High-throughput metabolite profiling of cell media for improved antibody production utilizing a dual separation/mass spectrometry system*

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Introduction

Chinese hamster ovary (CHO) cells are often used for commercial production of antibodies. Optimization of cell culture medium and feeds is required to obtain maximum product yield. Metabolomics analysis allows for quick determination of nutrient limitations or metabolite buildup during the cell culture. A semi-targeted workflow was designed to confidently measure critical nutrients, while allowing for the discovery of previously unidentified metabolites that affect growth and antibody yield.

Technological and methodological innovation

A dual UHPLC separation system coupled to an Orbitrap™ mass spectrometer was used to increase analysis throughput of a time-course study of 5 different feeds, while also maximizing metabolome coverage. This dual LC system combines RP and HILIC separation for the detection of hydrophobic and polar metabolites from the same sample. AcquireX intelligent acquisition maximized the number of metabolites interrogated by MS/MS, resulting in more confident metabolite annotations.

Results and impact

By combining RP and HILIC, we reproducibly analyzed a larger number of metabolites. Efficiently overlapping the two separations resulted in 30% decrease in analysis time. This increased throughput did not compromise reproducibility. Nutrient depletion could be easily measured and correlated to improved titer. This dual LC/MS system was successfully used for the analysis of cell media and provided robust metabolic indicators of cell growth and antibody yield and possible optimization strategies.

Poster 069: P069 *The development of selective sorbent for isolation of catecholamines and their metabolites from biological fluids*

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Introduction

The role played by catecholamines (CAs) and their main metabolites (homovanillic and vanillylmandelic acid) in different physiological processes and various disorders [1, 2] attracts interest in many areas of medical research. Thus, simultaneous determination of CAs and their metabolites in biological fluids using fast and reliable analytical procedure is the efficient way for diagnosis, therapy and prognosis of related diseases [3, 4].

Technological and methodological innovation

Taking into account several challenges usually encountered during isolation and detection of CAs and their metabolites, the aim of this study is the development of solid-phase extraction sorbent for simultaneous separation and enrichment of CAs together with corresponding acidic metabolites from biological fluids in single step. The development of the sorbent is based on combination of non-covalent and semi-covalent molecular imprinting [5] method to form binding sites for CAs and the acids.

Results and impact

Despite the fact that the study is in progress, the optimization of molecular imprinting procedure has been completed by varying cross-linking monomers, solvent system and the ratio of functional monomers to cross-linker. As a result, eight polymers based on methylenebisacrylamide as cross-linker have been prepared and tested. Preliminary single point batch extraction experiments have shown rather high capacity of these polymers for the analytes in comparison to control polymers (up to 7:1).

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Poster 070: P070 *Data processing workflow to study the exposome by suspect screening*

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Introduction

The characterization of all factors, chemical or not, to which an organism is exposed during his life, could be in part addressed by non-targeted metabolomics approaches. Biomarkers detected from this kind of methodology could be particularly interesting to better characterize the exposure to xenobiotics. However, one of the main challenges of nontargeted approaches to study the chemical exposome is to differentiate metabolites of xenobiotics from all other metabolites.

Technological and methodological innovation

One solution to highlight xeno-metabolites is suspect screening, as previously applied for pesticides [1]. It is based on a nontargeted acquisition of data, followed by a screening of suspect compounds that are expected to be present in samples. Annotation is achieved using databases of known xenobiotics, their known or putative metabolites but for which no standard is available. To deal with the potentially huge amount of matches, an advanced data processing workflow has been developed.

Results and impact

A large but representative database was built from metabolites of xenobiotics. Annotated features from this database are curated according to several parameters: chromatographic signal, signal/noise, blank comparison, potential annotation of an endogenous metabolite, isotopic pattern (mono-charged, monoisotopic, halogenated ions, etc.), targeted MS/MS, MS3 for conjugated metabolites. Following identifications at least at level 3, statistics can be performed on features of identified metabolites.

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Poster 071: P071 *Development of an untargeted approach based on isotope profiling of metabolic networks by high resolution mass spectrometry*

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Introduction

The study of metabolism has become a major research challenge for understanding the operation of biological systems (cells, tissues, organisms). A relevant approach is to study the topology and operation of metabolic networks mainly based on 13 C isotope labelling strategies. For this purpose, the MetaToul-MetaboHUB platform has developed different approaches for a wide range of applications, including biotechnology and human health, such as measuring metabolic fluxes and the isotopic profiling based to follow the incorporation of 13C atoms into metabolites through targeted analysis of central and energetic metabolisms and amino acid metabolism.

These current targeted approaches are very effective but do not allow to highlight unsuspected metabolic pathways not initially targeted. For this reason and to increase the coverage of metabolome and the understanding of the biological systems, the platform wishes to develop an untargeted approach based on isotopic profiling. This approach aims at the characterization of the metabolic networks of a biological organism without a priori knowledge.

Technological and methodological innovation

The implementation of this workflow is based in particular on the development of a software for automated data integration adapted to non-targeted analyses with 13C labelling. For this purpose, the preliminary step consists of an evaluation of pre-existing tools allowing the establishment of specifications. Then, the software developed by the platform according to these specifications will be integrated in emzed, an open source toolbox for rapid and interactive development of LCMS data analysis workflows in Python language in collaboration with ETH Zürich.

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Poster 072: P072 *Improving lipid annotation coverage using intelligent precursor selection software on an Orbitrap-based mass spectrometer*

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…

Lipid profiling provides valuable information to identify disease states and other physiological changes. A common approach used in lipid profiling by LC-MS is to identify lipid species by their MS/MS spectra prior to extraction of precursor information for relative quantitation between conditions. The novel intelligent data acquisition strategy, AcquireX, on the Thermo Scientific™ Orbitrap ID-X™ Tribrid™ mass spectrometer automatically excludes background ions from the MS/MS acquisition and prioritizes MS/MS precursor selection on sample relevant compounds, resulting in deeper lipidome coverage. Here, Bovine liver total lipid extract (Avanti Polar Lipids) was used to demonstrate the utility of the workflow. Samples were separated using reversed-phase conditions on a C30 column with mass spectral data acquired on an Orbitrap ID-X Tribrid MS. Data analysis was performed using Thermo Scientific™ LipidSearch™ 4.2 software to identify lipid molecular species based on acquired MSn fragmentation spectra.

The AcquireX data acquisition strategy was used to automatically generate background exclusion and/or compound inclusion lists that were updated iteratively for replicate injections, reducing redundant data collection and triggering more unique lipids for fragmentation. As a result, a significantly larger number of lipids (>40%) could be detected compared to a conventional data-dependent MS/MS approach.

In addition, the acquisition of neutral loss-triggered CID-MS3 and product ion-triggered CID-MS2 improved the confidence in the lipid annotations. For example, the use of fatty acid neutral loss-triggered MS3 fragmentation with triglycerides allowed to distinguish multiple co-eluting isomers with different fatty acid chain lengths based on their respective MS3 data.

Poster 073: P073 *Comparison of normalization techniques for untargeted metabolomics of urine samples.*

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Introduction

Urine is one of the most used matrixes for metabolomics, in particularly in toxicology since it represents one of the main detoxification routes. To overcome the natural and high variability of urine concentration that can hamper the results interpretation, many normalization strategies have emerged. Since there is no consensus on which normalization technique should be used or not, we evaluated the most commonly used techniques on a metabolomics dataset.

Technological and methodological innovation

Urine samples of 40 healthy volunteers (20 women and 20 men) were analysed by UPLC-HRMS. Before the analysis, samples were diluted to normalize creatinine or osmolality level or diluted by a common factor. Postacquisition normalization methods based on these parameters (in combination or not with PQN or MSTUS) were also assessed. Comparison of normalization techniques relied on their ability to reduce unwanted variability and to improve the performance of generated statistical models.

Results and impact

Compared with non-normalized results, data obtained from either osmolality or creatinine normalized samples yielded better results. Pre-acquisition normalization led to a better statistical discrimination compared to nonnormalized or post-acquisition normalized samples especially with specific dilution factors according to osmolality levels. MSTUS and PQN normalization did not improve statistical discrimination due to the suppression of part of the biological variability of interest.

Poster 074: P074 *Comparison of two sampling and extraction protocols to perform metabolomic studies on human adherent cells*

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Introduction

Different methods of cell sampling and metabolites extraction can be used when performing metabolomics analyses on adherent cells. However, these steps are critical and can notably impact the results. An appropriate design of the experiment is therefore necessary, particularly as regards the quenching of metabolic activity and the washing steps. These steps have to be fast and reproducible to prevent biases and unwanted variability in the detection of metabolites due to different factors (temperature change, cell stress…).

Technological and methodological innovation

We compared two sample collection workflows for performing targeted and untargeted metabolomics on adherent cells. The first one is a standard protocol involving 1 operator, widely used for such application and consisting in the washing and scraping of the cells within wells [1]. The second is a fast-quenching (FQ) protocol requiring 5 operators, which aims to block cell metabolism as fast as possible to minimize metabolic status disturbances, and optimizes the washing step to avoid a contamination by cell media [2]. Sampled were analyzed by LC-HRMS, IC-HRMS and NMR.

Results and impact

Samples obtained with the FQ workflow were shown to display less variability, suggesting that stopping cells metabolism fast enough allows to lessen experimental cells metabolome disruption, especially as regards central carbon metabolites which are prone to fast metabolic reactions. Blank samples obtained with the FQ method were also found to be less polluted, providing a valuable alternative to prevent metabolites concentration overestimation in targeted metabolomics, and preserve a maximal number of variables during the step of blank filtration, in untargeted metabolomics.

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Poster 075: P075 *Development of a System Suitability QC Sample for Nano-Electrospray Direct Infusion Mass Spectrometry Metabolomics*

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Introduction

Nano-electrospray direct-infusion mass spectrometry (nESI-DIMS) based metabolomics is well recognized for its capability for high throughput screening, high analytical sensitivity and low selectivity. In order to be adopted for applications other than academic research, for example into regulatory or clinical practice, standardised and robust quality assurance and quality control (QA/QC) procedures are required, as described in the MERIT guidelines[1].

Technological and methodological innovation

Presented is the development and testing of a system suitability QC (SSQC) comprising of a mixture of standards analysed using our current DIMS workflow[2]. For added confidence, in-source fragments were considered alongside multiple adducts, isotopes and database matching. Repeated analyses of the SSQC were then carried out in a multi-batch DIMS metabolomics study, facilitating in-depth exploration of the m/z accuracy, and m/z and intensity precision to allow acceptability thresholds to be set.

Results and impact

Of the ca. 77 standards analysed, 10-25 chemicals were reproducibly detected and annotated per assay[3]. Repeat analyses showed expected m/z precision to be <0.5ppm and intensity RSDs of 5-30%. Mass errors were higher than predicted with a range of +/10ppm, though the observed trend between mass error and m/z was strikingly repeatable across replicates. Future work will include tandem DIMS analysis of SSQC for level 1 identification[3].

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Poster 076: P076 *Evaluation of U-^{13}C Spirulina (Arthrospira platensis) for stable isotope assisted untargeted metabolomics and liquid chromatography-isotope dilution mass spectrometry (LC-IDMS)*

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Introduction

The use of commercially available microorganisms grown on labelled substrates has become an accessible source of multiple isotopes for the use in stable isotope assisted untargeted metabolomics (SIAM) and LC-IDMS [1]. However, in most cases the enrichment profile of the metabolites is not provided and therefore, the presence of unlabelled metabolites in the labelled extract introduces more variations in sample analysis, leading to false identification and inaccurate quantification.

Technological and methodological innovation

Commercially available U-^{13}C spirulina was investigated using untargeted metabolomics approach. ZIC-pHILIC-HRMS/MS was used for the analysis of the methanolic extracts of U-^{13}C, U-^{12}C spirulina (n=6) and 268 authentic standards in a single analytical run. Metabolite identification and SIAM were performed using Compound Discoverer by interrogating a species specific BioCyc database, mzCloud database and RTs of the standards and the use of stable isotope labelling algorithm.

Results and impact

226 metabolites were identified including amino acids, nucleotides, carbohydrates, organic and fatty acids and the relative exchange ratio [^{13}C/^{12}C] showed that 42% were fully labelled, 33% were partially labelled and 25% were unlabelled. The characterisation of spirulina has an added impact on its use; the labelled metabolites in the U-^{13}C extract enhance SIAM and LC-IDMS analyses, while the unlabelled isotopologues restrict their use as internal standards thus an adjustment method is needed.

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Poster 077: P077 *Increased throughput and coverage for the annotation of Saponins using a structurebased MSn approach on a Tribrid Orbitrap mass spectrometer*

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Introduction

Saponins are major components of Chinese medicines and exert various pharmacological effects such as cardiovascular protective activity and anticancer activity. The comprehensive annotation of saponins remains challenging because of the limited availability of authentic standards and the structural diversity of this class of compounds.

Technological and methodological innovation

Taking advantages of high resolution MS and MSn capability offered by the tribrid Oribitrap mass spectrometer (Thermo Scientific™ Orbitrap ID-X™ Tribrid™), we developed a product ion-dependent MSn data acquisition method in which MS^2 data is constantly collected and further followed by higher order FTMSn if sugar neutral loss are detected from the MS^2 data. The $MS^{n\ (up\ to\ 4)}$ spectral tree data were processed using Thermo Scientific™ Mass Frontier™ 8.0 and Thermo Scientific™ Compound Discoverer™ 3.1 softwares. Multiple databases were employed in the processing workflow including mzCloud™ spectral library, ChemSpider database, and custom databases for unknown saponin structure annotation.

Results and impact

As the proof of concept, methanol extracts from Sanqi were analysed. The collected MS^n tree data were first searched against the mzCloud MS^n spectral library in batch mode using the subtree search tool of Mass Frontier 8.0 software for identifying the saponin class compounds. The unknown compounds with exact MS^n spectral tree match against the library references were annotated. However, for most unknown compounds, only partial MS^n tree matched with the library references, resulting in only partial saponin basic structure annotation of these unknown compounds. The partial structure of unknown compounds could be annotated as the precursor structure which generated the MS3 spectrum of the saponin library reference and the unknown compound was annotated as one of saponin class compounds.

In addition, the novel structure ranking tools included in the Thermo Scientific™ Compound Discoverer™ 3.1 software together with Chemspider and custom database was used for final structure annotation of identified saponin class compounds. The structure-based MS^n workflow was able to annotate 127 saponin compounds.

Poster 078: P078 *Application of ^{13}C flux analysis in diagnosis of metabolic disorders*

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Introduction

Significant cost reduction of DNA sequencing results in discovery of multiple new gene variants. However, their pathogenicity and molecular phenotypes needs to be uncovered and verified by functional tests. Labeling with stable isotopes and targeted metabolomics can be used to measure metabolic fluxes within central carbon and sugar metabolism providing a unified functional assay capable of diagnosing multiple genetic defects.

Technological and methodological innovation

Targeted LC-MS/MS methods were optimized for detection of incorporation of ^{13}C labels into intermediates of sugar and central carbon metabolism. In silico MS/MS fragmentation was performed to predict massed of fragment ions for selective detection of isotopomers. Patient-derived fibroblasts were cultured until near confluency. After a shift to a medium supplemented with ^{13}C glucose the incorporation of isotopic label was followed for up to 48 hours.

Results and impact

The method allowed detection of ^{13}C incorporation into TCA cycle intermediates on positions consistent with action of pyruvate dehydrogenase and citric acid synthase. Significantly lower PDH-dependent flux into TCA cycle was observed for cells derived from patient with prior diagnosis of PDH deficiency.

The results show potential to use MS/MS to distinguish positional isotopomers of metabolites of central carbon metabolism and deconvolute fluxes through alternative pathways.

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Poster 079: P079 *Study on Intracellular Tracking of 13C isotope - labeled Volatile Cancer Markers*

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Introduction

Intracellular volatiles generated during the development or progression and intensification of cancer have the potential to be a significant indicator of cancer diagnosis. But it is difficult to track changes in intracellular metabolites simultaneously, especially when they are volatile. Therefore, we established a novel intracellular detection method of volatile cancer markers through isotopic labeling and estimation of the pseudo-steady state of intracellular metabolite flux.

Technological and methodological innovation

Targeted cancer cell was supplied with ^{13}C labeled glucose and confirmed that the internal metabolic system was sufficiently saturated to ^{13}C. This process was measured by nanoSIMS imaging of $^{13}C/^{12}C$ compared to $^{12}C/^{14}N$, and the isotopic pseudosteady state was calculated by extrapolating carbon flux in the cells. ^{13}C-saturated volatile metabolites were traced to find significant cancer markers that were significantly increased in cancer cells.

Results and impact

Metabolic flux analysis results indicate that 2-pentadecanone is derived from metabolic cascade from glucose to fatty acid biosynthesis. 2-pentadecanone was firstly identified as a potential pan-cancer marker. We also applied a novel analytical platform to trace intracellular metabolism-derived volatiles and established the relationship between volatiles and cancerspecific metabolism.

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Poster 080: P080 *Metabolomics profiling urine by Benchtop NMR*

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Introduction

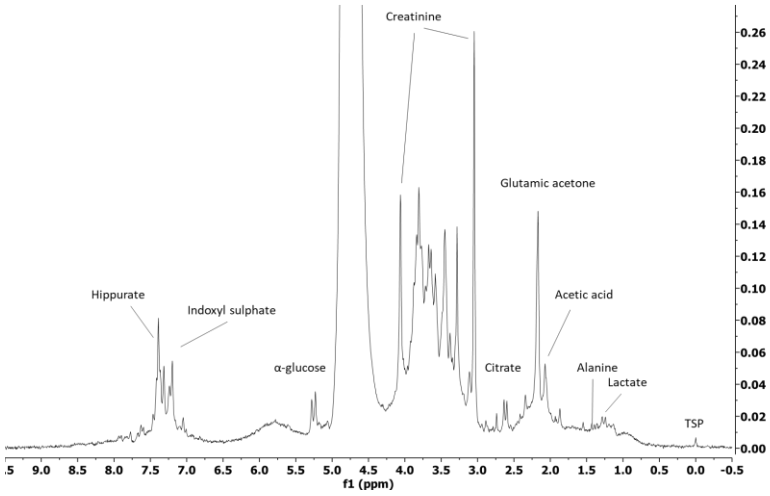
NMR is routinely used for metabolomics investigations of biofluids in order to detect and quantify potential disease biomarkers. The direct proportionality of the NMR signal amplitude to the concentration of the molecules in the sample allows for an absolute quantification without the need for demanding calibrations. Furthermore, the calibration of the method is independent of the composition of the sample or the solvent used for the preparation. [1]

Technological and methodological innovation

Benchtop NMR is a fast-developing field, where the main advantages compared to standard high field systems are portability, no maintenance requirement, easy-to-use and robustness. Moreover, benchtop NMR does not require deuterated solvents to work, making the sample preparation of biofluids very convenient. Therefore, the use of benchtop NMR for fingerprinting metabolomics in biofluids area is an emerging field of interest. [2-4]

Results and impact

The aim of this work is to show the potential of benchtop NMR for the detection of metabolomics in urine samples of common markers [3], in particular we will provide examples to demonstrate the resolution and sensitivity that can be expected from spectra measured in this type of equipment.



^{1}H NMR of urine sample.

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Poster 081: P081 *Multi-platform and multi-matrix analytical development*

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Introduction

The untargeted metabolomic approach, focuses on the study of a type of sampling by several analytical platforms [1] or by several samples by an unique analytical platform [2] . NMR and MS coupling to UHPLC require a specific sample preparation. Because clinical studies need a huge number of samples, for multi-platform and multi-matrix approaches, the preparation can be time consuming.

The goal of this study is to achieve a suitable and common sample preparation for NMR and MS in order to detect the maximum number of metabolites. Saliva, urine, and feces are considered in this study.

Technological and methodological innovation

Different solvents (acetonitrile, methanol, water) and solvent ratios were tested using 1H-NMR and UHPLC (HILIC and C18) coupling to Q-Orbitrap (ESI +/-), each protocol were evaluated. To evaluate the most suitable extraction protocole, metabolite numbers and coefficient of variation were calculated. Analytical method validation, precision, linearity and limit of detection, are performed. Venn diagram are drawn in order to show the multi-platform complementary.

Results and impact

The suitable sample preparation for urine matrix is water dilution, acetonitrile for saliva matrix and methanol/acetonitrile/water solvent for faeces matrix. Those extractions allow to detect 395 identified molecules (urine), 299 identified molecules (saliva) and 382 identified molecules. Analytical platforms are complementary around 40% of metabolites are detected by C18, 50% with the HILIC phase and 10% with 1H-NMR. Validation method parameter, linearity, precision have been evaluated in 1H-NMR and UHPLC-MS.

References

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Poster 082: P082 *A comparison between a targeted and an untargeted approach in the frame of a pilot biomonitoring study*

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Introduction

Exposure to environmental toxicants can be assessed through the determination of metabolites of chemicals in biofluids.

Targeted and untargeted approaches can be applied to characterize the "exposome", both with advantages and disadvantages [1]. The aim of this work was to compare the results of a pilot biomonitoring study obtained both with a targeted quantification of urinary mercapturic acids, metabolites of volatile organic compounds, and with an untargeted approach.

Technological and methodological innovation

Urine samples were collected from 67 adults: 38 non-smokers, 7 electronic cigarette smokers, and 22 traditional smokers. 17 mercapturic acids were quantified with a validated isotope dilution LC-MS/MS method, using a triple-quadrupole mass spectrometer [2]. Using the same chromatographic conditions, the untargeted approach was performed with a time-of flight operating in data dependent mode; data were processed using XCMS and the ANOVA test was used to detect significant features among groups.

Results and impact

Most analytes were quantified in all samples with the targeted approach. 3613 features were identified in the untargeted assay, among which only five mercapturic acids were present; seven mercapturic acids were not identified as a feature but detected inspecting the chromatograms; and four mercapturic acids were not detected at all in the untargeted approach. Despite its promising ability to characterize exposome, the untargeted approach may not detect chemicals present at low levels.

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Poster 083: P083 *Development and validation of a RPLC-MS/MS method for the quantification of ceramides in human serum of patients with CAD*

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Introduction

Ceramides are key-role compounds that regulate numerous central cellular processes, such as apoptosis, inflammation, etc. A plethora of studies have showed that changes on the concentrations of these bioactive lipids in serum are associated with different pathophysiological states, namely obesity, cardiovascular diseases, ovarian cancer, metabolic disorders. Four specific molecular ceramides are highly linked to coronary artery disease (CAD), enhancing the prediction of fatal outcomes.

Technological and methodological innovation

Different sample preparation methodologies were tested, with supportive liquid extraction (SLE) providing the optimum analytical results for the quantification of ceramides in human serum. Here, we present a targeted metabolic profiling for the rapid quantification of ceramides C16:0, C18:0, C24:0 and C24:1 in serum with RPLC-MS/MS.

Results and impact

SLE was found to be an effective sample preparation approach for the accurate and reproducible determination of molecular ceramides in human serum. The validated method is considered of having potentially clinical significance for the rapid diagnosis of CAD subjects enabling the establishment of valid references values in the specific population and the understanding of ceramides' biochemical role. The proposed method can be used in routine clinical analyses.

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Poster 084: P084 *AlpsNMR: an R package for fully untargeted NMR-based metabolomics workflow*

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Introduction

NMR-based metabolomics is widely used to explore metabolic fingerprints of biological systems [1]. While there are various computational tools developed for targeted metabolomics, there is a lack of open-source tools for fully untargeted NMRbased metabolomics, able to handle datasets from raw data to metabolite lists [2].

Technological and methodological innovation

Here, we present AlpsNMR (Automated spectraL Processing System for NMR), an R package that offers an automated workflow for untargeted NMR-based metabolomics analysis. AlpsNMR includes spectra loading, metadata handling, signal processing, integration, statistical analysis, and metabolite identification based on the Human Metabolome Database [3]. This tool allows non-experienced users to include this workflow in their metabolomics routines.

Results and impact

The AlpsNMR R-package, along with a guided tutorial including a test case [4], is freely available to download from https://github.com/sipss/AlpsNMR/blob/master/vignettes/tutorial.pdf under the MIT license.

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Poster 085: P085 *Costs and benefits of switching from vendor-based to open source pipelines for untargeted LC-MS metabolomics*

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Introduction

Data pre-processing of the LC-MS data is a critical step in untargeted metabolomics studies in order to achieve correct biological interpretations. The purpose of data pre-procesing is to reduce the complexity of the raw data, extract the main features and transform them in order to subsequently perform adequate statistical tests [1]. Several tools have been developed for pre-processing, and these can be classified into either commercial or open source software [2].

Technological and methodological innovation

In the present work, two methodologies, vendor vs open-source, for data pre-processing in untargeted metabolomics studies were compared. We highlight differences in these two pipelines using 369 plasma samples analyzed by LC-MS. Specifically, we have processed the data using Agilent software, representing a vendor pipeline. On the other hand, an open source methodology based on R was represented by the combination of several packages, namely IPO/XCMS [3], batchCorr [4] and RAMClustR [5].

Results and impact

Both vendor and open source methodologies have strength and weaknesses. However, we have shown that the open source methodology is the most suitable option for metabolomic studies with larger number of samples in multiple batches. However, this environment is also less intuitive, frequently with lower quality graphical output and with a distinctly steeper learning curve. We provide a detailed tutorial to help users of commercial software to start processing data through R-based methodology.

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Poster 086: P086 *Volatile Biomarker discovery in flatus by GC-MS and GC-IMS to diagnose digestive disorders and diseases.*

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Introduction

Little is known about the organic volatiles (VOCs) in flatus and their relation to health implications. Patients and healthcare professionals have observed that feces change its smells during gastro-intestinal diseases, suggesting that the identification of VOCs biomarkers could offer the potential for rapid identification of gastrointestinal diseases and disorders. The flatus complexity and the difficulties in the identification of minor metabolites still provide great analytical obstacles. [1 2 3 4 5]

Technological and methodological innovation

The aim of this work is to propose new general methodologies to investigate digestive disorders using VOCs found in human flatus. These methodologies are based on GC-MS and GC-IMS measurements focusing on the creation of analytical methodologies and bioinformatics pipelines for data pre-processing and multivariate analysis of VOCs. The flatus analysis is so new, that even the exploratory analysis of the composition for healthy individuals may be of interest for publication.

Results and impact

This study potentially offers novel and significant insights to understand the volatiles from flatus and any advance in this area may have a large impact if any association between VOCs and heath is found. Such use would lead to improved diagnoses and the development of tailored therapies with potential clinical benefits to the patients. Using the volatiles expelled from the human body, opens a new frontier in medical diagnostics due its inexpensive potential and being a non-invasive technique.

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Poster 087: P087 *MetExplore: Omics data analysis in the context of metabolic networks*

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Introduction

Metabolism of an organism is composed of hundreds to thousands of interconnected biochemical reactions responding to environmental or genetic constraints. This metabolic network provides a rich knowledge to contextualize omics data and to elaborate hypotheses on metabolic modulations. Nevertheless, performing this kind of integrative analysis is challenging for end users without sufficiently advanced computer skills since it requires the use of various tools and web servers.

Technological and methodological innovation

MetExplore [1] is a well-established sustainable freely available project maintained since 2010. It is a web server (www.metexplore.fr) dedicated to the analysis of genome scale metabolic networks, with special care taken to allow analyzing metabolomics data in the context of these networks.

MetExplore offers a free all-in-one online solution composed of interactive tools for metabolic network curation, network exploration/visualization [2] and omics data analysis.

Results and impact

Today, MetExplore contains 297 public metabolic networks and 1196 private networks representing 404 organisms. MetExplore has 846 registered users and was used for 39 national and international trainings.

The server is used to tackle many scientific questions, particularly MedDay Pharma uses MetExplore to study neurological diseases such as hepatic encephalopathy [3] and CAFSA syndrome [4].

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Poster 088: P088 *BARSA: BidimensionAl nmR Spectra Annotation*

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Introduction

Metabolomic studies usually aim at comparing metabolic profiles of organisms exposed to different stimuli (e.g. control vs xenobiotic-exposed animals). NMR is one of the main analytical techniques used to generate metabolic profiles. Automated identification of discriminant metabolites based on 1D spectra is a major bottleneck for biomarker discovering, mainly because of peaks overlapping of many metabolites, whereas two-dimensional (2D) NMR can provide more specific structural information.

Technological and methodological innovation

2D NMR spectra provide resonance separation along a second dimension, facilitating identification of individual chemical components [1]. We developed an automated algorithm for metabolite identification based on several 2D NMR sequences using the R language. BARSA compares peak lists of 2D spectra of biological samples to those of pure compounds included in an in-house cured database. Criteria can be applied to exclude metabolites for highly confident minimizing of false positive metabolites.

Results and impact

BARSA algorithm was assessed using a mix of 23 standard compounds. Annotation was based on individual or combined sequences (2 to 5). Sensitivity and specificity were computed for several thresholds based on presence probability and unicity of peak annotation. Highest values were obtained by combining 4 sequences and a 0.5-threshold on presence probability. BARSA overcomes 1D NMR limitations and enables automated annotation of complex NMR spectra with high confidence on identified metabolites.

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Poster 089: P089 *Assessing the impact of physicochemical parameters on the predictive capabilities of thermodynamics-based stoichiometric approaches under mesophilic and thermophilic conditions*

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Introduction

Metabolic engineering in the post-genomic era exploits novel methods in metabolomics and fluxomics. Flux distributions are either experimentally measured (metabolic flux analysis, ^{13}C-MFA [1]) or simulated (flux balance analysis, FBA [2]), by using constraint-based models. However, the optimal solutions do not guarantee thermodynamically-feasible metabolic fluxes – applying thermodynamic constraints has been suggested, but in-depth comparisons and evaluation of these approaches are lacking.

Technological and methodological innovation

Towards this end, the recently published matTFA toolbox [3] was extended to include a broader range of physicochemical parameters and more suitable adjustment equations (2^6 combinations). Additionally, an approach based on max-min driving forces (MDF) [4] was used for comparison. Available experimental results (bioprocessing parameters, ^{13}C-MFA-derived fluxes and metabolomics data) for Escherichia coli and Thermus thermophilus were used to assess the predictive capabilities of these methods.

Results and impact

Whilst a high level of reliability at the fluxomics level (similarity to ^{13}C-MFA) was achieved for all the combinations, the quality of predicted metabolite concentrations values varied significantly. Adjusting the calculations to the growth conditions (e.g. t = 37 °C) improved the correlation with metabolomics data from 5% to 41%. In addition, frequently neglected limitations of 13C-MFA/FBA were analysed, suggesting the need for standardisation and cross-validation in the field.

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Poster 090: P090 *Uniting metabolomics data processing and highly confident annotation across six MS instrumental set ups: MetaboScape 5.0*

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Introduction

By combining different platforms, complementing their respective strengths, the gap between high-throughput and in-depth analysis strategies can be closed. MetaboScape 5.0 incorporates the feature extraction and ion deconvolution algorithms TReX 2D, 3D and 4D, integrates the processing-, dereplication- and unknown annotation-workflows for FIA-MRMS, LCMRMS, LC-ESI-TOF, and LC-ESI-TIMS-TOF in a single software. T-ReX² additionally covers MALDI-MRMS and MALDI-QTOF imaging data.

Technological and methodological innovation

The T-ReX feature extraction algorithms transform raw data from different instrument types into compound matrices of putative compounds, comprising ions and isotope patterns. Various tools for metabolite annotation seamlessly adapt to the annotation quality criteria each instrumental platform provides (accurate mass, isotope pattern (incl. fine structure), retention time, MS/MS spectra, and/or collisional cross section). Data acquired in different polarities can be merged for joint evaluation.

Results and impact

MetaboScape allows for molecular formula generation, in-silico fragmentation, CCS prediction, MSMS spectral and retention time matching across different platforms. This allows uniting high throughput workflows with the maximum of in-depth exploitation of all the mass spectral information for specific metabolites. As an example, it also enables to transport metabolite annotations from LC-ESI-TIMS-PASEF, confirmed by all 5 quality criteria, into MALDI imaging measurements for spatial metabolomics.

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Poster 091: P091 *Integrated workflow with quality control for large cohort and clinical metabolomics research using robust hardware and signal correction*

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Introduction

Metabolomics research relies on precision measurement of statistically powered sets of hundreds or thousands of samples. First, this requires robust analytical hardware with long term stability, capable of generating high precision data. Second, processing of large datasets may require additional mathematical correction to compensate for systematic changes in observed signals as samples interact with the analytical system affecting its performance.

Technological and methodological innovation

We present a fully automated software workflow for the automated feature-wise correction of intensity drifts based on quality control samples, improving data precision and statistical reliability. An interactive and intuitive visualization provides rapid review of intensity drifts and their corrections as well as detection of statistical outliers.

Results and impact

We investigated the long-term stability of an LC-HR-QTOF system by measuring a batch of more than 1000 urine samples and monitoring the effect of data acquisition on MS ion source contamination, detector aging and sample degradation. A new run-order signal drift correction reduced the relative standard deviation of feature intensities within sample groups measured across replicate quality control measurements. This increased the number of analytes which meet the requirements of an RSD below 20%.

Poster 092: P092 *Automatic annotation in untargeted metabolomics: a proof of concept protocol based on recurrent experiments*

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Introduction

Untargeted LC-MS metabolomics detects thousands of signals in each experiment. Some of them refer to real measured metabolites, but others, known as non-biological signals, can be associated to a wide range of factors, such as background ions, chemical contaminants or informatic artifacts, among others. It is broadly recognized that one of the major bottlenecks in the metabolomics workflow is metabolite identification, which is time-consuming and usually has a considerable failure rate.

Technological and methodological innovation

A large number of metabolites are detected recurrently in different studies on the same sample type when using the same analytical method. Knowledge acquired in previous experiments thus allows to accelerate the identification process of new studies and enables to focus on novel and/or still unknown metabolites. We propose to implement functionality required to annotate signals in a specific system and to build a reference database facilitating annotations of subsequent studies.

Results and impact

Currently we are listing signals associated with specific compounds, but also yet unidentified ions detected in the system. We think that in the future we will be able to provide a catalog of metabolites detected with a specific analytical method in a specific biological matrix. The effectiveness of this pipeline is now evaluated using a series of different standard mixtures and next we plan to apply this on complex biological matrices.

Poster 093: P093 *Metabolomics results interpretation using recommender system embedded in metabolic network visualization*

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Introduction

The visual and interactive exploration of metabolic networks, usually aiming to reconstruct metabolic scenarios explaining experimental data, is mainly driven by expert knowledge not necessarily modelled in the network. It is thus difficult to fully automatize the reconstruction of metabolic scenarios and the selection of the most-relevant ones. On the other hand, manual exploration is usually tedious without a priori filtering, given the complexity and size of genome-scale metabolic networks.

Technological and methodological innovation

In order to reduce the information overload, we recently developed a recommendation system, highlighting relevant compounds to investigate, based on their connections to markers identified by metabolomics [1]. In order to ease its use in the reconstruction of metabolic scenarios, we embedded it in an interactive network visualization [2]. We shifted from the traditional overview browsing model for large networks to an incremental expansion of users' initial focus [3], empowered by AI support.

Results and impact

Our approach, focused on expert interaction with their data, is freely available through MetExplore [4]. By highlighting parts of a compound's neighborhood that can lead to known markers and by providing integrated document retrieval from PubMed, our metabolic exploration framework assists the reconstruction in a comprehensive way. We used it to extend recently published mechanistic scenarios, adding parts of the network which relation to the condition under study is supported by the literature.

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Poster 094: P094 *Technological developments and opportunities with Workflow4Metabolomics[1]*

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Introduction

Metabolomics data analysis is a complex, multistep process, which is constantly evolving with the development of new analytical technologies, mathematical methods, bioinformatics tools and databases. The latest online Workflow4Metabolomics infrastructure [1] provides new upgrades for LC-MS, GC-MS and NMR pipelines, including preprocessing, quality control, statistical analysis and annotation tools. W4M also proposes new community resources promoting open science in metabolomics.

Technological and methodological innovation

W4M major updates include: a new MSMS pipeline; MS extraction workflows based on the XCMS3.0 R package[2]; data filtering and visualization steps based on Analytic Correlation Filtration[3] and Between-table correlation tools; metabolite databases downloader; a contaminant identifier, and utilitarian tools. New training resources through the Galaxy Training Network (GTN) are proposed and W4M is announcing the opening of its GitHub repository to gather metabolomics tools and libraries.

Results and impact

W4M's improvements increase raw data input management and LC/GC-MS workflow efficiency. We highlight how current advances contribute to open data analysis practices worldwide. In addition the W4M organization on Github repository aims to review code, annotate tool and propose a showcase for contributors from the metabolomics world.

References

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Poster 095: P095 *Taxonomically informed metabolite annotation enhances confidence in specialized metabolomes annotation*

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Introduction

Specialized metabolites, are by definition strongly linked to the taxonomical position of the producing organisms. Considering taxonomy when exploring natural products thus appears as an evidence. In 1804 De Candolle postulated that *1) Plant taxonomy would be the most useful guide to man in his search for new industrial and medicinal plants and 2) Chemical characteristics of plants will be most valuable to plant taxonomy in the future.* [1] We adhere to De Candolle's postulate and propose computational solutions to establish their validity.

Technological and methodological innovation

We build a benchmarking dataset constituted by >2000 entries composed of structures, their biological source and associated experimental MS fragmentation spectra. This dataset encompasses structures and biological sources representative of the known specialized metabolome space. Experimental spectra were acquired on the main MS platforms (Orbitrap, ToF, QQQ, etc.) We wrote a set of scripts allowing to proceed to the automated taxonomically informed metabolite annotation process.

Results and impact

We show that the consideration of taxonomic position in the metabolite annotation process leads to a systematic improvement (x1.5 to 7 fold increase in the F1 score) of state-of-the-art in silico metabolite annotation solutions (ISDB-DNP, Sirius, MSFinder) outputs.[2] This increased confidence in metabolite annotation efficiently improves the natural products drug discovery process. We will discuss the principles and implementation as well as practical applications of such approach.

References Max 5

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Poster 096: P096 *Improving prediction of essential genes using context-specific metabolic network ensembles*

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Introduction

Context specific metabolic networks [1] are automatically reconstructed networks based on experimental (mainly transcriptomic data) with the aim of modeling and predicting the specific metabolic behavior of different tissues, cells or conditions [2]. However, some of the limitations of the techniques are: 1) there is no single resulting network which best fits the data in contrary to what is returned by most methods; and 2) different parameters can generate slightly different networks with no objective way to assess which one is better. We hypothesize that predictions using the ensemble of the best fitted networks can improve on average the predictions made by a single model.

Technological and methodological innovation

In order to validate this hypothesis, we developed an extension of iMAT [3] that allows us to explore in a flexible way the set of optimal networks that can be generated from a given transcriptomics dataset. Using data from Yeast under aerobic conditions, we generated thousands of optimal metabolic networks for different parameter settings. We used the ensemble of networks to predict gene essentiality and we evaluated the precision and recall with different network ensemble strategies.

Results and impact

Analysis of the predictions made with the ensemble of networks show that ensembles can improve precision and recall of the predicted essential genes. In addition, different trade-offs between precision and recall can be achieved using different ensemble strategies.

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Poster 097: P097 *Showcasing Longitudinal Metabolomic Datasets: Accessible, Fast and Simple*

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Introduction

The increasing number of longitudinal metabolomics studies enable time-resolved exploration of individual molecular changes over days, weeks, or even years, as well as insights into metabolic responses to specific perturbations. Adding the dimension of time significantly increases the complexity of metabolomics data. For an in-depth exploration of these timeresolved datasets, tools for comprehensive presentation and visualization of data and statistical results are crucial.

Technological and methodological innovation

To this end, we are developing a reusable computational framework, which integrates a pipeline covering the steps from time-course data pre-processing to interactive visualization of results. This R-based framework enables efficient data exploration of time-resolved metabolic features for non-data scientists and can be easily customized to any longitudinal metabolomics study. The output is depicted within an intuitive web-based graphical user interface, which can be accessed through any browser.

Results and impact

Our framework combines multiple modules to visualize data and statistical results. Within our advanced search module, the user can discover metabolites of their interest or find similar metabolic temporal patterns. Further modules facilitate a quick interactive visualization of molecular dynamics by time course plots, as well as providing a systems overview of metabolic changes in dynamic networks. An example use case of our framework can be found at http://humet.helmholtz-muenchen.de/.

Poster 098: P098 Developing methods for targeted metablomics by LC-MS

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Introduction

Mass spectrometry is increasingly used as the key metabolomic profiling technology. Hyphenation with an appropriate separation method increases sensitivity and the ability to discriminate metabolites. Liquid chromatography has many advantages, but also one key disadvantage – highly polar metabolites are challenging to retain well. Reversed-phase chromatography is best for non-polar or semi-polar metabolites; HILIC is also widely used, but is less robust than RPLC.

Technological and methodological innovation

We have evaluated in depth combining existing LC-MS techniques: reversed-phase, ion-pairing, and amine derivatization. While these techniques have all been used for metabolomics, we have carried out an exhaustive comparison of retention times and effects on sensitivity for a direct comparison of these techniques, for a library of around 500 metabolites.

Results and impact

We have developed methods for targeted metabolomics, but the data about the chromatographic properties would also be relevant to untargeted profiling. We have established recommendations for combinations of the different LC-MS approaches; using all three gives maximum coverage, but ion-pairing LC-MS and amine derivatization and RPLC-MS are clearly complementary, and combining just these two covers a wide range. We demonstrate the method in a biological model of healthy ageing.

Poster 099: P099 *The SMRT dataset for machine learning-based metabolite retention time prediction.*

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Introduction

Retention time (RT) is an orthogonal property that enables confident metabolite annotation when combined with mass spectrometry information [1]. However, current approaches for RT prediction in liquid chromatography–mass spectrometry (LC-MS) lack sufficient accuracy due to limited available experimental data [2].

Technological and methodological innovation

We introduce the METLIN's [3] small molecule retention time (SMRT) dataset [4], a publicly available dataset covering the experimentally acquired retention time of 80,038 small molecules using reverse-phase LC. We assessed the potential of the SMRT dataset by applying machine learning to predict and use RT for metabolite annotation.

Results and impact

Results showed a good capability to rank the correct molecular identity among the top 3 candidates based on predicted RT. Results also showed that machine learning-based prediction is limited by the number and similarity level of molecules in the training set to those in the validation or a real case set. We anticipate that the SMRT dataset will enable the community to apply machine learning or first principles strategies to generate better models for RT prediction.

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Poster 100: P100 LC-MS/MS data analysis with xcms

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Introduction

The xcms software [1] is a well established R package for the preprocessing of LC-MS-based untargeted metabolomics data. By extensive refactoring the package and re-using functionality and mass spectrometry (MS) data representation strategies from the MSnbase package [2] for proteomics data analysis, xcms gained native MS/MS support.

Technological and methodological innovation

We recently added support for LC-MS/MS data in xcms: for data dependent acquisition (DDA) data, MS2 spectra can be identified and extracted for each detected chromatographic peak or feature. For data independent acquisition (DIA) data such as SWATH data, xcms allows to perform chromatographic peak detection on MS2 data and reconstruct MS2 spectra. MS1 chromatographic peaks are matched to MS2 based on peak shape correlations.

Results and impact

The recent changes in xcms, particularly the support for LC-MS/MS data analysis, facilitate an improved compound annotation for untargeted metabolomics experiments.

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Poster 101: P101 *Targeting MDM2-dependent serine metabolism as a new therapeutic strategy for liposarcoma*

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Introduction

Well-differentiated and de-differentiated liposarcomas (LPS) are characterized by a systematic amplification of the MDM2 oncogene that encodes a key negative regulator of the p53 pathway. The molecular mechanisms underlying MDM2 overexpression, but sparing wild-type p53, in LPS remain poorly understood. Here, we show that the p53-independent metabolic functions of chromatin-bound MDM2 are exacerbated in LPS and mediate an addiction to serine metabolism that sustains nucleotide synthesis and tumor growth.

Technological and methodological innovation

To investigate the role of MDM2-mediated regulation of serine metabolism in liposaroma cells, we performed stable isotope tracing experiments to determine the metabolic fate of exogenous serine or glucose. Our data indicate that pharmacological and genetic inhibition of MDM2 impairs de novo serine synthesis and impacts on nucleotide metabolism in LPS cells.

Results and impact

Treatment of LPS cells with Nutlin-3A, stabilized p53 but unexpectedly enhanced MDM2-mediated control of serine metabolism by increasing its recruitment to chromatin, likely explaining the poor clinical efficacy of this class of MDM2 inhibitors. In contrast, inhibition of chromatinbound MDM2 or interfering with de novo serine synthesis, impaired LPS growth both in vitro and in clinically-relevant Patient-Derived Xenograft models. Our data indicate that targeting MDM2 functions in serine metabolism represents an efficient therapeutic strategy for LPS.

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Poster 102: P102 *Biofluid Applications in Benchtop Low-frequency 60 MHz NMR: A Metabolomics Investigation*

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Introduction

High-frequency (HF) ^{1}H NMR analysis has been successfully developed to be applied in metabolomics investigations, and offers significant bioanalytical advantages for such applications. However, HF NMR analysis also presents some disadvantages such as high cost and size, which limits their accessibility to many researchers and precludes regular use in health centres. Surprisingly, few metabolomics studies using low-field spectrometers (LF) have been published to this day and remains to explore[1,2].

Technological and methodological innovation

The objective of this work was to apply and compare LF and HF 1H-NMR metabolomics approaches to the study of type 2 diabetics (T2D) urine samples versus controls, as well as evaluates the capabilities of LF 2D cosy spectra on urine samples in term of metabolites resolution.

Results and impact

T2D samples distinguish from controls and several metabolites are linked to this separation. These results are observable with both HF and LF spectra. We concluded that under the same preprocessing conditions, it is possible to attain comparable results for both HF and LF. Linear correlations were then performed between 1D and 2D intensities of several metabolites and led to good coefficients values attesting of the validity of 2D LF spectra. LF spectrometers could make for excellent tools to perform metabolomics analyses.

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Poster 103: P103 *Analysis of Penillium sclerotiorum metabolome by molecular networking*

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Introduction

Insects are the most represented and diversified living organisms on earth.1 They are associated with a very large diversity of microorganisms. Whereas associations are well studied within the Apocrites,2 few publications are dedicated to termites/microorganism interactions outside of trophobiosis context3. A collection of 130 microorganisms isolated from 12 species of termite workers collected in French Guiana was studied and the metabolome of each strain has been explored.

Technological and methodological innovation

The ethyl acetate crude extracts (1 mg/mL) from each strain were separated by RPLC on a C18 column and a water/acetonitrile gradient. The metabolites were analyzed using data dependent analysis mode on a Q-ToF (Agilent Technologies). Each crude extract was also tested for their antimicrobial activity on human and insects pathogens. Both information was combined in MetGem software4 to construct a molecular network allowing the annotation of metabolites family with potent antimicrobial activity.

Results and impact

The crude extract of the fungus BSNB-CN111, identified as Penicillium sclerotiorum, showed an activity of 32 µg/mL against a human pathogen (Tricophyton rubrum, fungus) and showed a unique metabolite cluster of interest. Two metabolites were identified through the MS2 pattern as ochrephilone and sclerotiorin.5 In-depth analysis of molecular network from BSNB-CN111 specific molecules allows us to identify several other known compounds as well as new putative analogues.

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Poster 104: P104 *FoodomicsGR*

National research infrastructure for the comprehensive characterisation of Foods

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Introduction

National infrastructure FoodOmicsGR_RI aims to coordinate the work of 17 research teams from 8 Greek Universities/Research Centers in a network that aims toward 1) method development and protocol validation for the comprehensive characterization of foods and 2) support intervention studies that will provide data on health claims/nutritional value of foods. FoodOmicsGR_RI combines food/nutrition science with the most advanced analytical techniques, bioinformatics and field/application sciences.

Technological and methodological innovation

Key objective is the generation of databases of food content to promote the assessment of nutritional value, geographical origin and traceability of local produce. Key to these, is the establishment of protocols for metabolomics and proteomics analysis and omics data combination. State of the art UPLCHR-MS, UPLC-MS(MS), GC-MS(MS), NMR, ICP-MS, NGS technologies are employed to map unique sample sets (various vintages, experimental cultivations etc) with detailed metadata and background knowledge.

Results and impact

FoodOmicsGR_RI has so far established 14 protocols [1-5] for the analysis of wine, olive oil, honey, dairy and other food products. Metabolomics (both un-targeted and targeted modes) along with proteomics, genomics and metallomics data classify foods according to geographical origin (various products), vintage, cultivation methods (e.g. wild vs fed fish, game vs bred animal, bee products by feeding modes) authenticity/quality (adulteration) control and assisted numerous nutritional studies.

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www.foodomics.gr

Poster 105: P105 *Bioavailability of dietary long chain-PUFAs in the rat retina: mapping by MALDI imaging mass spectrometry*

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Introduction

Dietary EPA and DHA are recommended to protect against age effects in the retina1. Therein, these PUFAs are elongated into very long chain PUFAs (VLC-PUFAs; C ≥28), allowing normal retinal functions and structure2. Nevertheless the spatial distribution of fatty acids in the retina in response to dietary omega-3 supplementation has never been explored thoroughly, although it would participate to the prevention of neuroinflammatory diseases.

Technological and methodological innovation

In this study, Wistar rats were fed with equivalent doses of EPA-containing phospholipids (PL), EPA containing triglycerides (TG), DHA containing PL and DHA-containing TG incorporated in their diets as source of lipids during 8 weeks. Quantitative and spatial organization changes in phosphatidylcholine (PC) and phosphatidylethanolamine (PE) species in the retina were determined by LC-MS/MS and MALDI-ToF-MS imaging following the different dietary intakes.

Results and impact

We found an increase in DHA content in the retina, particularly double DHA-containing PC and PE and an increase in VLCPUFAs following PL-EPA and TG-DHA rich diets. VLC-PUFAs-containing PC were detected in the photoreceptor layer. All supplemented diets triggered spatial organization changes of DHA in the photoreceptor layer around the optic nerve. These data provide new insights in fatty acids distribution in the rat retina after dietary omega-3 PUFA supplementation.

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Poster 106: P106 *Colorectal Cancer: Biomarkers and Effect Size*

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Introduction

Colorectal cancer kills more than 700.000 persons each year worldwide1. Nevertheless, its diagnosis is still largely based on invasive tissue sampling and gaps remain in the understanding of its pathogenesis, with complex combinations between lifestyle, genetics, epigenetics, chronic inflammation (IBD) and microbiota. In addition to existing screening methods, global metabolic profiling can help both to diagnose and to understand the various states of the disease2,3.

Technological and methodological innovation

We studied the active and remission states (n=17 each) of colorectal cancer by comparing them to respective healthy control samples (n=19 and n=17). To do so, an optimized and validated (NIST SRM 1950) comprehensive GC×GC-(HR)TOFMS method we developed was used. It includes an in-house QC system, data processing and identification. We looked for potential biomarkers and applied a measure called effect size4,5.

Results and impact

We found 22 significantly altered metabolites in the active state that were able to discriminate efficiently (ROC AUC of 0.86, sensitivity and specificity of 0.78 and 0.73). 10 of them had signals significantly returning to normal, healthy levels in the remission samples and were therefore spectific to the active state. Since it is rarely employed in metabolomics and separation science, we discuss the interest and methodological aspects of effect size.

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Poster 107: P107 *Inflammation signaling by cardiac stromal exosomes*

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Introduction

Cardiac remodeling changes the interactions between myocytes and stromal cells. Polyunsaturated fatty acids (PUFAs) regulate the post-myocardial infarct inflammation (1) or remodeling (2). PUFAs give rise to inflammatory or resolving mediators (3). Exosomes are important paracrine vectors, exerting a therapeutic effect on the damaged heart (4). Few is known about the lipid mediator content in exosomes. We analyzed in depth the PUFAs contents in cells and in exosomes of the cardiac stroma.

Technological and methodological innovation

To identify the mechanisms of macrophage polarization by cardiac stromal cells, we analyzed in depth the PUFA contents in cells and in exosomes of the cardiac stroma. As clinical trials assessed the effects of supplementation with PUFAs in secondary prevention of cardiovascular disease (5), we used conditioned media of cultured heart fibroblasts and Mesenchymal Stem Cells (BM-MSC) supplemented with EPA and DHA. The inflammatory and pro-resolving lipids were analyzed by MRM HPLC-MSMS.

Results and impact

We analyzed the lipid mediator signature of the cardiac stromal cells (fibroblasts, myoblasts) or BM-MSC and of the exosomes released by them. We studied the impact of EPA and DHA supplementation on these signatures. The two PUFAs strongly modified the mediator profiles in different ways. The differences between these cells and exosomes fingerprints associated to their high sensitivity to PUFA supplementation, make exosomes to a probable inflammatory regulator involved in the heart remodeling.

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Poster 108: P108 *Assessment of Preterm Infant's Nutrition by Untargeted Human Milk Lipidomics*

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Introduction

Human Milk (HM) is the gold standard for newborn nutrition. Preterm infants whose mothers are unable to provide sufficient own mother's milk (OMM), receive pasteurized donor human milk (DHM). We hypothesize that storage, processing, and differences due to gestational age and between individuals are reflected in compositional changes of HM. In an untargeted liquid chromatography-mass spectrometry based approach we assess the lipidomic signatures of OMM and DHM before/after pasteurization.

Technological and methodological innovation

An experimental procedure was developed for achieving an adequate sample throughput suitable for clinical studies including a single phase extraction using methanol and methyl tert-butyl ether [1] followed by UPLC-TOFMS analysis of milk extracts with a total runtime of 16 min. The suitability of Quality Control – Support Vector Regression for batch effect compensation was demonstrated for lipidomics applications and a novel strategy for data-dependent acquisition of MS/MS spectra was developed.

Results and impact

The experimental procedure allowed the identification of 280 lipids and metabolites of different classes including DAGs, TAGs, oligosaccharides, FAs, and PCs in HM collected at different gestational ages and lactation periods. We identified significant changes in HM fingerprints before and after pasteurization as well as between DHM and OMM. Results will be useful to adjust DHM treatment and storage protocols for Human Milk Banks to optimize nutrition for preterm infants receiving DHM.

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Poster 109: P109 *Exploring the use of GC-CI-MS for stable isotope labeling in metabolomics*

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Introduction

Metabolomics is nowadays a well-established branch of the omics field. Due to the chemical diversity of naturally occurring metabolites, metabolomics is rich in analytical instrumentation and configurations, being GC-MS and LC-MS the most common techniques.

With the introduction of chemical derivatization, its high chromatographic resolution and low background noise, GC-MS-based metabolomics has evolved into a powerful analytical platform for multiple types of targeted and untargeted metabolomic experiments, and fluxomics.[1,2]

Technological and methodological innovation

The most widely used ionisation method in GC-MS is electron impact ionization (EI), a hard ionisation strategy that generates highly reproducible fragmentation spectra. However, softer ionization techniques based on chemical ionisation (CI) are alternative methods, where molecular ions of analysed compounds are kept (mostly) intact. GC-EI MS has become a platform of choice for stable isotope tracing studies and fluxomics approaches. [2] The coverage of GC-EI MS is comprehensive enough to cover most central carbon metabolism thanks to the use of chemical derivatization. Interestingly, the use of GC-CI MS for stable-isotope tracing is largely unexplored. [3]

Results and impact

In here, we have studied the suitability of GC-CI-MS for stable-isotope tracing using multiple analytical configurations based on low-resolution and high-resolution mass spectrometry. We prove that isobutane is an ideal ionization gas by its ability to yield intact protonated molecular ions [4], and coupled to cutting-edge highresolution GC-MS instruments, currently provide the best configuration for isotopologue quantification. In addition, we have developed an R-package called isoSCAN capable of processing GC-CI MS data from stable-isotope labeling studies.

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Poster 110: P110 *Development of a targeted urinary metabolic profiling technique for inflammatory bowel disease using Liquid Chromatography Mass Spectrometry with Electrospray Ionization Quantification.*

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Introduction:

The inflammatory bowel diseases (IBD), ulcerative colitis (UC) and Crohn's disease (CD) are characterised by chronic gastrointestinal inflammation. Both of which may cause debilitating symptoms. Diagnosis involves radiological and endoscopic procedures. In the last decade, multiple metabonomic studies have given insights into both UC and CD; several biofluids have been studied1,2. However, findings have been inconsistent, and few offer clinically useful quantitative data.

Technological and Methodological Innovation:

A comprehensive literature review (with the expansion of relevant biochemical pathways) was carried out to identify consistently reported metabolites for incorporation into a fully quantifiable and targeted assay. A selection of these metabolites was validated for use in predicting IBD with a combination of univariate and multivariate techniques using 1H-NMRacquired urine data. A finalised panel was then taken forward for UPLC assay development and validation in a clinical cohort.

Results and Impact:

42 metabolites were identified for the IBD metabolite panel: these have been taken forward for assay development. Analysis of existing urinary data from IBD patients/controls showed that the specified panel of metabolites improved the predictive score and significance of the models when compared to global profiling (from p=1 to p=0.003). Following development, the assay will be validated on a de novo cohort of IBD patients to quantify metabolic perturbations associated with disease and therapy.

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Poster 111: P111 *CEU MASS MEDIATOR: THE TOOL TO ANNOTATE COMPOUNDS IN CE-MS FOR METABOLOMICS*

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Introduction

CEU Mass Mediator (CMM) is an online tool that provides services for metabolite annotation and identification such as the simultaneous querying of metabolomic databases and the subsequent scoring of the query results using an expert system[1]. Capillary electrophoresis coupled to mass spectrometry (CE-MS) is a complementary tool to add to the toolkit of multiplatform untargeted metabolomics[2]. This technique is ideal for the analysis of ionic and polar compounds in complex sample matrices[3].

Technological and methodological innovation

Recently, ROMANCE software tool allowed to convert migration time (MT) into the effective mobility (μEff)[4]. However, there are no on-line databases providing services to annotate compounds from CE using MT or μEff. A new service to provide a search over the databases using m/z and MT or μEff values has been created. Experimental data from 500 compounds both pure reference standards or spiked into human plasma has been collected using a CE system coupled to a TOF-MS equipped with an ESI source.

Results and impact

The new service allows users to annotate compounds from CE-MS experiments[5]. The database contains experimental data obtained in CEU-San Pablo and Geneva University. It is designed to enable the incorporation of new data (submissions are welcome). It is prepared to process information from different polarities (direct and inverse), internal standards (paracetamol and methionine sulfone by now) and buffers (formic and acetic acid by now). It intends to be the main database for CE-MS annotation.

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Poster 112: P112 *Improved Metabolite Identification in a Single Injection with SWATH® Acquisition for Untargeted Metabolomics Workflow*

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Introduction

Comprehensive metabolite identification with MS/MS library spectral matching can be problematic for data dependent acquisition (DDA) workflows as it often requires multiple injections for each sample. SWATH® Acquisition, a Data Independent Acquisition (DIA) method, with optimized variable windows, provides a powerful workflow requiring only a single injection per sample for each polarity. In addition to capturing product ion spectra for all ionizing analytes, SWATH Acquisition also provides the option of quantitation at either the MS or MS/MS level allowing a comprehensive qualitative and quantitative analysis of metabolites in complex biological samples like plasma.

Both the DDA and SWATH Acquisition methods compared in this study were single injection workflows, using the same simple RP chromatography with a 20 minute run time per injection. The speed of the TripleTOF® 6600 QTOF system allows for the top 20 selection in DDA or 20 variable SWATH acquisition windows in DIA analysis with a cycle time of 651 msec for both the DDA and SWATH Acquisition methods. The choice of top 20 or 20 SWATH Acquisition windows has previously been demonstrated to provide high quality comprehensive coverage1. In the trade off of cycle time with comprehensive coverage and data quality this cycle time still permits for quantitation with more than 9 points across a peak of 6 seconds providing accurate and reproducible integration.

CONCLUSIONS

A simple 20 minute RP-LC method was used to acquire DDA and SWATH acquisition data on an extracted human plasma sample using a TripleTOF 6600 System. The top 20 DDA method was able to identify 476 of the features on the basis of precursor mass and MS/MS spectral matching. The 20 variable window SWATH acquisition method resulted in an additional 215 features being identified, a 45% increase, for a total of 671 compounds identified in a single injection. This study has shown that variable window SWATH acquisition can be a useful for improving compound identification in untargeted metabolomics.

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Poster 113: P113 *High-throughtput single-step sample preparation coupled to targeted LC-MS/MS approach for extended coverage of human plasma metabolome and lipidome*

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Introduction

Expanding coverage of the polar metabolome and lipidome essential for generation of well-founded hypotheses from untargeted assays. Traditionally, polar metabolite extraction is performed using methanol and lipid extraction using biphasic liquid-liquid extraction either with MTBE, chloroform or dichloromethane. In this work, we evaluated single-step extraction methods for polar metabolome and complex lipid profiling, using high-coverage targeted LC-MS/MS methodology.

Technological and methodological innovation

Single step methods using isopropanol (IPA) and butanol:methanol (BuMe) solvent mixtures were compared against the goldstandards for lipid (biphasic MTBE) and for polar metabolite (MeOH:H2O) extraction. The polar metabolome and complex lipids coverage, signal intensity, reproducibility and protein removal efficiency were used as criteria to evaluate the method performances. HILIC-based ESI-MS/MS approach targeting complex lipids and polar metabolites was used for the analysis.

Results and impact

The developed high-throughput single step sample preparation (IPA or BuMe) using HILIC-MS/MS profiling has allowed for the detection of 600 lipid species (glycerolipids, cholesterol esters, sphingolipids, glycerophospholipids, free fatty acids) and 140 polar metabolites (including amino acids, carboxylic acids, nucleotides, etc) in human plasma, in a reproducible manner. The high coverage and reproducibility of this single-step approach permits its application in large-scale population studies.

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Poster 114: P114 *In vivo isotopic tracing experiments reveal lactate as an oxidative substrate for brown/beige adipose tissues*

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Introduction

Activation of "healthy" energy dissipating brown/beige adipose tissues represents attractive therapeutic strategy against obesity and age-related metabolic disorders1. Traditionally described for their ability to burn glucose and lipids, our previous work suggested that lactate may constitute an alternative substrate for brown/beige adipose tissues 2-4, opening a novel field in the control of their metabolic activity. We herein aim at finely characterizing lactate utilization in these tissues.

Technological and methodological innovation

Metabolomics experiments were performed on the supernatant of primary adipocytes using nuclear magnetic resonance, to determine kinetic variations in metabolite concentrations. In vivo isotopic tracing experiments using 13C-lactate intra-peritoneal injection in mice and analyses of 13C incorporation in metabolites by mass spectrometry enabled us to trace lactate utilization and its metabolic fate in brown/beige adipose tissues.

Results and impact

We demonstrated that brown/beige adipocytes can uptake lactate through the monocarboxylate transporter-1 and oxidize it. In vivo 13C-tracing experiments reveal that circulating lactate significantly feeds the oxidative metabolism of brown adipose tissues, more importantly than in white adipose tissues. These findings open novel perspectives for the control of brown/beige adipose tissues activity and for their role in systemic lactate homeostasis that may be disturbed in age-associated diseases.

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Poster 115: P115 *LipostarMSI: Comprehensive and Vendor-Neutral Data Analysis Platform for Mass Spectrometry Imaging*

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Introduction

Mass spectrometry imaging (MSI) is used for studying the distribution and alterations of the metabolome within biological tissues [1, 2]. The rich, multidimensional data produced by MSI, combined with the lack of comprehensive software solutions that cover the entire workflow needed for a complete MSI data analysis, poses a major challenge preventing its full exploitation. Here we introduce LipostarMSI: new vendor-neutral software for comprehensive targeted and untargeted MSI data analysis [3].

Technological and methodological innovation

To evaluate the image analysis and automated identification capabilities of LipostarMSI, data (MSI and MS/MS) was acquired from rat liver and human biopsies using the innovative DDA-imaging method recently described [4]. All data analysis and identification was performed using LipostarMSI (Molecular Horizon Srl, IT). LipostarMSI is the only integrated software package to-date capable of supporting both comprehensive high-resolution MSI data analysis and analytes identification.

Results and impact

Applications to human biopsies enabled lipid species accumulation within tissues to be visualised and unambiguously identified, providing insight into the metabolic hallmarks of different tumours. LipostarMSI provides a powerful platform for comprehensive MSI data analysis, greatly streamlining biochemical data interpretation. Its use will pave the way to establish a data-driven histology exploitation of high resolution MSI omics and to achieve unprecedented functional interpretation.

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Poster 116: P116 *SWATH® Acquisition Allows a Deeper Level of Comprehensive Metabolite Quantitation*

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Introduction

The field of metabolomics and metabolic profiling faces a large challenge, in accurately identifying and quantifying hundreds to thousands of metabolites in a single run. Generally, quantitative metabolomics is performed on triple quadrupole or QTRAP® systems in a targeted manner by multiple reaction monitoring (MRM) for enhanced sensitivity and selectivity. Internal standards are often used to enhance quantitative accuracy. SWATH acquisition, a data independent acquisition (DIA) technique, is well adopted in quantitative proteomics1, but still not commonly used in quantitative profiling of metabolome. Variable window SWATH acquisition has been shown to identify a higher number of metabolites compared to the traditional data dependent acquisition (DDA) approach, thus enabling broader metabolome coverage2. Here, SWATH acquisition is used for quantitation of selected metabolites using the MS/MS data, for reduced interferences, improved signal-to-noise and deeper metabolite quantitation (Figure 1). The use of MS/MS fragments for metabolite quantitation provides better selectivity, and ultimately increased sensitivity compared to simply relying on the precursor ion for quantitation. A SWATH acquisition map contains MS and MS/MS information of every detectable metabolite in the sample and is therefore a digital archive of the sample. This reduces the need to go back and re-run samples; data can just be re-mined as the hypothesis evolves.

CONCLUSIONS

Variable window SWATH Acquisition provides good quality quantitative data for metabolites in complex matrices. Using the full scan MS/MS data provides both confidence in identification and quantitation data that is less prone to issues with interferences.

SWATH acquisition measures MS and MS/MS spectra of every detectable metabolite in the sample, providing a digital archive of the sample that can be easily remined.

Due to many coeluting species in complex matrices, using only the MS spectrum and retention time is often not sufficient for metabolite identification. MS/MS information is necessary to obtain further structural knowledge about the metabolite. MS/MS quantitation of metabolites often leads to lower detection limits due to significantly improved signal to noise ratios vs MS data. Measuring the whole MS/MS spectrum allows selection of the best fragments for metabolite quantitation.

SCIEX OS software combines comprehensive qualitative and quantitative data analysis, making data processing easier and more efficient.

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Poster 117: P117 *Community-based urine sampling methodology and biomarker technology for assessment of dietary exposure*

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Introduction

Obtaining objective, self-reported dietary exposure information from individuals is challenging due to the complexity of food eating patterns and the limitations of self-reported monitoring tools (1). This hinders research to associate dietary intakes with health outcomes. Measurement of biomarkers in urine samples can provide additional objective estimates of food intake (2). We developed methodologies for home urine collection, yielding samples rich in terms of targeted biomarker measurements.

Technological and methodological innovation

Urine samples were collected at home using a postal bespoke urine collection kit. We used flow infusion electrospray ionisation high resolution MS to generate a non-targeted metabolome fingerprints. Random forest (RF) classification models were used to determine the extent of sample stability. Targeted metabolite quantification was performed using Ultra-High Performance Liquid Chromatography MS using reverse phase (C18) and ZIC-pHILIC columns (3-4).

Results and impact

The home collection and postal transport method was highly acceptable by free-living volunteers. Statistical analysis of both metabolome and selected dietary exposure biomarkers in spot urines collected and stored under different temperature regimes showed that they were compositionally similar to control urines with immediate freezing. This urine sampling methodology and biomarker technology appeared to be suitable for routine use and may provide a scalable, cost-effective means to assess diet.

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Poster 118: P118 *Simultaneous quantification of urine metabolites to allow comprehensive assessment of dietary exposure*

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Introduction

Metabolites derived from foods are found in human urine after consumption and their measurement could provide objective indicators of dietary intake (1). For comprehensive dietary exposure assessment, analytical methods will need to provide quantification simultaneously of the structurally diverse mixture of target metabolites present at a wide range of concentrations. We developed an extendable biomarker panel allowing simultaneous quantification of a comprehensive panel of dietary biomarkers.

Technological and methodological innovation

Urine sample analysis was performed by liquid chromatography triple quadrupole mass spectrometry (LC-QqQ-MS) with HILIC (Hydrophilic Interaction Liquid Chromatography) and Reverse Phase (RP) chromatography. Initial analysis of analytical characteristics (ie, peak shake, asymmetry, ionisation efficiency) allowed for selected reaction monitoring parameters for 59 biomarkers to be optimised. The selected biomarker panel has been used in a series of dietary intervention and nutritional epidemiological studies to report on dietary behaviour.

Results and impact

We established and validated a dietary exposure biomarker analytical panel by extensive analysis of analytical characteristics, which can be extended in the future to cover further food items.

We demonstrated a quantitative relationship between biomarker concentration in urine and exposure levels for commonly consumed foods in the UK. Integration of self-reported dietary recording tools with biomarker technology will allow more accurate assessment of dietary exposure.

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Poster 119: P119 *Utilizing the semantic web for kinetic modeling of metabolic disease pathways*

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Introduction

Currently, a vast amount of biomedical data is captured in various databases, which have limited capabilities to interact with each other. The knowledge in these databases could be used to explore if existing drugs can be repurposed for (rare) metabolic diseases, or if the synergies of drug combinations could lead to fewer side effects for patients [1]. Both of these use cases could be modeled in silico with the appropriate kinetic data, by applying semantic web technologies (e.g. RDF).

Technological and methodological innovation

Here, we present our approach to create pharmacologically compatible pathway for metabolic disorders. First, machine readable pathway models were created [2], which were uploaded to WikiPathways [3] converting the pathway data to the RDF format [4]. Next, we visited four kinetics databases and literature to identify relevant kinetic parameters and two drug-target databases to find corresponding inhibitors. Last, we created an RDF model for this data, compatible with the pathway models.

Results and impact

Our approach led to five new pathways relevant for metabolic disorders, which are supported by kinetic and drug-target information. Unfortunately. kinetic data could not be obtained for all metabolic interactions. However, when relevant data is captured in a semantic model, researchers can easily assess which interactions are missing data, shortening wet-lab time. Furthermore, adding data for other pathways is user-friendly, allowing others to extend and utilize our method.

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Poster 120: P120 *Untargeted Lipidomic analysis of bioactive lipids in epicardial adipose tissue and the effect of coronary artery disease status*

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Introduction

Epicardial adipose tissue (EAT) is an active endocrine organ that could contribute to the pathophysiology of coronary artery disease (CAD) through the paracrine release of proatherogenic mediators. We aimed to identify the specific untargeted lipidomic signature of EAT in CAD.

Technological and methodological innovation

Subcutaneous and EAT untargeted lipidomic analysis was performed in 25 CAD and 14 non CAD patients and compared to paired plasma lipidomic analysis of isolated VLDL and HDL. Lipidomics was carried out on a LC C18 column hyphenated to a Q-Exactive plus mass spectrometer, using both positive and negative ionization mode.

Results and impact

EAT and SAT had an independent lipidomic profile, with 95 lipid species differentially expressed and PE 18:1p/22:6 20-fold more expressed in EAT compared to SAT. CAD patients exhibited more ceramides($p=0.01$), diglycerides($p=0.004$), monoglycerides($p=0.013$) in EAT than non CAD patients. Conversely, they had lesser unsaturated TG($p=0.02$). Only 51 species were found in common between EAT and plasma lipoproteins. CAD is associated with specific lipidomic signature of EAT, unlike SAT.

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Poster 121: P121 *Using metabolomics to investigate the role of the gut microbiota in mediating the effects of diet on appetite in humans*

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Introduction

Obesity is a major global health issue; a third of the world is overweight or obese1. Using functional foods to enhance levels of satiating hormones glucagon-like peptide 1 (GLP-1) and peptide YY (PYY) is a promising therapeutic avenue. The gut metabolome largely mediates this hormonal response, but the role of the gut microbiota in generating this metabolome is largely unknown. Understanding how the microbiota influences the metabolome and thus appetite will facilitate targeted diet therapies.

Technological and methodological innovation

Gut fluid was collected from human volunteers using nasointestinal tubes after feeding a high protein and high fibre meal. Multiplatform analytical techniques generated metabolic profiles of the hydrophilic and the hydrophobic portion of the distal ileum and the proximal colon contents. This will be integrated with 16-S gut microbiome data, in-house gut hormone assays2 and visual analogue scales to measure appetite, investigating the role of the microbiota on diet-induced physiological changes.

Results and impact

The integration of metabolomic, microbiomic and gut hormone data provides a deeper understanding of the relationship between diet and the gut metabolome, and their combined impact on appetite. As well as providing novel insights into the therapeutic potential of appetite manipulation in antiobesity interventions, these results also highlight the importance of microbiota-directed patient stratification to maximise efficiency of treatment programs.

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Poster 122: P122 *Seeking new biomarkers of atherogenesis in an atherogenic-prone down-sized pig model and their relevance in a cardiometabolic risk human cohort using LC-MS/MS lipidomics*

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Introduction

The study aimed to specify the role of various dairy fats on the etiology of atherosclerosis, a vascular disease leading to myocardium infarct or stroke. This was evaluated both in an atherogenic-prone minipig model and in an at-risk cardiometabolic cohort of human volunteers given the same kind of fats for 2 years and 2 months, respectively. Plasma LC/MS untargeted lipidomics was used to identify predictive disease biomarkers in pigs relevant for humans, and dairy fat consumption indicators.

Technological and methodological innovation

43 atherogenic-prone minipigs and 169 human volunteers with a cardiometabolic risk (LDL-chol >1.6g/L) were divided into 4 groups. They were given respectively for 2 years (pigs) and 2 months (humans) 3 kinds of dairy fats or a control plant-fat diet. Untargeted LC-MS/MS lipidomics was performed on plasma collected at 0, 6, 12, 18 and 24 months in pigs, and 2 months in humans. Bifactorial statistical analyses were performed, and multiplex biomarker combination calculated with the NIPALS algorithm.

Results and impact

>300 lipid species in both pigs and humans scattered in >21 lipid classes were found. In pigs, a lipidome subset sensitive to diet peaked after 1 year, then partially returned to starting conditions, showing a resilience to the dietary intervention. We found 4 lipids in pigs that in combination were predictive of the atherogenic outcome both in pigs and humans, irrespective of time and diet. In humans, a dairy fat intake score using 16 lipids was calculated that could help in epidemiological studies.

Poster 123: P123 *MS-based targeted metabolomics of eicosanoids and other oxylipins: analytical variability and interlaboratory comparison*

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Introduction

Oxylipins are potent lipid mediators involved in numerous physiological and pathological processes and their quantitative profiling has gained a lot of attention [1]. To maximize the utility of the oxylipin profiling in clinical research it is now crucial (i) to assess its analytical variability; (ii) to determine its comparability between laboratories and (iii) to identify putative critical oxylipins. These three main challenges are addressed within the EU JPI HDHL*-Oxygenate project.

Technological and methodological innovation

To address the challenges stated above, a SOP was established by a reference laboratory for the MSbased targeted metabolomics of total oxylipins (free + esterified, ~160 oxylipins) in human plasma [2]. The intra- and inter-day variabilities of each oxylipin were assessed. Then, the SOP was transferred to 4 independent laboratories together with mixtures of internal standards, calibrants and 7 different pools of plasma to determine the comparability of oxylipin profiles between labs.

Results and impact

The cumulated intra-/inter-day variabilities revealed that 68 % of oxylipins (>LLOQ) have a CV<20%. The interlab-variability was low and dependent on the type of plasma analyzed. Overall, our results show that the MS-based profiling of total oxylipins in human plasma is a robust tool for clinical research. Moreover, the comparability of oxylipin profiles will allow generating large-scale databases allowing a better understanding of the relationships between oxylipins and human health.

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Poster 124: P124 *Migrating from PLS to Artificial Neural Networks – Adapting Interpretation Strategies*

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Introduction

Highly covariate data necessitates the use of machine learning (ML) methods for analysing metabolomics data. While PLS is most commonly used, in part due to its interpretability, nonlinear methods such as artificial neural networks (ANNs) are gaining traction. Historically, ANNs have been considered uninterpretable 'black boxes', yet their simplest architecture is structurally equivalent to PLS[1]. AIM: To migrate data visualisation techniques from PLS to ANNs and directly assess utility.

Technological and methodological innovation

Migration included both direct and adapted approaches. Model optimisation strategies were adapted to improve simultaneous interpretation of >1 hyperparameter. Visualisations of model scores and evaluation were directly migrated. Finally, variable contribution assessment was adapted, comparing the ANN Connection Weight Approach (CWA)[2] and Garson's Algorithm[3] to PLS coefficients and VIP, respectively. All workflows were developed in Jupyter Notebooks, ensuring transparency and interoperability[4].

Results and impact

All visualisation techniques successfully migrated to ANNs. The hyperparameter optimisation adaptation provided an additional strategy for PLS and greatly improved interpretation for ANNs. PLS coefficients and ANN CWA scores were highly correlated, providing similar results; however, VIP and Garson scores showed less alignment. These results not only open the door for ANNs to be directly applied to metabolomics analysis, but also more complex architectures such as multiblock integration.

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Poster 125: P125 *Identification of biomarkers in Attention Deficit with Hyperactivity Disorder (ADHD)*

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Introduction

Attention Deficit Disorder with Hyperactivity Disorder (ADHD) is a heterogeneous neurodevelopmental disorder affecting 35% of school-aged children and characterized by attention deficit, hyperactivity, and impulsivity1. Currently, the diagnosis is made mainly using cognitive tests with a significant risk of diagnostic errors2.

To date, no reliable biomarkers of ADHD were identified but such issue remains an important challenge for early diagnosis and appropriate patients follow-up.

Technological and methodological innovation

The use of animal models is relevant to study cerebral metabolome. One of the best-characterized and mostly used model is the comparison between SHR/NCrl rats (n=8) and WKY/NHsd rats (n=8).

The objective of this study is to identify metabolic biomarkers, centrally and peripherally, in SHR / NCrl rats. For this, ten brain regions were taken, as well as peripheral samples (blood, urine, and feces), then analyzed in LC-HRMS.

Results and impact

Multivariate analyses revealed that it was possible to discriminate each rats strain from the other based on their peripheral and central metabolomes. Based on the discriminant metabolites, we found alterations in specific metabolic pathways (lysine degradation pathway) in the brain metabolome but also in the periphery (arginine and proline metabolic pathway). In the future, metabolomics studies on clinical peripheral samples (urine and blood) will be carried out.

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Poster 126: P126 *Metabolic signatures of urinary schistosomiasis and pharmacometabolomics of praziquantel treatment efficacy in children from rural Côte d'Ivoire*

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Introduction

Urinary schistosomiasis is a neglected tropical disease that affects millions of children in the rural tropics. It results in fibrotic abnormalities in urogenital organs and impaired childhood development. The metabolic links between infection and morbidity have not been studied. Moreover, current efforts to create a pediatric formulation of praziquantel, the sole treatment, are complicated by the drug's inconsistent bioavailability, which may be impacted by host metabolic predispositions[1].

Technological and methodological innovation

We employed untargeted 1H NMR spectroscopy to investigate metabolic effects of urinary schistosomiasis and associated pathology in 344 infected and 42 uninfected children from rural Côte d'Ivoire[2]. Urinary profiles were analysed with infection, clinical examination and bladder ultrasound data[3] using standard multivariate statistics[4]. Analysis with matched praziquantel pharmacokinetic data[5] was used to identify host metabolic factors associated with praziquantel ADME and efficacy.

Results and impact

Infection resulted in a depletion in TCA cycle and microbial co-metabolites, which in turn were associated with infectioninduced bladder thickening. Pharmacometabolomic profiling demonstrated that both CYP-associated and microbial-metabolites predicted variation in bioavailability praziquantel for both enantiomers and the main drug metabolite and overall treatment efficacy. This suggests a role for gut microbial modulations to ameliorate both infection and praziquantel treatment outcomes.

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Poster 127: P127 *Metabolomics at the top: Characterizing hypoxic responses and chronic mountain sickness in the highest city of the world*

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Introduction

A significant proportion (5-10%) of individuals living at high altitude develops signs of maladaptation generally encompassed within the syndrome of chronic mountain sickness (CMS) which frequently terminates in cardiorespiratory diseases such as pulmonary hypertension and right or left heart failure. Our aim was to characterize metabolic adaptation to high-altitude including signs of CMS using LC-MS plasma metabolomics.

Technological and methodological innovation

Plasma from 19 individual living permanently at Lima (see level), 13 at Puno (3500m) and 29 at La Riconada (5100-5300m) were collected. 3 and 19 suffered from CMS in Puno and La Riconada, respectively. Untargeted plasma LC-MS metabolomics was performed, followed by PLS-DA statistical analyses to extract relevant features linked to altitude and CMS. Metabolites annotation was performed using our in-house database and MS/MS fragmentation.

Results and impact

Metabolomics profiles distinguished see level, intermediate and high-altitude dwellers. Preliminary results showed an impact of altitude on metabolites linked to NO metabolism, energy production, proteinogenesis and (mainly at 5300m) the redox status. CMS seemed to relate to various metabolic deregulations mainly related to vascular health, oxidative stress, proteinogenesis, muscle metabolism and possible neuronal function. Red blood cells lipidomics is under way to complete these first results.

Poster 128: P128 *INPUT OF DEEP PHENOTYPING IN THE METABOLIC SYNDROME STRATIFICATION*

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…

Introduction

Metabolic syndrome (MetS), defined as a cluster of cardiometabolic factors, is a public health challenge because of its growing prevalence. In the context of personalized medicine, new tools are necessary to bring additional knowledge about MetS etiology, better stratify populations and customise strategies for prevention. The objective of this study was to characterize the MetS phenotypic spectrum using complementary untargeted metabolomics platforms (HRMS, RMN).

Technological and methodological innovation

A case-control study was designed within the Quebec NuAge cohort1. Six complementary untargeted metabolomic/lipidomic approaches were performed on serum samples collected at recruitment and 3 years later. Procedures were set up to guaranty the inter-laboratory standardisation from sample preparation to data processing, performed using reproducible online Galaxy workflows. A full feature selection strategy was developed to build a comprehensive molecular MetS signature, stable over time.

Results and impact

A wide range of metabolites (lipids, carbohydrates, amino-acids, peptides…) reflecting subject stability and providing new insights about underlying mechanisms, were found to be modulated. An optimized reduced signature was proposed, allowing good prediction performances (12% misclassification, AUC=0.95, CI:[0.92-0.98]). These results demonstrated the interest of a multidimensional molecular phenotyping as part of the next generation of medicine tools in the frame of non-communicable diseases.

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Poster 129: P129 *The circulating metabolites in the progression to islet autoimmunity and type 1 diabetes*

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Type 1 diabetes (T1D) is a chronic autoimmune disease caused by specific destruction of the insulinproducing beta cell. Clinically pre-diabetic period in T1D is characterized by presence of beta cell specific autoantibodies. Earlier metabolomics study suggests specific metabolic disturbances before individuals precede to auto-immunity.

Here, we analyze circulating metabolites in a prospective series of plasma samples from children who progressed to T1D, children who developed islet autoantibody but did not progress to T1D during the follow-up (P1Ab) and matched controls (CTR) in the subset of samples from internationally recognized DIPP and DIABIMMUNE study cohorts. We found sphingomyelins and methionine to be persistently dysregulated in PT1D when compared to the P1Ab and CTR groups. Additionally, bile acids were found to be dysregulated in cases than the CTR during early infancy. We also found potential microbial metabolites including hydroxyphenyllactic acid, indole acetic acid, and 11-eicosenoic acid to be significantly downregulated at early age (3 and 6 months) preceding clinical T1D. Microbial community modelling obtained from the shotgun metagenomics data showed significant differences in microbial metabolic pathways such as fatty acids, Lmethionine, bile acids and amino acid biosynthesis among the cases.

Taken together, our study support findings from earlier studies and suggests novel metabolic signatures that characterize children who progressed to islet autoimmunity or overt T1D, which may be helpful in the identification of at-risk children before the initiation of autoimmunity.

Poster 130: P130 *Milk molecular species of triacylglycerols characterized by lipidomic approach in cows and goats fed diets supplemented with various lipid sources*

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Introduction

Lipid fraction is a major determinant of milk nutritional quality that can be modulated by nutritional factors such as lipid supplementation.

In a comparative study between cows and goats fed similar diets supplemented in lipids, we characterized the triacylglycerols (TAG) composition by lipidomic. This study was part of a trial aiming at characterizing milk lipid responses to diets inducing either milk fat depression or increase in milk fat secretion, with species-specific responses [1].

Technological and methodological innovation

The effects of diets containing no additional lipids (CTL) or supplemented with corn oil and wheat starch (COS), marine algae powder (MAP), or hydrogenated palm oil (HPO), on milk fat content and composition were studied in 12 cows and 12 goats conducted simultaneously in a replicated 4x4 Latin square design. Milk samples were collected over 2 consecutive milkings on d24 of each period. Lipids were extracted before TAG determination by LC-HR/MS. Data were subjected to ANOVA using R software.

Results and impact

In cows, milk fat content was lowered by COS (-45%) and MAP (-22%) and increased by HPO (+13%) whereas in goats, only MAP decreased milk fat content (-15%). Lipidomic analysis revealed TAG differences among species: 1/irrespective of diets, 16 were more abundant in cows and 23 in goats, 2/on COS, 28 were modulated in cows but not in goats and 3/on MAP, 13 were modulated in cows and 8 in goats. These new data demonstrate the TAG species specificities and their nutritional regulation.

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Poster 131: P131 *Genome-scale metabolic modeling of human CD4+ T cells reveals ceramides as metabolic signature of Th17 and regulatory T-cell differentiation*

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Introduction

T helper (Th) cells play a pivotal role in cell-mediated immunity. During the development, T cells undergo metabolic remodeling that is essential for orchestrating the action of other immune cells.

Technological and methodological innovation

In order to understand global metabolism during T-cell development, we developed genome-scale metabolic models (GEMs) for human Th1, Th2 and Th17 subsets and T-regulatory cells (iTreg) [1].

Results and impact

Meta-analysis of T-cell specific human transcriptomics datasets have identified 72 novel metabolic genes, corresponding to 355 reactions spanning various metabolic pathways. Reporter metabolites and pathway overrepresentation analysis suggested that T-cell activation induces gluconeogenesis, glutaminolysis, and lipid biosynthesis. Moreover, ganglioside (GA1, GMb) and N-acetylneuraminate (NANA) associated with sialyl-T antigen were significantly up-regulated in Th17 cells at 72 hours of initiation, while the glucosyl-, lactosyl- and galactosyl ceramides were down-regulated. On contrary, such trends were either reversed or absent in iTregs at this time-point. These results have been validated by LC-MS-based lipidomics. Our findings suggest that, ceramides are involved in the metabolic regulations and functioning of T-cells. It was also found that, Th subsets exhibit unique metabolic phenotype, already during early stages (72 h) of specification, thus playing a central role in guiding the fate of the cells. The findings from this study, provide a basis for modulation of human Th subsets crucial for immune responses under metabolically aberrant conditions and in immune-mediated disorders.

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Poster 132: P132 *How metabolomic data support an innovative read across approach for safety assessment in cosmetics*

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Introduction

In toxicology, read-across is one of the most commonly used alternative approaches for data gap filling in registrations submitted under REACH Regulation. However, a level of uncertainty remains present with current read-across approaches compared with traditional testing of the substance. Metabolomics is the -omics discipline, that is more closely related to biochemical pathways, and therefore could be helpful to fill the gap during read-across analysis for safety assessment.

Technological and methodological innovation

A new approach using metabolomics for read-across between a candidate molecule A only studied in vitro, and a reference molecule B previously investigated using traditional in vivo toxicity methods. Two in vitro models (Skinethic model and HepaRg cell line) were treated and studied by metabolomics. Extracts were then analyzed by NMR and UHPLC-HRMS, and data were processed using W4M[1]. Metabolic pathways were highlighted with Metexplore[2] and confronted with other toxicological data.

Results and impact

Molecule A yielded similar metabolomic profiles to reference molecule B in both models underlining similar mode of action. Indeed, metabolic networks minor modifications are purine catabolism, nucleotide interconversion, glycerophospholipid metabolism, valine, leucine, and isoleucine metabolism pathways. Metabolomics data obtained allow us getting a better understanding of the biological impact of both molecules on skin and liver and permit to validate this innovative read-across approach.

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Poster 133: P133 *LC-MS-based semi-targeted lipidomics method: Application to the discovery of lipid biomarkers in diabetes*

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Introduction

Diabetes has been reported with significant lipid alterations1, but potential lipid biomarkers are still unknown due to the limitation of analyzing method for lipids2. To investigate different classes of lipidomic changes in diabetes patients, a LC-MS-based semi-targeted lipidomics was developed to monitor concentration changes in 273 lipids with confirmed identification. Time restricted feeding (TRF) and daily caloric restriction (CR) study was compared to explore biomarkers related to diabetes.

Technological and methodological innovation

In TRF study, participants were asked to restrict eating between 8am and 4pm whereas in CR study, participants were asked not to alter the temporal distribution of energy intake. Plasma from pre and post-intervention were extracted with chloroform: methanol (2:1) with 13C-labelled internal standards added. Samples were loaded on LC-MS with the developed method. Statistical analysis such as uni- and multivariate analysis was used to discover concentration changes in various lipids and potential biomarkers.

Results and impact

A new LC-MS-based semi-targeted lipidomics method was developed with good reproducibility and repeatability. The method is sensitive enough to detect subtle changes in 18 lipid classes, including TG, DG, Ceramides and so on. 273 lipids were identified in two studies with this method. Based on t-test (FDR corrected), PCA and OPLS-DA results, a number of significantly changed lipids were determined in two studies, and these can be potential biomarkers for type 2 diabetes.

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Poster 134: P134 *Optimization of fecal NMR-based metabolomics to study the developing infant gut*

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Introduction

While substantial efforts have been made to optimize and standardize fecal metabolomics for studies in adults1, the development of a standard protocol to analyze infant feces is still lagging behind. Research of the infant fecal metabolome is gaining interest since it contributes to our functional understanding of the complex diet-microbiota interactions in the gastrointestinal tract, and its impact on host health2.

Technological and methodological innovation

Fecal samples from five infants were subjected to different preparation conditions in order to examine the impact of extraction solvent, dilution ratio, homogenization method, filtration and duration of centrifugation on the 1H NMR metabolite profile3,4. An optimized protocol for 1H NMR spectroscopic analyses was defined and directly implemented on samples collected from infants at 8 weeks, 4 and 9 months postpartum.

Results and impact

Diet has a profound impact on the gut metabolome, with mostly milk oligosaccharides derivatives present in 8 weeks and 4 months samples, and short-chain fatty acids in 9 months samples. The application of the proposed protocol developed for NMR-based metabolomics of infant feces will ensure robust data generation and improve comparability between laboratories. In combination with microbiome research, this can provide further inside in the influence of nutrition on the infant gut health.

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Poster 135: P135 *Biomarkers involved in glioma development in Drosophila melanogaster model*

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With a prevalence of about 5 to 8 cases a year per 100,000 people, human malignant glioma is the most common type of primary brain tumors in adults. They represent 2,000 to 3,000 new cases a year in France and constitute an important cause of suffering and mortality. If genetic alterations involving signaling pathways or epigenetic processes have been identified as causing gliomas, few effective therapies are currently available which leads to a poor prognosis. Tumor cells reprogram their metabolism to ensure their energy demands and molecules necessary for their proliferation (nucleotides, lipids, amino acids ...). These metabolic pathways may constitute new therapeutic targets.

In order to study these brain tumors, we have developed in the laboratory a model of glioma in Drosophila melanogaster that results from the activation of two signaling pathways (PI3K and EGFR) known for their involvement in glioma. The metabolic signatures of third instar larvae dissected brains developing a glioma were analyzed by NMR spectroscopy in liquid phase and HR-MAS and by multivariate statistical analyzes and compared to controls. Our original approach, combining solution state NMR and HR-MAS NMR, successfully identified several glioma biomarkers. Some of them are involved in the Krebs cycle and in the biosynthesis of amino acids. Some metabolites have already been demonstrated in studies of human oncogenesis or glial cell proliferation, which supports the use of the Drosophila melanogaster model to study human glioma.

Poster 136: P136 *Differential Mobility Separation Enhances the Quantification of Lysophosphatidic Acid in Plasma*

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Introduction

Lysophosphatidic acid (LPA) is a phospholipid signaling molecule in the class of lipid mediators, functioning by signaling through G-Protein coupled receptors or nuclear receptor proteins. LPA has been associated with a wide range of biological processes.

Accurate LPA measurement in plasma has proven difficult. A bottleneck for accurate quantitation of LPA in plasma using current analytical methods is the chemical interferences. The most abundant LPA fragments are typically the best fragments for quantification, however the most intense MRM transitions for LPA have strong matrix interferences when monitored in plasma. There is an MRM transition that has good specificity in plasma however it has much lower signal intensity.

Here, SelexION Technology is used for the quantification of LPA 18:1 in plasma which removes the matrix interferences observed when measuring plasma sample, allowing the use of the higher intensity MRM transitions for quantitation of LPA with much greater confidence.

Conclusions

A method has been developed here for the quantitation of Lysophosphatidic acid (LPA) in plasma using SelexION Technology for reducing matrix interferences and increasing method robustness. The method was applied to measure endogenous LPA in a series of plasma samples. With this method, a concentration of ca 60 mM in human plasma samples was determined, which is in line with the reported values in the literature.

Poster 137: P137 *The Ichem'Algae ANR project: Untargeted chemotyping of algae bank by GC-MS and non invasive vibrational spectroscopy*

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Introduction

The Ichem'Algae research project aims to develop an integrated analytical methodology to investigate the poorly known microalgae chemical diversity. The main objectives consist in a rapid fractionation approach combined with non-invasive and highly resolutive signatures methods for the development of a descriptive/predictive model and a a rapid screening of the partner algae bank.

Technological and methodological innovation

The analytical approach will combine non-invasive (FTIR/Raman spectroscopy, HRMAS) and highly resolutive methods (GCMS, HR NMR). The fractionation process will be performed using centrifugal partition chromatography, the produced fractions will be selected by GCMS [1] according to their metabolic signatures. The selected fractions (presenting high diversity or specific fractionation profiles) will be extensively explored using CPC-NMR based CARAMEL approach [2].

Results and impact

First results have demonstrated the ability of the GCMS to snapshot the chemical diversity of the analyzed strains, before and after dereplication using simple extraction methods (solvents with a gradient scale of polarity). The resulting fractions could be classified according to the solvent polarity. It also appeared clearly that most of the chemical diversity could only be characterized after dereplication (many signals were detectable only when investigated in contrasted polarity fractions).

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Poster 138: P138 *Prolonged Exposure to Milk Casein Results in Depressive Behaviour, Impaired Brian Development and Altered Metabolism in Wistar Rats*

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Introduction

Early-life nutrition influences brain development and gut microbiota composition with health repercussions later in life. Cow formula milk contains high concentrations of A1 b-casein and its proteolytic breakdown releases bcasomorphin 7, a peptide with morphine like properties1. Longer exposures to dietary casein, with delayed weaning, induce depressive phenotypes in rats, altering the gut microbiota and urinary metabolic profiles2. A nonbCM7 releaser (A2 b-casein) can potentially prevent this.

Technological and methodological innovation

Rats were fed different concentrations of casein from postnatal day 21 to 26. Depressive behavior was evaluated with the forced swim test (FST)3. Urine, brain and gastrointestinal contents were collected for metabolic and microbial analyses on PND26. In a second study, animals were fed for the same period either with water, commercialized cow milk (A1 b-casein) or A2 b-casein milk. Depression was again evaluated with the FST, while metabolic profiles of brain and urine were measured by 1H NMR.

Results and impact

The administration of casein-rich milk resulted in increased immobility time in rats, indicating depressive phenotypes. Urinary metabolic profiles reveal distinct metabolic phenotypes with major differences in bacterial metabolism. A2 b-casein milk appears to attenuate the development of depressive behaviours and to induce different brain and urinary metabolic profiles, stressing the importance of bCM7. This study can impact how current formula milk is designed and improve long term infant health.

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Poster 139: P139 *Assessment of the Substrate Biodegradability in Anaerobic Co-digestion using a Metabolomic Approach*

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Introduction

Anaerobic digestion (AD) is a sustainable process for the treatment of wastewater sludge (WAS) by the action of microorganisms[1]. WAS degradability can be enhanced by mixing it with other substrates richer in carbon, such as garden grass (GG) or fish waste (FW)[2]. Typically, AD performance is assessed by indirect measurements (i.e., pH, biogas production)[3], while the direct monitoring of metabolite degradation in AD using metabolomics instrumentation has never been used.

Technological and methodological innovation

Mixtures of WAS with either FW or GG (0/100, 25/75, 50/50, 75/25, and 100/0) were prepared and digested in batch reactors for one month. Samples were collected at 3 time-points and analysed with HPLC-MS spectrometry. The acquired data were processed with XCMS Online[4], and further analysed with Principal Components Analysis (PCA) and Common Components Analysis (CCA)[5]. This is the first time AD is monitored using untargeted HPLC-MS metabolomics and chemometrics.

Results and impact

Relevant metabolites were descriptive of FW or GG rather than WAS, and mainly corresponded to modified metabolites, such as decarboxylated amino acids and oxidized fatty acids. In both digestion experiments, concentrations for most metabolites were found to decrease over time. Finally, GG did not improve the biodegradability of WAS, whereas a synergistic effect was observed for FW, this effect being the highest for 25% FW. In conclusion, metabolomics results a promising tool to monitor AD.

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Poster 140: P40 *Comparative metabolomics in Mamiellales*

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Introduction

Mamiellales are cosmopolitan marine photosynthetic unicellular eukaryotes that dominate the picoeukaryotic plankton in coastal areas. Theses picoalgae exemplify the ecological success of miniaturized eukaryotic cells, displaying a simple cellular organization and a high surface-volume ratio, which is likely to confer advantages in nutrient poor environment. Phytoplanktonic eukaryotes have representatives in five of the six super-groups found in eukaryotes [1] where Chromalveolates dominate in terms of diversity and abundance over Archaeplastida [2], the green lineage to which Mamiellales and land plants also belong. Considering the diversity and the pivotal ecological role of the pico-phytoplankton they have been extensively studied to understand their past evolutionary history. However, how their ancient genetic divergence and ecological features correlate to their metabolomic profiles remains unknown.

Technological and methodological innovation

Here, we provide a straightforward approach to compare metabolomes of divergent algae, among which six Mamiellales (Ostreococcus, Bathycoccus, Micromonas and Mantoniella) three Prasinophytes (Pyramimonas and Nephroselmis) a core Chlorophyte (Picochlorum) and two Chromalveolates (Pavlova and Phaeodactylum), with a last common ancestor tracing back to the last common eukaryotic ancestor. Algae cells were collected on filters and solubilized in ethyl acetate for analysis by UHPLC/ESI-MS² (Orbitrap). Metabolite polarity ranges from polar to neutral lipids and pigments. Top ten most abundant metabolites from each species were identified on the basis of MS² spectra. Comparison and multivariate analyses were performed to identify Mamiellales specific metabolites.

Results and impact

We developed a simple workflow for lipids and pigments analysis, the method appeared to be suited to discriminate algae at the species and order level on the basis of lipid content. The method covers a broad range of lipids from neutral (TAG, DAG, MAG) to polar lipids (MGDG, DGDG, SQDG, DGTA/S, PG, PI, PC, PE). Chemotaxonomic analyses performed both on total ions and on top 10 metabolites exhibit the same pattern indicating most abundant constituents of algae are also robust biomarkers of algae delineation. Chemotaxonomies were surprisingly consistent with gene phylogenies suggesting metabolome divergence reflects evolutionary divergence [3].

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Poster 141: P141 *Deciphering B .methanolicus metabolism for a one carbon-based production platform*

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Introduction

One-carbon (C1) compounds such as methanol, with its low price and the possibility to be produced from renewable energy sources, are attractive feedstock alternatives for microbial fermentation. The gram-positive Bacillus methanolicus is a natural methylotroph (i.e. able to grown on a C1 compound as its sole energy and carbon source) and it has been described to produce large quantities of glutamate and lysine in methanol at high temperature, making it a good candidate for biotech applications.

Technological and methodological innovation

Here we conducted a system level analysis of B. methanolicus to understand its highly efficient methylotrophic metabolism, using modelling and omics analysis, particularly an instationary 13C-metabolic flux analysis using intra and extracellular pools of central metabolites in both methylotrophic and non-methylotrophic conditions.

Results and impact

Overall we have obtained insight into the metabolism and underlined key pathways that characterize this decisive C1 catabolism. Broadening the knowledge on natural methylotrophy will lead to metabolism improvement and to establish B. methanolicus as a promising cell factory, as well as help build synthetic methylotrophy in other model organisms.

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Poster 142: P142 *A Community-led Initiative to Develop and Promote Quality Assurance and Quality Control in Untargeted Metabolomics Research: the metabolomics Quality Assurance and quality Control Consortium (mQACC)*

metabolomics Quality Assurance and quality Control Consortium (mQACC)

Introduction

mQACC [1] was established in February 2018 and has a central mission to communicate and promote the development, dissemination, and harmonization of best practices in quality assurance (QA) and quality control (QC) in untargeted metabolomics. The formation of the consortium was a result of the National Institutes of Health-funded Think Tank on Quality Assurance and Quality Control for Untargeted Metabolomics Studies meeting in 2017, where participants identified long-term priorities for QA/QC efforts.

Technological and methodological innovation

Three key priorities are being driven forward: 1) publish a workshop report [2]; 2) document the complete experimental procedure for untargeted metabolomics; and 3) identify 2–3 reference materials to be developed. The consortium currently comprises over 65 representatives from North America, South America, Europe, and Asia, including instrument manufacturers, commercial laboratories, and government/academic stakeholders. mQACC is currently addressing its initial priorities and expanding these efforts.

Results and impact

mQACC pursues the following objectives: 1) to identify, catalog, harmonize, and disseminate QA/QC best practices; 2) to establish mechanisms to enable the metabolomics community to adopt QA/QC best practices; 3) to promote and support systematic training in QA/QC best practices; and 4) to encourage the prioritization and development of reference materials applicable to metabolomics research. We will discuss current progress in relation to the objectives and will also highlight how the metabolomics community can get involved in this important initiative.

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Poster 143: P43 *Analysis of Endophytic Colletotrichum sp. strains by MALDI-TOF mass spectrometry and t-SNE Molecular Networking*

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Introduction

From the 197 endophytes cultivated endophytic microorganisms from 6 species of the French Guianese palm tree Astrocaryum sciophilum, 42 strains were genetically identified as Colletotrichum. Protein fingerprints by MALDI-TOF allowed deciphering species variability. LC-MS2 data were processed to generate a Molecular Network (MN) using MetGem software to explore chemical diversity. Complete structural identification led to an unprecedented series of new metabolites designated as Colletamide.

Technological and methodological innovation

Protein fingerprints were acquired by MALDI-TOF after optimization of extraction method, clustered and were highly correlated to cytotoxic activities of respective ethyl acetate extracts whereas no correlation with ITS sequencing was obtained. MetGem software [1] was designed to generate MN using innovative t-SNE visualization preserving the local distances between related groups of spectra (clusters). Combination of NMR, CD and DP4 calculations [2] were used for complete structural analysis.

Results and impact

Protein fingerprints were precisely reported on the variability between strains at sub-species level and were fully correlated to the cytotoxicity. MN predictions were fully confirmed after isolation of cytotoxic pentacyclopeptides and cytochalasins (µM range). Isolation and complete identification of four new metabolites bearing the identical decadienamide moiety were performed. This methodology allows targeting interesting microbial extracts in the search for new bioactive metabolites.

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Poster 144: P44 *Fungal endophyte exo-metabolites alter the morphology and metabolome of the plant pathogen Fusarium graminearum: an LC-MS based metabolomics approach to unravel the biocontrol effect*

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Introduction

Fusarium graminearum (FG) is a predominant fungal pathogen of cereals, which also produces secondary metabolites, i.e. mycotoxins, that harm human and animal health [1]. We previously showed the potential of two endophytic fungi: Epicoccum nigrum (EG) and Sordaria fimicola (SF) to inhibit growth and mycotoxin production of FG [2]. In this study, we used metabolomics to elucidate the potential mechanisms involved in their biological control.

Technological and methodological innovation

We optimized an in vitro assay using tissue plate inserts to assess the biological effects of EG and SF exo-metabolites on FG. Changes in growth characteristics of FG exposed to the exo-metabolites were monitored using automated high-resolution multispectral imaging. We further investigated the metabolome profile changes of FG after exposure to the exo-metabolites using untargeted metabolomics on both reversed-phase and HILIC to elucidate potential mechanisms of action.

Results and impact

We showed that EG and SF exo-metabolites altered the morphological and growth characteristics, as well as the overall metabolome of FG. Using multivariate analyses, we selected metabolic features that could potentially elucidate the biochemical changes undergone by FG in response to the presence of exo-metabolites. This study shows that apart from competition of live fungi, EG and SF exo-metabolites themselves exert metabolic action against FG which could explain their biocontrol activity.

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Poster 145: P145 *Evaluation of micro Pillar Array Columns (µPAC™) Combined with High Resolution Mass Spectrometry for Lipidomics*

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Introduction

As an alternative to conventional packed bed nano LC columns, micromachined chip columns known as micro Pillar Array Columns (µPAC™) are emerging. Their separation capacities are illustrated here in the context of a lipidomics workflow.

Technological and methodological innovation

The high permeability and low 'on-column' dispersion obtained by the perfect order of the separation bed makes µPAC™ chromatography specific. The peak dispersion coming from heterogeneous flow paths in separation bed is eliminated, therefore components remain more concentrated during separation resulting in better separation performance (1). The freestanding nature of the pillars also leads to lower backpressure, allowing a high operational flow rate flexibility with good peak capacities (2).

Results and impact

Performance of the column is illustrated with the analysis of human blood plasma lipids.

Using a 200cm long µPAC™ column in combination with HRMS high lipidome coverage can be obtained. All major lipid classes are detected and their locations are shown on the chromatograms. Next to the inter-class separation, the methodology provides intra-class separation based on number of carbons and degree of saturation in the fatty acid side chains. Furthermore, isomeric lipids can be resolved(3).

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Evaluation of Micro-Pillar Array Columns (µPAC) Combined with High Resolution Mass Spectrometry for Lipidomics. LC

GC Spec. Issues 30.6 (2017) 6-13. [1] First author et al. YEAR. Journal. Volume and pages

Poster 146: P146 *Chemical mappings for the flowers of Abeliophyllum distichum using metabolomics tools*

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Introduction

Protection of the sovereignty of biological resources in each country is important due to the recent entry into force of the Nagoya protocol. The purpose of this protocol is the fair and equitable sharing of benefits arising from the use of genetic resources [1], thereby contributing to the conservation and sustainable use of biodiversity. With this protocol in force, research using domestic native species became a key issues.

Among the various Korean's plants, Abeliophyllum distichum (Oleaceae), which comprise of not only one species but also one genus and grown only in the Korean Peninsula, has high scarcity value [2]. Due to the effectuation of the Nagoya Protocol, value of this plant increases in process of time. But to date, no study has been reported for metabolites and the activities of A. distichum flowers. At the present, five variants have been reported; white miseon (A. distichum Nakai); pink miseon (A. distichum for. lilacinum Nakai); ivory miseon (A. distichum for. eburneum T. B. Lee); blue miseon (A. distichum for. viridicalycinum T. B. Lee); round miseon (A. distichum var. rotundicarpum T. B. Lee) [3]. The variants were classified based on only morphological characteristics expecially on the color of petals and sepals or shape of fruits. Accordingly phytochemical investigation and chemical mapping for the variants should be valuable.

Technological and methodological innovation

NMR spectrosopy, LC-MS, GC/MS, and IR are mainly used as metabolomics tools. Each analytical method has its own advantages as well as disadvantages such as limitation of target analysis, reproducibility, sensitivity, and so on. This study conjugated NMR, LC/MS, and GC/MS to reinforce each strength and make up for each weakness in complete identification of all metabolites and quantification [4]. And major metabolites of A. distichum flowers were isolated through repeated SiO2, ODS, and Sephadex LH-20 column chromatography and identified based on extensive analysis of 1D- and 2D-NMR and MS. Also Head Space-Solid Phase Micro Extraction-Gas Chromatography/Mass Spectrometry (HS-SPME-GC-MS) analysis was performed to profile volatiles for chemical mapping.

Results and impact

Twenty-nine metabolites including four new ones were isolated from A. distichum flowers through repeated normal, reverse-phase, and Sephadex LH-20 column chromatographies and their structures were determined using classical spectroscopic methods such as NMR, IR, MS.

NMR, UHPLC-tripleTOF-ESI-MSMS, and GC/MS based metabolomics study was performed to understand chemical differentiation among five variants of this flowers. From NMR, LC/MS, and GC/MS analyses, 50 primary metabolites, 58 secondary metabolites, and 145 hydrophobic primary metabolites were identified, respectively. As a result, primary and secondary metabolites showed different patterns among all variants according to morphological characteristics. Also metabolic flux analysis of five variants was confirmed through correlation analyses of the primary and secondary metabolites.

From this results, it was confirmed that various patterns of morphological characteristics such as the shape of fruits and color of sepals and petals were derived from the variety of primary and secondary metabolites. Especially, relative contents of flavonoids and phenylethanoid glycosides of two colored variants (pink and ivory miseon) are much higher than those of white variants (white and round miseon). Among colored flowers, pink miseon has more phenylethanoid glycosides and fewer flavonoids than pink miseon. It suggested that phenylethanoid glycosides and flavonoids get involved in color of petals. In addition, 66 volatile components of this plant identified by HS-SPME-GC/MS also showed unique patterns according to morphological characteristics, especially color of petals.

Metabolomics, NMR, LC/MS, and GC/MS data, showed high significance to understand chemical differentiation of the morphological characteristics among five variants of this flowers.

Poster 147: P147 METABOLOMIC INSIGHT IN THE RESPONSES OF STREAM BIOFILMS TO THE HERBICIDE DIURON AND ITS MODULATION BY ENVIRONMENTAL FACTORS

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Introduction

In the context of global change, aquatic ecosystems face multiple stressors that may endanger their sustainability and associated ecosystem services. It is therefore crucial to better understand how these pressures interact and modulate the responses and further the tolerance of aquatic organisms, in order to gain knowledge on the resilience of these ecosystems. In particular, regarding the increasing aquatic pollution, there is a need to establish the causality link between the exposure to chemicals and the effect(s) in order to propose monitoring and remediation solutions [1]. To tackle this challenge, the (xeno)metabolomic approach aims to characterize all (chemical) exposures (i.e. xeno-metabolome, xenobiotics as well as their products from phase I and/or II metabolism) in addition to exposure-induced effects on cellular biomolecules (i.e. endo-metabolome) [2,3]. In this global change context, stream biofilms -as a complex assemblage of algae, fungi, bacteria, protozoa - are increasingly used because of their relevance to investigate the impact of multiple environmental stressors at the community level (function and structure), as they also play a key role in aquatic ecosystems (e.g.primary production) [4].

Technological and methodological innovation

By using a (xeno)metabolomic approach, this study aims to investigate the influence of environmental confounding factors (temperature, seasonality, flow, photoperiod) on the impact of the chemical stress on stream biofilms. To this end, stream biofilms colonized at a reference site were exposed to the herbicide diuron - a model compound for photosynthesis inhibition- at the laboratory under different controlled conditions. The metabolomics responses were assessed for the different tested conditions through organic extraction by using accelerated solvent extraction followed by injection on UPLC-ToF system (Ultra-Performance Liquid Chromatography — Time of Flight Mass Spectrometry, Xevo G2-S ToF, Waters). As a first step, data were processed in MzMine2 in order to identify relevant features regarding exposure to diuron. The modulation of these signals by environmental confounding factors will be soon investigated. In addition, the photosynthetic activity was also assessed as a functional response of the biofilms supported by the metabolic changes triggered by diuron.

Results and impact

The data analysis is still ongoing. Nevertheless, by using the PlantCyc database, the first processing of the data in Mzmine2 allowed the identification of two plant-specific fatty acids, the docosapentaenoic acid and the eicosapentaenoic acid. These are two omega-3 fatty acids that play a major in the sustainability of the trophic chain and so ecossystemic servies since they are difficult to produce by higher trophic level (i.e. high metabolic cost). Although their identities remain to be confirmed through the injection of the standards, our result showed a strong inhibition of the production of the docosapentaenoic acid in the exposed biofilms (500 fold).

This study will provide a better understanding of the metabolic alteration associated with the inhibition of the photosynthesis activity, as increasing knowledge on the metabolic responses of heterotrophic communities (bacteria, fungi) following diuron exposure. By investigating confounding factors, it would help to identify metabolic pathways that might be involved in the adaptation/tolerance of autotrophic and heterotrophic aquatic communities. It will finally support the discovery of biomarkers for the monitoring of water quality in the global change context.

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Poster 148: P148 *The Power of MS/MSALL Acquisition for High-Throughput Metabolomics Studies*

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…

Introduction

One of the main goals of researchers in the field of untargeted metabolomics is to analyze a large number of samples and obtain the most useful information in the shortest time with limited sample preparation. The utility of the direct infusion or "shotgun" approach for high-throughput metabolomic and lipidomic profiling analyses using the infusion MS/MSALL acquisition approach in conjunction with fast sample introduction by flow injection analysis (FIA) has been demonstrated1,2.

This work investigates the performance of an automated processing for semi-quantitation and direct structural identification utilizing the MS/MSALL data, in support of metabolomics studies.

CONCLUSIONS

Earlier FIA-based metabolomic profiling5 approaches utilized just the HR MS scans and therefore they were not sufficient for a successful metabolite identification. The shotgun direct infusion approach combined with the data independent acquisition strategy collecting HR MS/MS from all precursors makes a more specific identification of analytes and the quantitation at the MS and MS/MS levels possible.

Curated and annotated Accurate Mass Metabolite Spectral Library provided unique conditions for a quantitation method as well as served as a reference source for an automated library search. Forty three endogenous metabolites were studied to establish the precision and accuracy of the method comprising both MS and MS/MS data. With the low CV values (around 3%), the MS/MSALL technique allows to uncover small changes in the metabolome.162 metabolites were identified in urine sample through the automated spectral processing and library search pipeline.

The MS/MSALL data collection strategy is well suited for high-throughput metabolomic profiling studies since it is fast and it offers semi-quantitative results complemented with a compound identification based on HR MS/MS spectral match.

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Poster 149: P149 *Exploring a volatomic-based strategy to study Lavendula semiochemicals*

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Introduction

Lavender (Lavandula angustifolia) and lavandin (Lavandula x intermedia) are emblematic plants of Provence and are widely used for their essential oils in perfumery, cosmetics and aromatherapy [1]. However, since few years, lavender fields suffered from a severe decline in France due to the propagation of yellow disease. This disease is associated with the presence of stolbur phytoplasma, which is transmitted by Hyalesthes obsoletus, a sap-sucking insect [2]. Consequently, a dramatic decrease has been seen in the production of French lavender and in essential oil yields.

Technological and methodological innovation

To better understand chemical defenses of Lavendula species, we proposed a volatomic approach. To highlight changes in Volatile Organic Compounds (VOCs), we developed an analytical strategy based on dynamic headspace extraction (DHS), followed by Automated Thermal Desorber-Gas Chromatography-Mass Spectrometry (ATD-GC-MS) to characterize VOCs emitted by lavender aerial parts. In addition, stored VOCs were extracted with hexane and then analyzed by GC-MS.

Results and impact

This is the first time such an approach has been used to study emitted and stored VOCs from lavender in open field. Several compounds were found as metabolomic markers of yellow disease infection. For instance, the PLS-DA score plot performed on lavandin plants showed a significant separation on two groups (13.3% of misclassification and P-value < 0.05). Among the discriminating compounds, lavandulyl acetate and (E)-β-farnesene were significantly more emitted by asymptomatic plants, while (Z)-3-hexen-1-ol acetate and 1,8-cineole were significantly more emitted by symptomatic plants.

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Poster 150: P150 *Plasma metabolomics to identify biomarkers of foie gras quality in mule duck*

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Introduction

"Foie gras" of duck is a traditional product in France. The liver weight (LW) and the technological yield (TY) when cooking are the main characteristics of liver quality. The TY must be greater than70% [1]. The LW was previously modeled with body weight and feed intake [2]. The TY was already modeled but the predictors required analyzing liver characteristics [2 and 3]. The objective of this study is to identify non-invasive biomarkers of the TY with 1H-NMR analysis of mule duck plasma.

Technological and methodological innovation

The experimental designed is clearly described in [2]. Briefly 65 mule ducks were overfed during 6 to 12 days. Plasmas and livers were sampled every two days. The LW and TY were measured. 1H-NMR spectra of plasma were acquired on a NMR 600 MHz Bruker Avance III HD. The spectra were transformed into a table of 239 buckets with WorkFlow4Metabolomics [4] and into a table of 87 metabolites with ASICS [5]. Then O-PLS regressions were performed with SIMCA-P+ software to identify the biomarkers of LW and TY.

Results and impact

With bucket data, the individual plots of O-PLS drew good regression of LW (R2X=0.795, R2Y= 0.314) and TY (R2X= 0.805, R2Y= 0.979). In the axes that were correlated to LW and TY, respectively 12 and 13 buckets had a VIP-value up to 1.5. The identification of peaks and the analyses of metabolite data are in progress. The non-invasive biomarkers that will be identified will have to be validated with other lab methodology with the same samples and with other animals.

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Poster 151: P151 *Effect of organic fertiliser on metabolic profile of hydroponically cultivated tomato*

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Introduction

Global production of tomatoes is growing rapidly. Simultaneously, organic waste from farms and greenhouses accumulates and pollutes aquatic ecosystems and atmosphere [1]. Recycling of waste fractions might reduce environmental impact of food production industry. However, an effect of organic fertilization on tomato fruit quality and plant development on metabolite level is not fully investigated. Former studies focused on answering how organic fertilizers affect biomass production and classic fruit quality parameters [2,3]. Metabolic status of roots receiving organic fertilizer has not been explored comprehensively, primarily because of difficulties during root extraction from soil. Hydroponic cultivation allows assessment of root metabolic and ionic status through analysis of root tissue and xylem sap. Xylem sap is a rare target for metabolomic studies of tomato [4,5]. However, it can reflect metabolic processes taking place in roots.

Technological and methodological innovation

We developed hydroponic cultivation system allowing growth of tomato plant on organic and mineral fertilizers with recirculation of nutrient solution. Influence of fertilizer composition on tomato fruit quality and metabolic profile of mature tomato plants were investigated.

The study is distinctive in terms of combination of cultivation aspects such as hydroponic growing, organic fertilization, and research subjects – root, xylem sap and fruit metabolomes.

Results and impact

We performed metabolomic and ionic analysis (by GC-MS and IC) of fruits, leaves, roots and xylem sap of tomato plants growing in hydroponic cultivation system with recirculation of nutrient solution. Organic and mineral fertilizers were used in combination with specific plant growth promoting bacteria culture. Results shows that organic waste can be used as fertilizer in tomato production for long-term cultivation. Presence of organic fertilizer enhanced nitrogen and amino acid metabolism in tomato roots, but reduces content of antioxidants (e.g., ascorbate, chlorogenate). Addition of the plant growth promoting bacteria positively affected content of sugars in xylem sap and fruits. Combination of the bacteria and the organic fertilizer resulted in a distinct metabolic profile, which was supplemented by an increased fruit yield.

Our results contribute to the limited knowledge of tomato root metabolism and provide a ground for further discussions.

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Poster 152: P152 *TACKLING THE ANTIOXIDANT METABOLOME OF WHITE WINE*

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Introduction

The reputation of great wines is synonym to the stability of their aroma flavour of young wines while developing specific varietal nuances during aging. For dry white wines, oxidation further relates to the actual worldwide problem of premature oxidation, which concerns cellar worthy white wines within just a few years after the vintage. However, understanding the various molecular mechanisms potentially involved, which can impact the transient wine composition, requires that top-down analytical strategies are implemented.

Technological and methodological innovation

In this presentation, we will show how two sets of combined analytical strategies involving on the one hand FT-ICR-MS and electrochemical oxidation of bottled wines, and on the other hand LC-QToF-MS of quinone-derivatized wines can bring unprecedented molecular signatures of dry white wines antioxidant metabolome.

Results and impact

FTICR-MS revealed the broad diversity of sulfur-containing compounds, which appear to be the most sensitive to oxidation, whereas nitrogen-containing compounds were mostly formed upon oxidation [1]. The electrophilic derivatization of up to 92 wines specifically unravelled the pool of native nucleophilic compounds potentially involved in the trapping of transient oxidized species, which include an important proportion of amino acids and peptides containing amino acids that are known to have antioxidant properties (Val, Leu, Ile, Pro, Trp, Cys and Met) [2].

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Poster 153: P153 *Metabolomics approach reveals disruption of metabolic pathways in the marine bivalve Mytilus galloprovincialis exposed to WWTP effluent*

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Introduction

Conventional wastewater treatment plants (WWTP) discharge multiple organic contaminants in aquatic environments [1]. A current challenge in ecotoxicology is to assess the effects of such multi-contamination on marine organisms. Environmental metabolomics can be helpful to meet these expectations [2]. In this study, we elucidated the effects of a WWTP effluent on the marine mussel Mytilus galloprovincialis through a non-targeted metabolomics approach.

Technological and methodological innovation

Mussels were exposed for 7 days in controlled laboratory conditions to a vehicle or to a WWTP effluent extract (WEE) corresponding to an environmental dilution of 5%. Metabolic fingerprints were generated by LC-HRMS and processed to highlight the impacted metabolites in male and female mussels. The WWTP effluent extract was characterized, based on a suspect screening approach of 80 contaminants, in an attempt to provide a first understanding of the relationship between observed effects and contaminants.

Results and impact

We highlighted key molecular events triggered by the WEE exposure (modulation of amino acids, purine and pyrimidine metabolism, Krebs cycle, etc.) which could lead to adverse outcomes on individual (reproduction, energy metabolism, osmoregulation, etc.). A sex-specific response was also demonstrated showing the importance to consider sex in the experimental design. Finally, 42 contaminants were detected in the WWTP effluent extract and could be related to the observed effects.

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Poster 154: P154 *Molecular networking as a novel approach to unravel chemical diversity of Dinophysis spp. from French coastal waters*

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Introduction

The dinoflagellate Dinophysis is the main threat to shellfish farming in France, as it is responsible for gastrointestinal illness (Diarrhetic Shellfish Poisoning) caused by the consumption of shellfish contaminated by two families of the toxins it produces: okadaic acid (OA) and their analogues dinophysistoxins (DTXs) and pectenotoxins (PTXs). Only four regulated toxins are routinely monitored by targeted chemical analysis by LC-MS/MS while Dinophysis spp. produce many other toxins[1].

Technological and methodological innovation

To identify unknown toxin analogues, we used a novel approach (Molecular Networking, MN) based on the untargeted analysis of mass spectrometry fragmentation data obtained by liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS/MS)[2]. In this study, MNs were created from cultures of several species of Dinophysis isolated from French coastal waters. Before obtaining informative networks, an optimization of data-dependent LC-HRMS acquisition conditions was conducted [3].

Results and impact

The comparison of 42 different conditions allowed us to focus on maximizing know-toxin detection with characteristic MS^2 and to provide a global overview of Dinophysis spp. metabolic diversity[4]. The peak picking step (MZmine 2) was mandatory to visualize well-resolved isomers. MN revealed the presence of new putative analogues of PTXs in some species of Dinophysis. Thus, MN provided a more complete and structured overview of the chemical and toxin diversity produced by harmful algae.

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Poster 155: P155 *Fast quantitative 2D NMR for targeted and untargeted lipidomics and metabolomics.*

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Introduction

Nuclear magnetic resonance (NMR) is a well-known and powerful analytical technique for the analysis of metabolic mixtures, both for untargeted studies involving a bucketing approach and for the targeted quantification of well-defined metabolites. Commonly, all studies are carried out relying on 1D 1H NMR, however sample complexity often limits the efficiency of 1D NMR in such applications, due to ubiquitous peak overlap.

Technological and methodological innovation

Using 2D NMR experiments overcomes this problem as it allows a better separation between overlapped resonances while yielding quantitative data, provided that appropriate analytical protocols are used. Besides, the experiment duration can be reduced by applying fast acquisition methods [1]. The general workflow to acquire fast quantitative 2D data in the "omics" context will be illustrated on three representative experiments: UF COSY, ZF-TOCSY with non-uniform sampling (NUS), and HSQC with NUS.

Results and impact

After providing some recommendations on how to apply this protocol [2], its implementation in the case of both targeted and untargeted metabolomic studies will be illustrated through recent examples. These include the absolute quantification of major metabolites in plant extracts with ultrafast COSY [3] or an untargeted lipidomic study of animal biofluid extracts with a variety of fast 2D methods [4].

Funding:

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Poster 156: P156 *4D-Lipidomics investigation of in C. elegans daf-2 mutants related to ageing and longevity*

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Introduction

The gene daf-2 was one of the first genes described to extend the lifespan in the model organism Caenorhabditis elegans. daf2 encodes for the sole homolouge of the insulin-like growth factor 1 (IGF-1) receptor in C. elegans. daf-2 mutants show different metabolic adaptations, including changes in lipid metabolism. Here, a 4D-Lipidomics workflow was applied to investigate characteristic changes in the complex lipidome of C. elegans wildtype vs. daf-2 mutants.

Technological and methodological innovation

Comprehensive coverage of detected lipids with a corresponding MS/MS spectrum is required for confident lipidome characterization. With the timsTOF Pro system (Bruker) utilized here, this is realized by the unique PASEF (Parallel Accumulation Serial Fragmentation) acquisition mode. This scan mode offers the possibility to generate clean MS/MS spectra by trapped ion mobility separation (TIMS) of chromatographically non-resolved isobaric lipids at high acquisition speeds.

Results and impact

An integrated workflow for evaluating 4D-Lipidomcics data will be presented. Comparing lipid extracts from C. elegans wild type and mutants enabled the pinpointing of characteristic lipids and their confident identification. Merging information from PASEF MS/MS spectra acquired in positive and negative mode provided complementary information on lipid headgroups and fatty acid side chains. By matching measured CCS values to predicted values increased confidence in lipid assignment even further.

Poster 157: P157 *Lipid profiling to identify changes in lipid metabolism in Caenorhabditis elegans upon starvation*

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Introduction

One of the most fundamental challenges for all living organisms is to sense and respond to alternating nutritional conditions in order to adapt their metabolism and physiology to promote survival and achieve balanced growth. We used UPLC-UHR-ToFMS based lipid profiling to examine temporal regulation of metabolism during starvation in wildtype Caenorhabditis elegans and in animals lacking the transcription factor HLH-30.

Technological and methodological innovation

We performed lipid profiling on a UPLC-UHR-ToF-MS using a previously described RP based method [1]. Employing data dependent acquisition (DDA) we collected over 150.000 MS2 spectra across the entire sample set. In order to be able to annotate lipids we created a workflow within the R statistical language, which performs exact mass searches, library MS2 matching using in silico databases as well as other functions to annotate detected lipid features.

Results and impact

We detected 4063 lipid features in positive and 2258 in negative ionization mode, which remained after normalization and filtering (detected in all QCs and RSD < 30%). Out of these 2068 were putatively annotated on the MS1 level and 427 on the MS2 level in positive ionization mode, and 955 and 118 in negative mode respectively. Our findings show that starvation alters the abundance of hundreds of lipid species in a temporal and HLH-30-dependent manner. Specifically, cardiolipins show changes exclusively in wildtype animals but not in hlh-30 mutants.

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Poster 158: P158 *High speed untargeted 4D-Lipidomics LC-MS/MS workflows with Parallel* Accumulation Serial Fragmentation (PASEF)

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Keywords: Lipidomics, SRM 1950, Plasma, LC, MS, IMS, CCS

Poster 159: P159 *Electrospray Ionization and samples complexity in Meta-metabolomics: a biomarker or a suppressed ion?*

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Introduction

Electrospray Ionization is one of the most used ionization techniques for LC-MS-based metabolomics[1]. However, it presents several drawbacks, e.g. the ion suppression phenomenon, causing ion intensity decrease[2]. More the sample is complex, higher is the occurrence of the phenomenon. Thus, studying samples with different complexities may lead to consider some biologically non-significant molecular traces as markers of discrimination. This is due to ion suppression occurring in complex samples.

Material and Methods

The issue is reported in an environmental context[3,4]. The study is performed on control non-spiked sediment samples and sediments spiked with a complex biopesticide; Bacillus thuringiensis israelensis. Meta-metabolome (endometabolome + xenometabolome) is extracted with QuEChERS method, then analyzed by LC-QToF in order to perform untargeted metabolic profiling, to discover the biomarkers of exposure. This to understand the pesticide impact on spiked sediments compared to control sediments.

Results and Discussion

Results revealed several markers with lower intensity in the spiked group. They were co-eluting with multi-charged xenometabolites. Hence, these markers are either less concentrated due to a biological impact, or suppressed by the co-eluted molecules. Thus, to discriminate between biomarkers and suppressed ions, samples are diluted and analyzed. In fact, as dilution decreases the ion suppression, suppressed features are no more significantly discriminant between the two groups of samples.

Keywords

Meta-metabolomics; Electrospray Ionization; Ion suppression; Biomarker; Liquid Chromatography-Mass Spectrometry

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Poster 160: P160 *Experimental strategy to discover new bioactive lipopeptides produced by gut microbiota: unknownknown approach by LC-HRMS.*

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Introduction

An irritable bowel syndrome is the most common gastrointestinal disorders and is characterized by visceral pain. An alteration of the interactions between the microbiota, the colon and the nervous system has been described. Due to the lack of a clear gut microbiota dysbiosis profile and a functional redundancy of bacteria within the microbiota, our main goal is to identify the bacterial compounds involved in pain regulation.

Technological and methodological innovation, results and impact

In a previous study we demonstrated that a probiotic bacteria, Escherichia coli Nissle 1917, produced analgesic lipopeptides related to GABA1. The aim of our current study is to identify lipopeptides from the gut microbiota and not only from a single bacteria using an unknown-known approach by LCHRMS. First, we developed a lipopeptide-specific extraction method using synthesized lipopeptide standards. Extraction of the standards with or without mouse colonic tissue as a matrix revealed that the method previously described to extract endocannabinoids2 by solid phase extraction with addition of heptane lavage and ethyl acetate liquid/liquid extraction possessed the best yield with a minimal matrix effect. Extracts were analyzed on a LC-QTOF Xevo G2XS system (Waters) using positive- and negative-ionization mode. The mass accuracy and the study of the fragmentation spectra will allow us to identify new families of lipopeptides. We will present in details our experimental strategy, the different soft used, their benefits and limits.

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Poster 161: P161 *Novel UHPLC-MS method for detailed analysis of lipid species using a scheduled MS/MS acquisition approach for improved metabolite annotation*

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Introduction

Lipids are a vast group of biologically important molecules, which are structurally very similar within its classes. Despite the demand for fast UHPLC assays, long separation times decrease ion suppression occurring during the ionisation process, thus increasing sensitivity and specificity of the analytical assay. This approach allows the discovery of new molecules normally masked or eliminated by ion suppression and provides a greater number of MS/MS mass spectra for lipid annotation.

Technological and methodological innovation

We developed a new UHPLC reversed-phase assay utilizing C30 stationary resins for the analysis of lipids present in biofluids and tissues. To maximise the number of compounds with MS/MS data we introduced a 'Scheduled MS/MS acquisition approach'. This utilized several m/z windows spread over the chromatogram. As a comparison, a 15-minute reversed-phase C18 assay was applied with multiple biofluids and mammalian liver samples. Data were processed using Compound Discoverer 3.1 and LipidSearch 4.2.

Results and impact

A 30-minute C30 assay showed a 2.4x increase in the number of compounds detected compared to the 15-minute C18 assay. Using a single MS/MS file 15,000 compounds were detected and 1100 compounds were annotated applying LipidSearch on average for 30 min C30 assay. Using this novel assay will maximize the amount of data gathered from a single injection analysis and reduces the time required to collect these data compared to the commonly applied multiple injections with unique precursor windows [1].

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Poster 162: P162 *Ammonium fluoride as suitable additive for HILIC-based LC-HRMS metabolomics*

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Introduction

The use of Hydrophilic Interaction LIquid Chromatography (HILIC) in metabolomics still does not reach the level of reliability of reverse phase (RP) chromatography. One of the main limitation is compound ionization, due to the limited ionization properties of the common additives used in this chromatography (ammonium formiate/acetate). Further additives are reported in literature to improve compound ionization in both RP [1] and HILIC [2, 3], for example, ammonium fluoride (AF).

Technological and methodological innovation

In this work, we tested the use of AF as additive for HILIC chromatography in a metabolomics framework. A concentration of 2 mM of AF was added in both HILIC mobile phases (ACN and H2O); its performance has been evaluated performing multiple comparative tests [4] versus a well-established ammonium acetate HILIC method. We, then, used the AF method in two real-case metabolomics experiments (urine and blood), to verify also the repeatability and the robustness of the approach.

Results and impact

AF showed sharp ionization and signal-to-noise increase in analytical standards' injections. Peak shape and repeatability improved in all tested conditions. The HILIC AF metabolomics experiment resulted in more discriminant performances than their RP counterparts [5], concluding to its relevance for urine/serum applications. Using stronger additives (like AF) improves the performance and applicability of HILIC's methods in metabolomics for extended metabolome coverage.

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Narduzzi et al. SUBMITTED

Poster 163: P163 *Metabolomics profiles of Annona species cultivated in Egypt by using bioassay-guided fractionation process as antiproliferative agent.*

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Introduction

Tropical and subtropical trees of *Annona* sp. have constituents of high medicinal value for pharmacological research and drug development and show many characteristic features such as antitumor, antioxidant and antimicrobial activities. Metabolomics was employed to functionally characterize natural products to distinguish differences between varieties[1]. Natural products are therefore encoded to be bioactive and of high interest in the drug discovery field[2].

Technological and methodological innovation

The present study was carried out on *Annona* tree species collected in Giza governorate, Egypt: *A. atemoy*a; *A. glabra*; *A. muricata*; *A. squamosa* and *A. Abdel razek*. Screening of *Annona* sps. samples of was determined by MTT assay on five cancer cell lines (MCF-7, HepG2, HCT, Caco, and T47D) which revealed a varied potency (IC_{50}) amongst them [1,3]. Then, further assays such as cell cycle analysis were performed to investigate the anti-cancer effect. Metabolomic analyses were performed using LC-MS/MS systems[4].

Results and impact

Our study was aimed to assess the inter-relationships among six *Annona* species by using 6 SCoT and 6 ISSRs primers were taken for DNA fingerprinting were polymorphic having 45.16 and 35.29 % polymorphism, respectively. GC-MS-based plant metabolomics was employed to compare 78 differential chemical volatile oil profiles of six *Annona* sps and metabolomics bioassay-guided fractionation process by structural analysis *via* HPLC-ESI-MSn, UPLC-HESI-MS/MS as antiproliferative activities of five cell lines.

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Poster 164: P164 *Targeting Esterified Oxylipins – Optimized Sample Preparation for LC-MS Analysis*

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Introduction

Several eicosanoids and other oxylipins are potent lipid mediators and are believed to act predominantly in their free, i.e. non-esterified, form. However, a major portion of oxylipins is found to be bound in lipids, e.g. phospholipids. Only little is known about the biological activity of these esterified oxylipins. To understand their role in health and diseases wellcharacterized, reliable and reproducible sample preparation procedures for their quantitative determination are needed.

Technological and methodological innovation

Esterified oxylipins are commonly quantified as a sum of free and bound oxylipins by means of liquid chromatography-mass spectrometry, however, current sample preparation procedures differ considerably and optimization data are missing. We present a detailed procedure for quantification of total oxylipins in biological samples, comprising extraction of lipids and removal of proteins, base hydrolysis to saponify lipids and solid phase extraction using a C8/anion exchange mixed mode cartridge [1].

Results and impact

It is highlighted that each sample preparation step has a direct impact on the apparent oxylipin concentration and the optimization of each step is crucial to ensure reproducibility and reliability. Moreover, special attention has to be paid to artificial formation of epoxy fatty acids through autoxidative processes. Based on the presented methodology the biological role of esterified oxylipins can be reliable investigated and could pave the route for their use as biomarkers for diseases.

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Poster 165: P165 *Stability of total oxylipins – influence of plasma generation and long-term storage*

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Introduction

Eicosanoids and other oxylipins have raised strong interest in clinical studies regarding their potential use as biomarkers in health and disease [1]. The modulation of the oxylipin profile is commonly investigated in response to pharmaceutical or dietary intervention. However, the outcome of such clinical results can be influenced by (unsuitable) sample handling, e.g. sample generation, storage and sample preparation, leading to artificial formation or degradation of oxylipins.

Technological and methodological innovation

We employed a comprehensive liquid chromatography-tandem mass spectrometry (LC-MS/MS) method covering over 160 analytes [2,3] to investigate the effects of sample generation and storage on the pattern of total (free + esterified) plasma oxylipins [4]. We examined the impact of different storage temperatures and times which are present in routine clinical practice on the total oxylipin levels in plasma and assessed the long-term stability of total oxylipins at -80°C during a period of 15 months.

Results and impact

We show that the levels of total oxylipins are stable during the transitory stage of plasma generation and only long delays increase the concentrations. The storage of plasma at -80°C for 15 months merely increased oxylipins formed autoxidatively. Conclusively, total oxylipins are robust during plasma generation and long-term storage allowing the generation of biologically meaningful oxylipin patterns.

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Poster 166: P166 *Understand the metabolic crosstalk in photosymbiosis*

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Introduction

Symbiosis between single-cell heterotrophic hosts and microalgae is a widespread and ecologically important lifestyle in the oceanic plankton1,2. Yet, the functioning of this interaction, specifically metabolite exchanges between the photosynthetic symbionts and their host, remains largely unexplored; mainly because of the technical challenges inherent to the study of these microorganisms. Our main objective is to elucidate the metabolic services provided by the symbionts and highlight the metabolic controls exerted by the host.

Technological and methodological innovation

We propose an approach involving the use of stable isotopes coupled with metabolomics (fluxomics) to identify the photosynthates produced by microalgae, in their free-living stage and in symbiosis. Combining these data with subcellular imaging is crucial for deciphering the metabolic crosstalk between partners. Thus, we chose to couple fluxomics analysis with nanoscale isotope imaging (NanoSIMS) to visualize and quantify the flux of 13C-labeled carbon at the subcellular scale between the host and microalgal cells.

Results and impact

Two symbiotic models involving two different types of symbionts are presented here: the freshwater Paramecium and the marine radiolarians, respectively. First results suggest both active 13C-metabolite transfer from the photosynthetic symbionts to the host, but also host de novo synthesis based on these transferred C-compounds. These new data will be of major interest as the knowledge derived from these model organisms can be applied to better understand other associations found in diverse environments.

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Poster 167: P167 *Molecular networks as a metabolomic tool to link traditional uses to biological activities and potential valuation of Polynesian plants*

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Introduction

Cosmetopoeia regards the traditional use of plants for body care [1]. In French Polynesia, plant-based preparations like monoi are used for hair and skin care [2] Six extracts of differing polarity from plants Bidens pilosa L., Calophyllum inophyllum L., and Fagraea berteroana A. Gray ex Benth. were chosen for having interesting cultural uses and in vitro biological activities [3]. The extracts chemical composition was studied by UHPLC-ESI-HRMS to find correlations with observed bioactivities.

Technological and methodological innovation

The combined ethnobotanical, biological, cosmetic and metabolomic data of this study enables a dual analysis of the molecular network obtained. Firstly, by tentatively matching regions of the network to common bioactivities of the phytoextracts, in vitro and in cellulo. Secondly, by comparing the mass spectra of known and active compounds from B. pilosa, with that of a less studied plant species, F. berteroana to enrich its known phytochemistry.

Results and impact

Preliminary results showed a hundred metabolites common to all three species, such as flavonoids, as well as compounds known for possessing cosmetic activities of interest to this study. The former could partially explain the extracts in vitro anti-inflammatory and antioxidant activities.

Thus, this metabolomic data gives further insight into the mode of action of the phytoextracts on cellular targets, justifying their traditional Polynesian use and stating hopeful future cosmetic valuation.

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Poster 168: P168 *A New Tool in Metabolomics: SWATH® Acquisition Analysis for Global Profiling and Quantitation*

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Introduction

Data independent acquisition (DIA) workflows are well adopted in quantitative discovery proteomics, but still not commonly used in discovery metabolomics. Data dependent acquisition (DDA) techniques are heavily employed in the field of metabolomics and workflows on mass spectrometers have been adapted so that as much data as possible can be captured. Researchers were limited by the speed of their QTOF mass spectrometers meaning a multiple injection workflow. Also, the stochastic nature of data dependent workflows often means MSMS of low abundant metabolites are often missed. Here, it is described how DIA enables the identification of a higher number of metabolites for untargeted metabolomics workflows compared to traditional DDA approaches thus enabling a broader profile of the metabolome.

Technological and methodological innovation

Urine and plasma were processed according to standard extraction protocols. Urine was diluted with water at a ratio of 1:4 (v/v) and centrifuged for prior analysis, whilst plasma was extracted 1:4 (v/v) with ice-cold methanol allowing for protein precipitation. Separation was performed on reverse phase chromatography.

DDA and DIA specific settings were chosen and evaluated. The data were acquired on a QqTOF mass analyzer. For the DDA acquisition, we selected the top 5, 10, 15, 20 and 25 precursor ions for MSMS. For DIA, we applied 15, 20 and 30 mass windows with either fixed window (fw) or variable window (vw) widths. Results were evaluated by the highest number of identifications and coverage of metabolites in plasma and urine extracts.

Results and impact

At the DDA level, the data demonstrate a significant improvement of metabolite coverage at the MSMS level when comparing the top5 to the top25 DDA method. We show over 100% increase of metabolite coverage in plasma extracts by increasing the number of selected precursor ions for DDA acquisition from top5 to top25. This result highlights the capability of the QqTOF mass analyzer for fast MSMS acquisition, which allows for the fragmentation of a large number of precursors in a single DDA cycle, leading to a larger number of metabolites identified.

In the second part of this study, we evaluated the DIA strategy with various fixed (fw) and variable window (vw) sizes with similar cycle time in a plasma extract. Increasing the number of fixed windows resulted in ~30% gain in metabolite coverage. Using the variable window method resulted in a ~70% gain in metabolite coverage.

We finally applied these experimental approaches to common matrices used in metabolomics studies, namely urine and extracted plasma. We show that a DIA approach applying 20 variable windows can identify up to 55% more metabolites than a traditional top20 DDA acquisition (in a urine extract). More confident MSMS based identifications lead to higher quantifiable metabolites in a metabolite expression experiment, which at the end allows better understanding of the biology. When comparing the performance in extracted plasma it can be observed that applying a DIA approach with 20 variable windows allows significant gains in metabolite coverage (around 55%) versus the top20 DDA acquisition, similar gains as seen in the urine extract.

Data Independent Acquisition Improves Metabolite Coverage over Traditional Data Dependent Techniques for Untargeted Metabolomics

Poster 169: P169 *How to automate boring lipidomic extraction?*

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Introduction

Lipids are ubiquitous biomolecules essential to all life, found in every cellular type, ranging from the human body and vegetal organisms, down to bacteria. They have many different functions in cell structuration, energy storage and signalling. Mass spectrometry (MS) coupled with liquid chromatography or gas chromatography is mainly used for global and specific analysis of lipids. But before their analysis, there is an important and time consuming step of sample preparation including liquid-liquid extraction (LLE) and solid phase extraction (SPE).

Technological and methodological innovation

To circumvent this point, a TECAN robot has been acquired to automate the sample preparation. Due to the specificity of lipid extraction, the robot needs a lot of optimisation. The optimisation was done on three major points: water contamination, accuracy and repeatability of dispense volume and the loss of lipids. Two systems have been set up on the robot and different parameters have been tested with these systems. In order to determine which, one was more appropriate for our uses, depending on the previous key points.

Results and impact

This presentation will show part of adaptation we had to perform to validate fatty acid, neutral lipid and phospholipid profiling. Lipidomic's results obtained with a complete automated sample preparation, including LLE steps, will be presented for liver and plasma sample. This process can be applied on large cohort of clinical samples.

Poster 170: P170 *Development of lipidomic profiling by SFC-HRMS*

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Introduction

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

Technological and methodological innovation

Due to the high structural diversity of lipid, a complete lipidomic profiling of biological matrices remains a challenge. In this context, we develop an untargeted lipidomic approach by using supercritical fluid chromatography high resolution mass spectrometry. The optimization of the separation and the detection of lipid species were performed on pure standards and then on liver lipid extract from mice exposed to a control or a high fat diet.

Results and impact

These analyses allowed the building of a homemade lipid data bank. An automatic process was then developed using MSDial software to produce the relative quantification of lipids species belonging to the 18 class of lipids. The optimization of this method will be present with the first results obtained on liver disease model and the limit of the use of MSDial will be exposed. First results obtained reveal the high efficiency of this method for the profiling of more than 600 lipids.

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Poster 171: P171 *Parsimonious ^{13}C Metabolic Flux Analysis*

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Introduction:

When a biological system is incubated with a ^{13}C-labeled substrate, ^{13}C propagates to metabolites throughout the metabolic network in a flux and pathway-dependent manner. ^{13}C Metabolic Flux Analysis (^{13}C MFA) integrates measurements of ^{13}C enrichment in metabolites to identify the flux distributions consistent with the measured ^{13}C propagation[1]. However, there is often a range of flux values that can lead to the observed ^{13}C distribution[2]. Hence, ^{13}C MFA might be unable to reduce the solution space towards a unique solution either in large metabolic networks or when small sets of measurements are integrated.

Technological and methodological innovation:

We developed parsimonious ^{13}C MFA (p^{13}CMFA), an approach that runs a secondary optimization in the ^{13}C MFA solution space to identify the solution that minimizes the total reaction flux. Furthermore, flux minimization can be weighted by gene expression measurements[3].

Results and impact:

As proof of concept, we demonstrated the potential of p^{13}CMFA by estimating intracellular flux distributions from ^{13}C-resolved metabolomics and transcriptomics data in a Human Umbilical Vein Endothelial Cells (HUVECs). p^{13}CMFA allows for the first time a seamless integration of ^{13}C data with transcriptomics data. Even more, p^{13}CMFA potentially paves the way for integrating ^{13}C resolved metabolomics with multiple layers of omics to compute genome-scale flux maps.

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Poster 172: P172 *The development of microbore UHPLC-MS assays to enhance sensitivity of untargeted metabolomic analysis of mammalian biofluids.*

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Introduction

To date, there has been limited research into ultra-high performance liquid chromatography-mass spectrometry assays for untargeted metabolomics analysis of biofluid samples of low volumes including dried blood spots and tears (<20µL) Demands for these assays are growing. Currently, most biofluids achieve satisfactory results by using standard methods applying 2.1mm internal diameter (ID) columns, but these methods do not provide the optimal sensitivity needed for samples of low volumes.

Technological and methodological innovation

Three metabolomics assays were developed (C18 reversed-phase lipidomics, C18 aqueous reversed-phase and HILIC) using human plasma and urine along with 2.1mm and 1.0mm ID UHPLC columns of the same stationary phase composition and particle size. Data comparisons quantified the influence of column ID on assay sensitivity, reproducibility and chromatographic peak width. The 1.0mm ID column assays were next evaluated using a time-series of plasma, urine and tear samples collected during a pig study.

Results and impact

Overall, reducing column ID saw a sensitivity increase (number of compounds detected, peak area). Similar reproducibility levels were seen. The decrease in flow rates used for UHPLC columns may be a large contributor to any unforeseen results including increased UHPLC peak widths. Comparisons of the three biofluids using the 1.0mm ID assays allowed for the metabolite characterisation of tear samples and understanding of the correlation of metabolite relative concentrations between biofluids.

Poster 173: P173 *MeTaQuaC: Quality Control Measures for Targeted Metabolomics Studies*

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Introduction

Optimized and validated targeted mass spectrometry profiling methods enable comprehensive routine applications such as the analysis of larger cohorts. However, such studies require consistent processing and reliable instrumentation to minimize technical variance and interference, including systematic and random errors. Consequently, multiple and reproducible quality controls (QCs) are required to verify data conditions with respect to quality assurance (QA) measures.

Technological and methodological innovation

Here we present MeTaQuaC, an extensive, easy-to-use and free quality control R package designed for targeted data acquired using Biocrates' kits and which is complementary to Biocrates' MetIDQ software. It combines several visualizations and statistics into an HTML report. These include, for instance, evaluation of measured and missing values, positional irregularities with respect to acquisition sequence or well plate coordinates as well as sample and metabolite variability and reproducibility.

Results and impact

MeTaQuaC enables detailed utilization of QA measures as provided by standardized kits and currently supports Biocrates' AbsoluteIDQ® p400 HR Kit and MxP® Quant 500 Kit. With defined, reproducible and viable QCs, it aids in verifying data consistency and quality or, if necessary, in identifying patterns of interference as well as removing low quality metabolites or samples, thereby increasing confidence in data and subsequent analysis. MeTaQuaC is available under MIT open source license [1].

References

[1] https://github.com/bihealth/metaquac