metabomeeting 2024

26 - 28 November | Liverpool, UK

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Organising Committee

Primary organiser
Howbeer Muhamadali
Lecturer, Metabolomics and Biotechnology
Centre for Metabolomics Research (CMR),
University of Liverpool, UK

Organiser
Roy Goodacre
Professor of Biological Chemistry
Centre for Metabolomics Research (CMR),
University of Liverpool, UK

Organiser
Warwick Dunn
Professor of Analytical and Clinical Metabolomics
Centre for Metabolomics Research (CMR),
University of Liverpool, UK

Department of Biochemistry, Cell and Systems Biology (BCSB)
Institute of Systems, Molecular and Integrative Biology (ISMIB)
University of Liverpool

Conference Venue

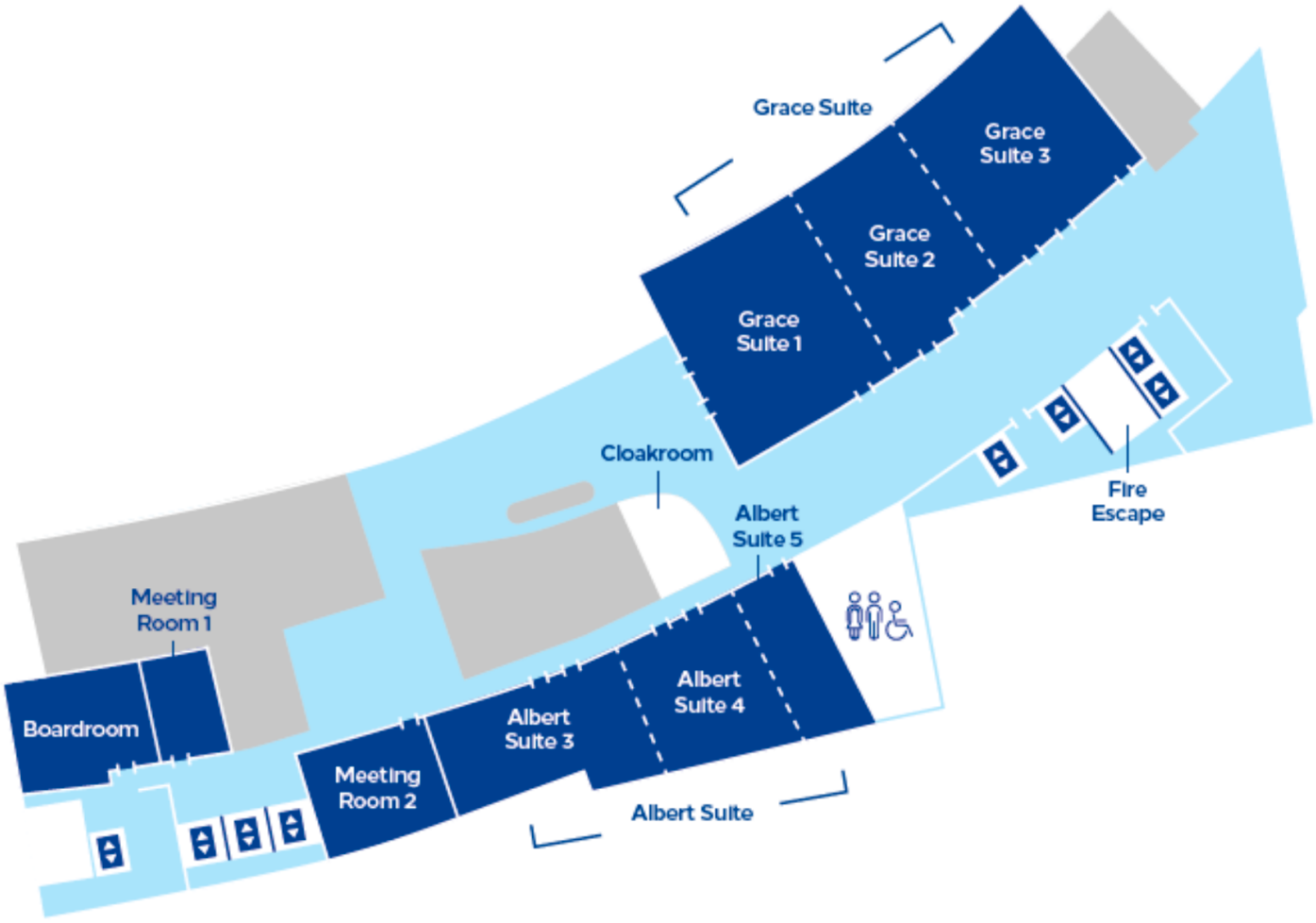
ABOUT THE HOTEL

Built on the Old Customs House site with a design that represents the meandering River Mersey on the Albert Dock, Hilton Liverpool City Centre is situated in the centre of Liverpool ONE retail and leisure complex, providing easy access to transport links and cultural attractions.

Hilton Liverpool City Centre
3 Thomas Street Way,
Liverpool,
L1 8LW
United Kingdom

Venue Map

The conference area (see map below) is situated on the first floor of the Hilton Hotel. To reach this area, go up the stairs directly after entering the hotel through the main entrance.



FOR A VIRTUAL TOUR OF THE VENUE PLEASE CLICK **HERE**

Conference Dinner

ABOUT

The Merseyside Maritime Museum forms part of Liverpool's historic waterfront, housed in a former bonded warehouse on the north side of the Albert Dock. It once stored tobacco, wines and spirits. Located in the heart of the Albert Dock, it's the ideal location to explore and uncover the development of the world-famous port.

Maritime Museum
Royal Albert Dock,
Liverpool,
Merseyside
L3 4AQ

Walking route from the conference venue (Hilton Hotel) to the conference gala dinner venue (Maritime Museum).

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Scientific Programme

Metabomeeting 2024, University of Liverpool, 26-28th November 2024	
Tuesday 26th November	
9:00 - 11:00	Tea, Coffee & Refreshments
9:45 - 10:45	Photothermal Spectroscopy Corp Workshop, Multimodal Submicron Simultaneous IR+Raman microscopy with co-located fluorescence for life science applications. Roy Goodacre, Cassio Lima, Mustafa Kansiz
11:00 - 11:15	Welcome session Prof. Chris Goldring, University of Liverpool
11:15 - 12:00 Howbeer Muhamadali	11:15 Plenary 1, Data-Driven Framework for Metabolomic Epidemiology Prof. David Broadhurst, Edith Cowan University
12:00 - 13:00	Lunch
13:00 - 14:30 Metabolomics in Health and Disease Chair: Jules Griffin	13:00, The genetic architecture of the metabolome across several organs illuminates responses to nutritional and pharmacological challenges Marc-Emmanuel Dumas, Imperial College London, CNRS
	13:30, A gut microbiome-kidney-heart axis in human populations Kanta Chechi, Imperial College London
	13:45, Untargeted and targeted metabolomics of cerebrospinal fluid for the discovery of biomarkers of children's brain tumour relapse Alison Whitby, University of Nottingham
	14:00, LC-MS profiling of the urine metabolome in adverse early pregnancy outcomes Christopher Hill, University of Liverpool
	14:15, Oral supplement in older adults to support physical fitness and mental well-being. Alison Watson, Aberystwyth University
14:30 - 15:00	Tea, Coffee & Refreshments

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Scientific Programme

Metabomeeting 2024, University of Liverpool, 26-28th November 2024	
Tuesday 26th November	
15:00 - 16:30 Microbial metabolomics Chair: Dong-Hyun Kim	15:00, Metabolic Profiling of Antarctic Snow Algae Matthew Davey, Scottish Association for Marine Science
	15:30, Something smells….and it's antibiotic resistance Breanna Dixon, University of Manchester
	15:45, From toxic waste to beneficial resource: acetate boosts *E. coli* growth at low glycolytic flux Gosselin-Monplaisir Thomas, Toulouse Biotechnology Institute
	16:00, Maternal *Toxoplasma gondii* infection induces metabolic changes to the maternal foetal interface Hafiz Arshad, University of Glasgow
	16:15, Application of Infrared spectroscopy for rapid identification of antimicrobial resistance and study of carbon-deuterium kinetics at the single-cell level Sahand Shams, University of Liverpool
16:30 - 17:00	Tea, Coffee & Refreshments
17:00 -17:40 ECR poster flash session 1 Chair: Adam Burke	17:00, Rapid microbore metabolic profiling of human liver disease plasma samples using a multi-reflecting time-of-flight mass spectrometer Ana Sanchez Lorenzo, Imperial College London
	17:02, Applying Fourier-transform Infrared spectroscopy to characterise and differentiate Achromobacter and Pseudomonas biofilms Joel Doherty, University of Liverpool
	17:04, metabolomic profiles to explore biomarkers of adverse body composition parameters in non-small cell lung cancer patients Ziyi Zhong, University of Liverpool

Scientific Programme

Metabomeeting 2024, University of Liverpool, 26-28th November 2024	
Tuesday 26th November	
17:00 -17:40 ECR poster flash session 1 Chair: Adam Burke	17:06, Optimising synovial fluid preparation for gas chromatography- mass spectrometry metabolomics analysis for orthopaedic research Yumna Ladha, Keele University
	17:08, Implementation of a Semi-automated Sample Preparation Workflow for Comprehensive Serum Metabolomics Ines Castro, Universite de Lille
	17:10, Annotation and dereplication of oxylipins in human urine using LC-HRMS/MS and molecular networking: insights from the Valencian community Ángel Sánchez-Illana, University of Valencia
	17:12, Discrimination of periodontal bacteria utilising IR spectroscopy techniques Jawaher Albahri, University of Liverpool
	17:14, Valorising waste streams by extracting high value components for the food industry. Cristina Saladas, University of Liverpool, Suntory
	17:16, Influence of SiNPs in soybean plants: an NMR-based metabolomic approach Amanda Lemos Quintela, Unicamp
	17:18, Development of a semi-targeted metabolomics assay for analysing mammalian urine and faeces by GC-MS Shiva Jalili, University of Liverpool

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Metabomeeting 2024, University of Liverpool, 26-28th November 2024	
Tuesday 26th November	
17:00 -17:40	17:20, Automated extraction of adherent cell lines from 24-well and 96-well plates for multi-omics analysis using the Hielscher UIP400MTP sonicator and Beckman Coulter i7 liquid handling workstation Lauren Cruchley-Fuge, University of Birmingham
ECR poster flash session 1 Chair: Adam Burke	17:22, Future possibilities for the SEISMIC facility and overview of current research and development projects Jake Penny, University of Surrey
	17:24, MetAssimulo 2: a web app for simulating realistic 1D & 2D Metabolomic 1H NMR spectra Yan Yan, Imperial College London
	17:26, Assessing small polar metabolite detection using mass spectrometry imaging: a comparison of MALDI and LD-REIMS spatial technologies Georgia Lorentzen, Imperial College London
	17:28, No Time to Dye - portable surface-enhanced Raman spectroscopy for on-site detection of Sudan dye in palm oil Joe Stradling, University of Liverpool
	17:30, Profiling the dynamic metabolome of colorectal cancer cells following irradiation Aaron Kler, University of Liverpool
18:00 - 21:00	Drinks Reception and Poster Interaction, at Hilton Hotel

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Scientific Programme

Metabomeeting 2024, University of Liverpool, 26-28th November 2024	
Wednesday 27th November	
9:15 - 10:00 Warwick Dunn	9:15 Plenary 2, Network science and knowledge mining to make sense of metabolomics data Prof. Fabien Jourdan, INRAE-MetaboHUB
10:00 - 10:30	Tea, Coffee & Refreshments
10:30 - 12:00 Bioinformatics and Statistical approaches Chair: Warwick Dunn	10:30, Can attention-based deep learning models help predict molecular structure from MS fragmentation spectra? Ivayla Roberts, University of Liverpool
	11:00, SpectruMS: A cost-effective MS/MS foundation model learning the language of mass spectrometry Daniel Crusius, Pangea Botanica Germany GmbH
	11:15, IsoDesign: streamlining experimental design for ^{13}C-metabolic flux analysis Loïc Le Grégam, Université de Toulouse
	11:30, Network-based meta-analysis of metabolomics studies from large-scale public data repositories Cecilia Wieder, Imperial College London
	11:45, Class level annotation of untargeted MS1 metabolomics data using Gaussian graphical models Tom Rix, Imperial College London
12:00 - 13:00	Lunch
13:00 - 14:00 ECR session Chair: Alison Whitby	13:00, Development of mass spectrometry-based methods to identify novel antigens presented by the unconventional MHC-like protein, MR1 Edward Mathias, The University of Birmingham
	13:15, Developing a panel of urinary dietary biomarkers for the intake of foods commonly consumed in the UK Juliet Vickar, Aberystwyth University

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Metabomeeting 2024, University of Liverpool, 26-28th November 2024	
Wednesday 27th November	
13:00 - 14:00 ECR session Chair: Alison Whitby	13:30, The role of metabolomics in screening for malnutrition and frailty risk in UK older adults. Thomas Wilson, Aberystwyth University
	13:45, Development of large-scale LC-MS-based metabolomics to investigate dairy cow lameness. Ana Cardoso, University of Nottingham
14:00 - 14:30 ECR poster flash session 2 Chair: Ivayla Roberts	14:00, Chemical attributes of UK-grown tea- Dartmoor Estate Tea. Alina Warren-Walker, Aberystwyth University
	14:02, The workflow for building high-quality spectral libraries using UHPLC-HRMS applied for human metabolomics studies. Romina Pacheco-Tapia, Université de Lille
	14:04, Probing metabolic behaviour of bacteria at surfaces Daniel Smaje, University of Liverpool
	14:06, Dietary impact of frog holometabolomes: identification of pumiliotoxin analogues through in-depth metabolomic investigation *Oophaga vicentei* dermal and gut extracts. Elliot Murphy, Imperial College London
	14:08, Using Raman spectroscopy to monitor biological processes by simulated addition of glucose & lactate. Clark Gray, University of Liverpool
	14:10, A comprehensive computational framework for real-time monitoring and quality control of LC-MS-based metabolomics data acquisition. Ossama Edbali, University of Birmingham

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Metabomeeting 2024, University of Liverpool, 26-28th November 2024	
Wednesday 27th November	
14:00 - 14:30	14:12, Using OrbiSIMS as a label free approach to deciphering metabolic heterogeneity in glioblastoma. Aimee Humphreys, University of Nottingham
	14:14, Metabolome associations with cardiovascular ageing. Shamin Tahasildar, MRC Laboratory of Medical Sciences
ECR poster flash session 2	14:16, Metabolomic ageing clock monitors risks of cardiometabolic diseases. Manyi Jia, Imperial College London
	14:18, Simulated metabolic profiles unveil biases in pathway analysis methods. Juliette Cooke, INRAE Toxalim, Toulouse
	14:20, Developing analytical methods for the detection of thiols associated to axillary malodour. Amy Colleran, University of Liverpool
Chair: Ivayla Roberts	14:22, Aqueous humour metabolome analysis discloses altered arginine metabolism in Parkinson's disease. Joan Serrano, University of Barcelona
	14:24, Analytical techniques to study the formation and structure of homogentisic acid-derived pigment. Harriet Willett, University of Liverpool
	14:26, Unravelling Fungal-Plant Interactions through Untargeted Metabolomics and Mass Spectrometry Imaging Tools. Marina Ferreira Maximo, Institute of Chemistry, UNICAMP
14:30 - 15:00	Tea, Coffee & Refreshments

Scientific Programme

Metabomeeting 2024, University of Liverpool, 26-28th November 2024	
Wednesday 27th November	
15:00 - 15:45 Roy Goodacre	15:00 Plenary 3, The SEISMIC facility for spatially resolved single and sub-cellular omics Prof. Melanie Bailey, University of Surrey
15:45 -17:00 Technology Development & Molecular Imaging Chair: Catherine Winder	15:45, Targeting brain cancer with precision: Insights from single-cell-based metabolite profiling. Dong-Hyun Kim, University of Nottingham
	16:00, Applying Oxygen Attachment Dissociation (OAD) MS/MS to identify positional isomers in lipid biomarkers associated with ethanol toxicity. Emily Armitage, Shimadzu Corporation
	16:15, Unravelling metabolic dysregulation in head and neck cancer with laser-desorption mass spectrometry imaging. Jasmin Werner, Imperial College London
	16:30, Insights into the interaction of cells with plastics via label-free optical imaging methods. Cassio Lima, University of Liverpool
	16:45, Multi-view machine learning for integration of multimodal mass spectrometry data. Lukas Kopecky, Imperial College London
17:00 - 17:15	Tea, Coffee & Refreshments
17:15 - 18:00	MPF open meeting and discussion, OPEN TO EVERYONE
19:00 – 23:00	Drinks Reception & Gala Dinner at the Maritime Museum

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Scientific Programme

Metabomeeting 2024, University of Liverpool, 26-28th November 2024	
Thursday 28th November	
10:00 - 10:30	Tea, Coffee & Refreshments
10:30 - 12:00 Plant Metabolomics Chair: James McCullagh	10:30, Assessing the impact of nitrogen supplementation in oats across multiple growth locations and years with targeted phenotyping and high-resolution metabolite profiling approaches. Will Allwood, James Hutton Institute
	11:00, Identifying health-promoting chemical attributes of tea after different processing methods using metabolomics and machine learning. Amanda Lloyd, Aberystwyth University
	11:15, Metabolomic evaluation of coffee beans and their husks from organic cultivation in the Caparaó region, Brazil. Murilo de Oliveira Souza, Instituto Federal do Espírito Santo
	11:30, Metabolomic 'global' fingerprinting and data mining on flour. Pilar Martinez-Martin, Aberystwyth University
11:45 - 13:00	Lunch
13:00 - 14:30 Metabolomics in Pharma & Other Biological Systems	13:00, The MATCHING international ring-trial to evaluate the reliability of metabolomics for "chemical grouping" in regulatory toxicology. Mark Viant, University of Birmingham

Scientific Programme

Metabomeeting 2024, University of Liverpool, 26-28th November 2024	
Thursday 28th November	
13:00 - 14:30 Metabolomics in Pharma & Other Biological Systems Chair:	13:30, GC-MS based metabolome profiling of Antarctic marine organisms & lessons learnt in environmental omics. Adam Burke, University of Liverpool
	13:45, A direct comparison of anion-exchange chromatography-mass spectrometry (AEC-MS) and HILIC-MS for cellular metabolomics James McCullagh, University of Oxford
	14:00, Getting to know your roommate – NMR metabolomics as a tool to characterise the role of metabolism in symbiotic relationships. Horst Joachim Schirra, Griffith University
14:30 - 15:00	Tea, Coffee & Refreshments
15:00 - 15:45 Howbeer Muhamadali	15:00 Plenary 4, Biomarkers of Food Intake: how can metabolomics help. Prof. Lorraine Brennan, University College Dublin
15:45 - 16:30	Closing ceremony and prizes
16:30 - 17:00	Tea, Coffee & Refreshments

BIOGRAPHY

David Broadhurst is Professor of Metabolomic Epidemiology & Biosystems Data Science at Edith Cowan University, Perth, Western Australia. He has been an active member of the metabolomics community for over 25 years, living in UK, Ireland, Canada and now Australia. He did his PhD with Prof. Douglas Kell, at the Aberystwyth University and while at Manchester University he was part of the HUSERMET team that developed the gold standard for large-scale mass spectrometry-based clinical metabolomics. He has since worked as the lead biostatistician on many large-scale metabolomics studies across many areas of human health. In the metabolomics community he is probably best known for his work in quality assurance and promoting best practice in design of experiments, biostatistics and machine learning. In 2022 he was made a lifetime honorary fellow of the Metabolomics Society.

DAVID BROADHURST

DATA-DRIVEN FRAMEWORK FOR METABOLOMIC EPIDEMIOLOGY

EDITH COWAN UNIVERSITY, PERTH

PLENARY 1

TUESDAY 26TH NOVEMBER, 11:15 AM

ABSTRACT

Metabolomic Epidemiology has been defined as “the field of scientific enquiry involving the systematic use of epidemiological methods and principles to study population-based variation in the human metabolome as it associates with health-related outcomes or exposures”. Recent advancements in analytical technology and computational power, together with the maturation of facilities dedicated to large-scale service provision, have made metabolomic epidemiological studies more accessible to health researchers. Accordingly, this field has seen recent exponential growth. The nature of epidemiology methodology often necessitates large-scale multi-centre sample collection and analysis. Such study designs are often led by health researchers with limited understanding of the metabolome’s sensitivity to small variations in sample collection, biobanking, and transportation. Equally, project leaders may have limited understanding of the capricious nature of 'data-driven' (untargeted) metabolomic data acquisition resulting in potentially large bias and uncertainty in reported metabolite abundances. These factors, together with phenotypic characteristics that are known to significantly influence the human metabolome (e.g. age, sex, BMI) need to be investigated and mitigated before any actionable epidemiological interpretation of associations with a primary outcome or exposure. For untargeted metabolomics, this process is compounded by both the number of metabolites typically measured in a single study and the multivariate nature of metabolite interactions. Here I present a ‘data-driven’ framework for investigating sources of bias and variance in metabolomic epidemiology, referencing a recently completed multi-centre project, together with personal observations on the limitations of standard epidemiological models when applied to this kind of data.

BIOGRAPHY

Fabien Jourdan holds a PhD in computer science from the University of Montpellier. He is INRAE research director (DR) in the research laboratory TOXALIM, Toulouse (France). He is co-leading a team of 25 scientists (Team MeX "Metabolism and Xenobiotics") working on the metabolic impact of food contaminants on Human health. His main expertise is the development of computational solutions to model metabolism at the cellular or tissue level. Since 2009, he has coordinated the development of the MetExplore web server which is used by more than 1500 users worldwide to study omics data in the context of metabolic networks. Since 2021, he is director of French national infrastructure for Metabolomics and Fluxomics MetaboHUB. Former president of French-Speaking Metabolomics and Fluxomics Network (RFMF) he served as international Metabolomics Society secretary from 2020 to 2024.

FABIEN JOURDAN

NETWORK SCIENCE AND KNOWLEDGE MINING TO MAKE SENSE OF METABOLOMICS DATA

INRAE-METABOHUB

PLENARY 2

WEDNESDAY 27TH NOVEMBER, 9:15 AM

ABSTRACT

Metabolic modulation is a cornerstone cellular response to genetic or environmental stresses. This plasticity is going beyond central metabolism and may involve complex processes spanning several metabolic pathways. Hence, it is a key challenge to be able to decipher metabolic modulations in a systemic and global perspective.

The aim of the computational methods and tools which will be presented is thus to consider the full complexity of metabolism. To do so, all metabolic reactions the cell is able to achieve are gathered in a single mathematical model call "genome scale metabolic network". Based on this model it is then possible to identify metabolic specificities of different cell lines, predict metabolic behaviours, simulate metabolic responses to single or multiple knock-out and ultimately identify potential drug targets.

Finally, even if metabolic profiles and associated metabolic network modulations are very informative, their biological and physiological interpretation remain a challenge, requiring researchers to gather and connect various pieces of knowledge from a large range of resources. To aid in this task, we introduce FORUM: a Knowledge Graph (KG) providing a semantic representation of relations between chemicals and biomedical concepts, built from a large-scale federation of life science databases and scientific literature repositories. These associations allow to derivate new hypothesis from observed metabolites or anticipate which metabolites could be expected to be measured for a given disease.

BIOGRAPHY

Melanie Bailey is Professor of Analytical Science at the University of Surrey. She is Director of the Wolfson Centre of Excellence for Bioanalytical Science, which brings together expertise in "omics", biomarker discovery and imaging. This includes SEISMIC, a new BBSRC national facility for spatially resolved single and sub-cellular "omics" , new instrumentation for multimodal imaging at the UK National Ion Beam Centre and bulk metabolomics, proteomics and metallomics. She is a member of the international advisory board for The Ion Beam Analysis conference series, International Nuclear Microprobe conference series and the Bragg Institute. She is a Trustee of the Analytical Chemistry Trust Fund and ran the London Marathon to raise money for Analytical Science in 2024. She will commence a term as Editor in Chief of the Royal Society of Chemistry's longest running journal "Analyst" in September 2024.

MELANIE BAILEY

THE SEISMIC FACILITY FOR SPATIALLY RESOLVED SINGLE AND SUB-CELLULAR OMICS

UNIVERSITY OF SURREY

PLENARY 3

WEDNESDAY 27TH NOVEMBER, 3:00 PM

ABSTRACT

Single-cell analysis is a new and rapidly growing field that is shedding new insight into the cellular mechanisms behind the pathogenesis and treatment of infectious diseases and cancer. Cells, even within a single tissue, culture or biological fluid are highly heterogenous.Analysis of bulk material therefore only provides an average value, meaning that information is lost. Single cell transcriptomics and proteomics are already making major advances to understand infection, immunity and drug delivery. Single cell lipidomics is highly desirable to understand the endpoint of metabolism, but currently lags behind the other "omics" at single cell level. The SEISMIC facility for spatially resolved single and sub-cellular "omics" is a new multi-user BBSRC facility that is advancing single cell and sub-cellular measurements, for the benefit of bioscientists across the UK.

At SESIMC, we use capillary sampling to sample live cells or sub-cellular compartments under microscope observation. This method of sample capture can be coupled to chromatographic separation, which offers additional advantages in mitigating matrix effects and aiding peak assignment.We will show how live single-cell lipidomics can be achieved using capillary-sampled cells as well as microfluidics. We will also show single cell lipid profiles of cancer cells respond to drug and radiation treatment.

BIOGRAPHY

Lorraine Brennan a full professor and a PI in the UCD Institute of Food & Health and Conway institute. She is the Vice Principal for Research, Innovation and Impact for the College of Health and Agricultural Sciences. She leads a research group at the forefront of the application of metabolomics in nutrition research and the development of Personalized nutrition. She is an ERC awardee and is currently involved in three European Consortiums- MUSAE, PlantIntake and Promed-cog.
She served as Director of the European Nutrigenomics Organization for 5 years and led a number of important initiatives such as the development of an Early Career Network and expansion of membership of the organization. She is a member of the National Academies of Science Engineering and Medicine Standing Committee on Evidence Synthesis and Communications in Diet and Chronic Disease Relationships – advising the US NIH and USDA on future research areas of priority. She was a member of the Food2030 Expert group to advise the European Commission with the development of FOOD2030 and exploring and formulating possible future R&I policy recommendations and actions and assessing their potential impacts.

LORRAINE BRENNAN

BIOMARKERS OF FOOD INTAKE: HOW CAN METABOLOMICS HELP

UNIVERSITY COLLEGE DUBLIN

PLENARY 4

THURSDAY 28TH NOVEMBER, 3:00 PM

ABSTRACT

Metabolomics applications in Nutrition Research can grouped into one of the following: (1) Identification of dietary biomarkers for single foods or for dietary patterns (2) Applications to dietary intervention studies to help understand metabolic alterations (3) Study of diet-related diseases and (4) Precision Nutrition. With respect to dietary biomarkers and exposure there has been a proliferation of publications in this field: these biomarkers have the potential to act as objective measures of dietary intake thus overcoming some of the key issues with traditional assessment methods. To date, metabolomic profiling has been successful in identifying several putative biomarkers of food exposure. Similarly, use of combination of biomarkers can be employed to study dietary patterns.

While significant progress has been made to date in the application of metabolomics in nutrition research a number of key challenges remain: addressing these will be key to development of future opportunities.

Poster Presentations

Metabomeeting 2024, University of Liverpool, 26-28th November 2024	
Tuesday 26th November at 18:00 - 21:00, and during lunch and breaks on Wednesday 27th and Thursday 28th November	
1	Global metabolomic profiling of tumor tissue and paired serum samples to identify biomarkers for response to neoadjuvant FOLFIRINOX treatment of human pancreatic cancer. Sander Johannes Thorbjørnsen Guttorm, Oslo University Hospital
2	Valorising waste streams by extracting high value components for the food industry. Cristina Saladas, University of Liverpool, Suntory
3	Collaborative research efforts: analysis of ergothioneine and oxylipin lipids. Nigel Gotts, University of Liverpool
4	MetAssimulo 2: a web app for simulating realistic 1D & 2D metabolomic 1H NMR spectra. Yan Yan, Imperial College London
5	Optimising synovial fluid preparation for gas chromatography-mass spectrometry metabolomics analysis for orthopaedic research. Yumna Ladha, Keele University
6	Metabolome associations with cardiovascular ageing. Shamin Tahasildar, MRC Laboratory of Medical Sciences
7	PCA to reveal non-adherence and non-analytical outliers using global metabolomics and lipidomics in clinical settings. Katja Benedikte Prestø Elgstøen, Oslo University Hospital
8	Probing metabolic behaviour of bacteria at surfaces. Daniel Smaje, University of Liverpool

Poster Presentations

Metabomeeting 2024, University of Liverpool, 26-28th November 2024	
Tuesday 26th November at 18:00 - 21:00, and during lunch and breaks on Wednesday 27th and Thursday 28th November	
9	A comprehensive and in-depth characterisation of model-organism metabolomes. Ralf Weber, University of Birmingham
10	A comprehensive computational framework for real-time monitoring and quality control of LC-MS-based metabolomics data acquisition. Ossama Edbali, University of Birmingham
11	Automated extraction of adherent cell lines from 24-well and 96-well plates for multi-omics analysis using the Hielscher UIP400MTP sonicator and Beckman Coulter i7 liquid handling workstation. Lauren Cruchley-Fuge, University of Birmingham
12	Using OrbiSIMS as a label-free approach to deciphering metabolic heterogeneity in glioblastoma. Aimee Humphreys, University of Nottingham
13	Discrimination of periodontal bacteria utilising IR spectroscopy techniques. Jawaher Albahri, University of Liverpool
14	Metabolomic ageing clock monitors risks of cardiometabolic diseases. Manyi Jia, Imperial College London
15	PrecisionTox: Cross-species, multi-omics analyses as a new approach to chemical hazard assessment. Martin Jones, University of Birmingham
16	Dietary impact of frog holometabolomes: identification of pumiliotoxin analogues through in-depth metabolomic investigation *Oophaga vicentei* dermal and gut extracts. Elliot Murphy, Imperial College London

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17	Assessing small polar metabolite detection using mass spectrometry imaging: a comparison of MALDI and LD-REIMS spatial technologies. Georgia Lorentzen, Imperial College London
18	Building informative retention time and MS/MS libraries for reliable metabolite identification in UHPLC-MS metabolomics studies. Hannadi Almari, University of Liverpool
19	Anion-exchange chromatography-mass spectrometry (AEC-MS) for untargeted plant metabolomics. John Sidda, University of Oxford
20	Applying Fourier-transform Infrared spectroscopy to characterise and differentiate Achromobacter and Pseudomonas Biofilms. Joel Doherty, University of Liverpool
21	The workflow for building high-quality spectral libraries using UHPLC-HRMS applied for human metabolomics studies. Romina Pacheco-Tapia, Université de Lille
22	Implementation of a semi-automated sample preparation workflow for comprehensive serum metabolomics. Ines Castro, Université de Lille
23	Aqueous humour metabolome analysis discloses altered arginine metabolism in Parkinson's disease. Joan Serrano, University of Barcelona
24	Unravelling Fungal-Plant Interactions through Untargeted Metabolomics and Mass Spectrometry Imaging Tools. Marina Ferreira Maximo, Institute of Chemistry, UNICAMP
25	MetaboLights - open access metabolomics resource. Callum Martin, EMBL-EBI

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Tuesday 26th November at 18:00 - 21:00, and during lunch and breaks on Wednesday 27th and Thursday 28th November	
26	Differentiation between patients with Multiple Sclerosis (MS) and Clinically Isolated Syndrome (CIS) based on UHPLC-QTOF MS untargeted serum lipid profiling. Olga Begou, Center for Interdisciplinary Research and Innovation (CIRI-AUTH)
27	Chemical attributes of UK-grown tea- Dartmoor Estate Tea. Alina Warren-Walker, Aberystwyth University
28	LC-MS-based untargeted metabolomics of Bambara groundnut for food security in response to drought – root to seed. Sandra Martinez-Jarquin, University of Nottingham
29	Influence of SiNPs in soybean plants: an NMR-based metabolomic approach. Amanda Lemos Quintela, Unicamp (State University of Campinas)
30	Liverpool Annotation of metabolites using Mass sPectrometry Software (LAMPS) - an adaptable software for metabolite annotation using full-scan mass spectrometry data. Wanchang Li, University of Liverpool
31	Simulated metabolic profiles unveil biases in pathway analysis methods. Juliette Cooke, INRAE Toxalim
32	Future possibilities for the SEISMIC facility and overview of current research and development projects. Jake Penny, University of Surrey
33	Annotation and Dereplication of Oxylipins in Human Urine Using LC-HRMS/MS and Molecular Networking: Insights from the Valencian Community. Ángel Sánchez-Illana, University of Valencia

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Metabomeeting 2024, University of Liverpool, 26-28th November 2024	
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34	No Time to Dye - Portable Surface Enhanced Raman Spectroscopy for On-site Detection of Sudan Dye in Palm Oil. Joe Stradling, University of Liverpool
35	Analytical techniques to study the formation and structure of homogentisic acid-derived pigment. Harriet Willett, University of Liverpool
36	Comparison of different reversed-phase liquid chromatography stationary phases for untargeted and semi-targeted metabolomics assays. Catherine Winder, University of Liverpool
36	Investigating the metabolomic profile of a novel bacterial isolate grown on terephthalic acid (TPA) and ethylene glycol (EG) for the sustainable production of valuable chemicals. Hiya Deshpande, University of Nottingham
38	Profiling the dynamic metabolome of colorectal cancer cells following irradiation. Aaron Kler, University of Liverpool
39	Development of a semi-targeted metabolomics assay for analysing mammalian urine and faeces by GC-MS. Shiva Jalili, University of Liverpool
40	Metabolomic analysis of primary head and neck squamous cell carcinoma (HNSCC) tissue reveals changes to one-carbon metabolism that associate with the disease phenotype. Andy Southam, University of Birmingham

Poster Presentations

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41	Metabolomic profiles to explore biomarkers of adverse body composition parameters in non-small cell lung cancer patients. Ziyi Zhong, University of Liverpool
42	Dog and cat dried blood spot samples stored at -80°C for 3 years: consistency in biological insights and approaches to sample bridging. David Allaway, Waltham Petcare Science Institute
43	Using Raman spectroscopy to monitor biological processes by simulated addition of glucose & lactate. Clark Gray, University of Liverpool
44	Developing analytical methods for the detection of thiols associated to axillary malodour. Amy Colleran, University of Liverpool
45	IN-U-Bar: Improving Nitrogen - Utilisation in Barley for Scotland's green recovery – environmentally friendly grain production for the brewing and distillery industries. Will Allwood, James Hutton Institute
46	Rapid microbore metabolic profiling of human liver diseases plasma samples using a multi-reflecting time-of-flight mass spectrometer. Ana Sanchez Lorenzo, Imperial College London
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Poster Presentations

Metabomeeting 2024, University of Liverpool, 26-28th November 2024	
Tuesday 26th November at 18:00 - 21:00, and during lunch and breaks on Wednesday 27th and Thursday 28th November	
48	Fast scanning MRM quantitative lipidomics analysis Rebekah Sayers, SCIEX
49	Quantitative analysis and structural characterization of bile acids using the ZenoTOF 7600 system Ozbalci Cagakan, SCIEX
50	Quantitation and structural characterization of lipid mediators by high-resolution mass spectrometry Tom Ruane, SCIEX

Oral abstracts

Tuesday 26th November

Data-Driven Framework for Metabolomic Epidemiology

David Broadhurst

Abstract:

Metabolomic Epidemiology has been defined as "the field of scientific enquiry involving the systematic use of epidemiological methods and principles to study population-based variation in the human metabolome as it associates with health-related outcomes or exposures". Recent advancements in analytical technology and computational power, together with the maturation of facilities dedicated to large-scale service provision, have made metabolomic epidemiological studies more accessible to health researchers. Accordingly, this field has seen recent exponential growth. The nature of epidemiology methodology often necessitates large-scale multi-centre sample collection and analysis. Such study designs are often led by health researchers with limited understanding of the metabolome's sensitivity to small variations in sample collection, biobanking, and transportation. Equally, project leaders may have limited understanding of the capricious nature of 'data-driven' (untargeted) metabolomic data acquisition resulting in potentially large bias and uncertainty in reported metabolite abundances. These factors, together with phenotypic characteristics that are known to significantly influence the human metabolome (e.g. age, sex, BMI) need to be investigated and mitigated before any actionable epidemiological interpretation of associations with a primary outcome or exposure. For untargeted metabolomics, this process is compounded by both the number of metabolites typically measured in a single study and the multivariate nature of metabolite interactions. Here I present a 'data-driven' framework for investigating sources of bias and variance in metabolomic epidemiology, referencing a recently completed multi-centre project, together with personal observations on the limitations of standard epidemiological models when applied to this kind of data.

Tuesday 26th November

The genetic architecture of the metabolome across several organs illuminates responses to nutritional and pharmacological challenges

Maxime Rotival, Jessica Le Ven, Vaclav Zidek, Josef Zicha, Julien Chilloux, Antonis Myridakis, Santosh Atanur, Norbert Hübner, Timothy Aitman, Jeremy K. Nicholson, Michal Pravenec, Enrico Petretto, Dominique Gauguier, **Marc-Emmanuel Dumas**.

Abstract:

Genetic variants disrupting metabolic homeostasis play an important role in the pathogenesis of complex disorders. However, how genetic variants alter metabolic traits in a tissue-specific manner remain elusive. Here, we apply a systems genetics strategy to untargeted metabolomics to dissect the tissue-specific genetic architecture of metabolomic traits. Using a rat recombinant inbred panel as a model of human metabolic syndrome, we profiled the metabolome in liver, peritoneal fat, heart, aorta and brain by untargeted ultra-performance liquid chromatography coupled to mass spectrometry generating >16,764 metabolomic features per tissue, among which 2,072 were replicated across all 5 tissues. Tissue metabolites are highly heritable (H2>27%), mostly polygenic. We identified 4,131 metabolomic features under genetic control (mQTLs), with >48% of these being tissue-specific and >200 mapping to the Abcb4 transporter locus acting a master regulator of liver and serum phospholipids. Metabolite-phenotype correlation networks predicted the response to high-fat diet and anti-hypertensive drugs, opening avenues in understanding the genetic basis of metabolic flexibility.

Tuesday 26th November

A gut microbiome-kidney-heart axis in human populations

Kanta Chechi, Sebastien Fromentin, Antonis Myridakis, Rima Chakaroun, Sofia K. Forslund, Trine Nielsen, Judith Aron-Wisneswky, Gwen Falony, Sara Vieira-Silva, Julien Chilloux Kazuhiro Sonomura, Solia Adriouch, Karen Assmann, Lesley Hoyles, Laura Martinez-Gili, Francesco Pallotti, Petros Andrikopoulos, Francesc Puig-Castellvi, Hugo Roume, Nicolas Pons, Emmanuelle Le Chatelier, Benoit Quinquis, Nathalie Galleron, Magali Berland, Michael Olanipekun, Manyi Jia, Angelos Manolias, Bridget Holmes, Matthias Blüher, Luis Pedro Coelho, Kévin Da Silva, Soraya Fellahi, Pilar Galan, Boyang Ji, Aurélie Lampure, Hamida Mohamdi, Patricia Margaritte-Jeannin, Ana Luisa Neves, Edi Prifti, Andrea Rodriguez-Martinez, Christine Rouault, Joe-Elie Salem, Valentina Tremaroli, Eric Verger, Tue H. Hansen, Nadja B. Søndertoft, Christian Lewinter,Helle K. Pedersen, The MetaCardis Consortium, Peter D Mark, Jens P Gøtze, Lars Køber, Henrik Vestergaard, Torben Hansen, Jean-Daniel Zucker, Taka-Aki Sato, Serge Hercberg, Fredrik Backhed, Ivica Letunic, Jean-Michel Oppert, Jens Nielsen, Jeroen Raes, Ioanna Tzoulaki, Abbas Dehghan, Verena Zuber, Emmanuelle Bouzigon, Mark Lathrop, Parminder Raina, Philippe Froguel, Fumihiko Mastuda, Florence Demenais, Dominique Gauguier, Michael Stumvoll, Peer Bork, Oluf Pedersen, Karine Clément, S. Dusko Ehrlich, Marc-Emmanuel Dumas

Abstract:

Gut microbiome is a key determinant of human physiology and pathophysiology. Recently, we segregated gut microbial markers of ischemic heart disease (IHD) from its metabolic pre-morbidities whilst noting that majority of these alterations occurred in association with dysmetabolism as opposed to IHD, highlighting a key role for the gut microbiome in early stages of disease development. Here, we observe gut microbial functional signatures for circulating pro-atrial natriuretic peptide (proANP) levels in the asymptomatic metabolically healthy (AMH, n =275) individuals of the deeply-phenotyped European MetaCardis study. Natriuretic peptides including proANP are key regulators of fluid and pressure homeostasis via their effects on kidney function (i.e., eGFR). Consistently, the metabolomics signature of proANP derived from univariate analyses, and of gut microbiome derived from multivariate models, which included key microbial metabolites of phenylalanine-tyrosine pathway also exhibited associations with eGFR in the AMH individuals. Replication of the AMH gut microbiome-metabolites-phenome associations in the MetaCardis metabolically unhealthy individuals (n = 1602) revealed a depletion pattern suggestive of loss of protective gut microbiome features with disease development. Additionally, key metabolites associated with higher incidence of future cardiovascular disease in two external populations i.e., EPIC-Norfolk (n = 11, 966) and Canadian Longitudinal Study of Aging (n = 8,669) in Cox regression analyses. Crucially, we identify causal roles for genetically predicted levels of phenylacetylcarnitine and cinnamoylglycine in lowering eGFR whereas genetically predicted eGFR causally associated with lower circulating levels of phenylacetylglutamine, 3-(4-hydroxyphenyl)lactate, 4-cresyl sulfate and vanillactate. In addition, genetically predicted levels of vanillactate associated with higher incidence of heart failure in Mendelian Randomization analyses. Our work highlights the dynamic interplay between gut microbiome and host physiology, in particular the kidney-heart axis, where even subtle shifts in host metabolism result in detectable key alterations of the microbiome, making it a key determinant of disease-initiation and disease-trajectory.

Tuesday 26th November

Untargeted and targeted metabolomics of cerebrospinal fluid for the discovery of biomarkers of children's brain tumour relapse

Alison Whitby, Laudina Amugi, Clare Eynon, Dong-Hyun Kim and Madhumita Dandapani

Abstract:

Ependymomas, malignant paediatric brain tumours, have poor outcomes due to a 50% relapse rate. Most relapses occur within two years, suggesting persistent minimal residual disease (MRD) below the imaging detection threshold. Diagnosing MRD at end of treatment or diagnosing early relapse using a sensitive, minimally-invasive liquid biopsy may improve outcomes. Extracting metabolites arising from aberrant cancer metabolism from cerebrospinal fluid (CSF) is promising because of its proximity to tumour.

Liquid chromatography with tandem mass spectrometry (LC-MS/MS) of 25µl CSF extracted with 75µl methanol was used for untargeted metabolomics of ependymoma (n=33; 14 days post-surgery) and control (n=49; end of treatment leukaemia) CSF. Labelled standards were added to methanol for subsequent targeted metabolomics of 13 metabolites with high linearity ($R2>0.97$), precision ($CV\leq11\%$) and selectivity ($Rs>1.1$). Secondary ion MS (OrbiSIMS) surface analysis metabolomics of matched ependymoma formalin fixed tissue samples was used to infer the origin of the statistically significant CSF metabolites.

Eighteen metabolites were differentially abundant between ependymomas and controls, with 14 metabolites being increased in abundance in ependymomas. These included amino acids and derivatives (n=8), metabolites in one carbon metabolism (n=1), carnitine cycle (n=2), aerobic glycolysis (n=2), purine catabolism (n=2), ascorbate degradation (n=1) and creatinine synthesis (n=2). From those, betaine/creatinine ratio gave an area under the curve (AUC) of 0.95 (excellent) in receiver operating curve analysis; a cut-off ratio of 0.1769 had sensitivity and specificity of 90% and 88%. Targeted metabolomics confirmed the significance of these biomarkers. Six of the metabolites were detected in ependymoma tissue, namely creatinine, creatine, hypoxanthine, pyruvate, N-acetylhistidine and proline.

This is the first CSF metabolomics study in ependymoma. Ratios of CSF metabolites can be used as biomarkers to detect brain tumour residue post-surgery sensitively and specifically. This will be developed into a liquid biopsy assay for MRD and early relapse.

Tuesday 26th November

LC-MS profiling of the urine metabolome in adverse early pregnancy outcomes

Christopher J. Hill, Andrew S. Davison, Brendan Norman, Marie M. Phelan, Nicola Tempest & Dharani K. Hapangama

Abstract:

Background: Approximately 15% of all pregnancies are lost in the first trimester and a further 2% are ectopic (EP), the latter of which is the leading cause of maternal mortality in early gestation. Symptoms of pregnancy loss and EP are often indistinguishable but are also common in live normally sited pregnancies (LNSP). To date, there are no definitive biomarkers to differentiate LNSP from pregnancies that will not progress (non-viable or EP), defined together as combined adverse outcomes (CAO). Here, we investigate the urine metabolome to identify novel biomarkers of pregnancy outcome.

Methods: This study included 354 pregnant participants with abdominal pain and/or vaginal bleeding at ≤10 weeks of gestation. A urine sample was collected prior to final diagnosis of pregnancy outcome; LNSP, (n=160), miscarriage (n=93), pregnancy of unknown location (PUL, n=47), or tubal EP (tEP, n=54). Urine was analysed by LC-QTOF-MS, and metabolite annotation was performed using MassHunter and an in-house library of analytical standards. Relative metabolite abundances underwent statistical analysis (with FDR adjustment) using MetaboAnalyst and custom-built R scripts.

Results: Six metabolite abundances were found to differ significantly between the four pregnancy outcomes. Taurine was significantly higher in the LNSP group compared to miscarriage, PUL and tEP. When miscarriage, PUL and tEP were combined into a CAO group and compared to LNSP, eight metabolite abundances (adenosine, arginine, deoxyguanosine, histidine, hydroxylysine, hypoxanthine, L-2-aminoadipic acid, and taurine) were significantly different. Taurine demonstrated modest predictive accuracy (AUC=0.70, CI=0.64-0.75) for differentiating LNSP from CAO.

Conclusions: We have identified changes in the urine metabolome related to pregnancy outcome. Taurine levels are known to increase during pregnancy, but its utility as a biomarker of pregnancy outcome has not been explored previously. A diagnostic test to confirm LNSP and thus rule out potentially life-threatening outcomes would be invaluable in clinical emergencies.

Tuesday 26th November

Oral supplement in older adults to support physical fitness and mental well-being.

Alison Watson, Robert Nash, Pilar Martinez Martin, Alina Warren-Walker, Courtney Davies, Thomas Wilson, John Draper, Manfred Beckmann, Amanda J Lloyd.

Abstract:

Cucumbers have long been anecdotally credited with anti-inflammatory properties, though the specific active ingredient was not identified until recently. idoBR1, an iminosugar amino acid isolated from the fruits of certain cucumbers (Cucumis sativus, Cucurbitaceae), has been demonstrated to possess anti-inflammatory activity. IminoTech Inc., based in the USA, has developed a quality-controlled cucumber extract containing measured idoBR1 (Q-actin™), which has shown promising results in the treatment of osteoarthritis through oral administration.

In our study, we recruited a cohort of 49 middle-aged and older adults (ages 50-78; 16 men and 33 women) who were randomised to receive either Q-actin (two 50 mg gummies daily) or a placebo (two 50 mg gummies daily) for 12 weeks. Physical strength and finger dexterity were assessed using hand grip strength and the Nine-Hole Peg Test (9HPT), respectively. We also measured generic quality of life with the EQ-5D questionnaire, evaluated diet choices (Prime Diet Quality Score (PDQS)) and sleep quality (Pittsburgh Sleep Quality Index), and collected first-morning urine samples for chemical composition analysis using Flow Infusion Electrospray Ionisation Mass Spectrometry (FIE-MS).

The FIE-MS analysis was conducted with an Exploris 120 mass analyser coupled with a Dionex Vanquish UHPLC system (Thermo-Scientific), measuring ion intensities within the m/z range of 55 to 1200. The top-ranked features contributing to multi-dimensional scaling models were identified using re-sampling methods and p-values for False Positive Rates (FPR $\leq$ 0.05). Features that showed good classification performance in pairwise comparisons were further investigated. All p-values were corrected for multiple testing using the Bonferroni correction.

Tentatively identified melatonin derivatives were found to be elevated after Q-actin supplementation, suggesting a positive effect on sleep quality, which correlated with self-reported Pittsburgh Sleep Quality Index. The relative m/z-transition areas of melatonin and its two main biotransformation products in selected urine samples after Q-actin supplementation were examined following LC-MS/MS fragmentation on the Exploris-120, revealing interesting trends.

Tuesday 26th November

Metabolic Profiling of Antarctic Snow Algae

Matthew Davey, Scottish Association for Marine Science

Abstract:

Snow algae are found in snowfields across cold regions of the planet, forming highly visible red and green patches below and on the snow surface. In Antarctica, they contribute significantly to terrestrial net primary productivity due to the paucity of land plants, but our knowledge of these communities is limited. Here we provide data on the metabolic diversity of these snow algae communities from southern and northern locations along the Antarctic Peninsula. We use a mixture of metabolic fingerprinting using FT-IR and targeted analysis using GC-MS (MSTFA and FAME) and HPLC (Pigments). The temporal and spatial dynamics of the community metabolome will be presented, showing how the metabolome varies from samples collected in the field from 2014 to the present day at Robert Island. We will also present information on the photosynthetic and respiration rates these snow algae communities produce over a season (NCER net carbon exchange rate, ER ecosystem respiration, GEP gross ecosystem photosynthesis) using gas exchange IRGA techniques, and how these values compare to rates in other nearby moss and grass species. We will discuss logistics of sampling and data collection in remote regions for metabolomic studies and our morphological analysis of new isolates. These data show the complexity and variation within snow algae communities in Antarctica and provide valuable insights into the contribution they make to ecosystem functioning https://snowalgae.org/.

Tuesday 26th November

Something smells….and it's antibiotic resistance

Breanna Dixon, Drupad Trivedi, Waqar Ahmed, Stephen Fowler, Tim Felton

Abstract:

The emergence of antibiotic resistance is a growing public health challenge. One subset of particular concern are carbapenemase-producing Enterobacteriaceae (CPE). Conventional clinical methods for detecting CPE typically involve culture-based techniques with lengthy incubation steps, and the sensitivity afforded by these approaches has been debated. There is a need to develop rapid and accurate methods for the detection of resistance, for implementation into clinical diagnostics. With cellular phenotype closely linked to the metabolome, we hypothesised that the acquisition of resistance would result in fundamental differences in microbial metabolism. Recent studies have demonstrated differences in volatile organic compound profiles of susceptible and resistant bacteria. Accordingly, in this study we sought to characterise the volatile metabolome of Enterobacteriaceae isolates belonging to both CPE and non-CPE groups. Headspace volatiles were collected during 6h of incubation and were analysed using thermal desorption-GC-MS. Furthermore, we assessed changes in the volatile profile of isolates after subjection to increasing doses of antibiotic.

We performed robust feature selection and found that CPE isolates exhibit an altered volatilome compared to their susceptible counterparts both with and without antibiotic stress. This suggests that CPE may be distinguished from non-CPE in under 7h using volatile biomarkers. In addition, certain compounds were highly correlated with the degree of resistance, demonstrating potential as a means of susceptibility testing. These results show applicability for the detection of resistant pathogens using volatile organic compound analysis and reveal metabolites which can be probed for mechanistic insight into the resistant phenotype and the elucidation of therapeutic drug targets.

Tuesday 26th November

From toxic waste to beneficial resource: acetate boosts *E. coli* growth at low glycolytic flux

Thomas Gosselin-Monplaisir, Sandrine Uttenweiler-Joseph, Brice Enjalbert, Pierre Millard

Abstract:

Acetate is a major by-product of glycolytic metabolism in *Escherichia coli* and many other microorganisms. Traditionally considered a toxic waste product that inhibits growth and reduces the productivity of bioprocesses, acetate poses significant challenges in biotechnology. However, recent studies have revealed a more complex role for acetate: it can serve as a co-substrate with glycolytic nutrients and is a global regulator of *E. coli* metabolism and physiology. In addition, most studies on acetate metabolism in *E. coli* have been performed under conditions of high glycolytic activity, which differ from those encountered by *E. coli* in its environmental niches. This raises the question of what is happening when glycolysis is modulated. Here, we used a systems biology approach combining kinetic modeling, 1H-NMR and extracellular flux measurements, to explore the functional interplay between glycolytic and acetate metabolism in *E. coli*. Experimental and computational results show that reducing glycolytic flux enhances the co-utilization of acetate and glucose. This shift allows acetate to compensate for reduced glycolytic flux and stabilize carbon uptake. In this condition, acetate can even boost *E. coli* growth, thereby ruling out its perceived toxicity. To confirm this mechanism, we employed three strategies: chemical inhibition of glucose uptake, mutant strains with impaired glycolytic pathways, and alternative substrates with inherently low glycolytic flux. Our findings demonstrate that acetate enhances *E. coli's* robustness to glycolytic perturbations and acts as a nutrient that can promote microbial growth. By uncovering acetate's dual role as both a metabolic regulator and a nutrient, we create opportunities for enhancing microbial growth and robustness in biotechnology.

Tuesday 26th November

Maternal *Toxoplasma gondii* infection induces metabolic changes to the maternal foetal interface

Hafiz M. Arshad, Craig W. Roberts, Gareth Westrop, Margarida Borges

Abstract:

T. gondii infection during pregnancy can cause abortion or congenital disease. Events in the maternal-foetal interface, where immunological changes occur, are critical in determining the pregnancy outcome. Several studies cover the serum biochemical/metabolic changes following *T. gondii* infection, but limited information exists concerning changes to the placental metabolome or the foetus while in utero. For the first time, this study covers the metabolomic profile and potential underlying mechanisms in the maternal-foetal interface, the developing foetus and maternal serum in BALB/c mice in a *T. gondii* congenital infection model. Results demonstrate the highest number of metabolite changes in the maternal serum, however a subset of these changes to tryptophan degradation pathway, arginine metabolic pathway was also found in the maternal-foetal interface and the developing foetus. In addition, some metabolites from microbiome origin including indoxylsulfate and 4-guanidinobutanoate were changed compared with the controls, suggesting the potential of T. gondii to change the host microbiome. However, preliminary metagenomics analysis did not demonstrate such changes, albeit in a different model of *T. gondii* infection. Comparison of alterations of metabolites between the developing foetus and the brain from adult mice born to infected mothers was carried out to determine whether the changes observed in early foetal life were still evident in later life. The most significant finding of this study is that increased kynurenine levels are found in early foetal life. This metabolite was found to be increased in the brains of adult mice with congenital *T. gondii* infection, but not in uninfected litter mates exposed to maternal-immune activation. This suggests that raised kynurenine levels in foetuses in utero might be maternally derived and short lived, but ultimately endogenously produced in congenitally infected mice. This metabolite has been implicated in psychoneurological diseases, but the consequences of kynurenine exposure in these circumstances remain to be determined.

Tuesday 26th November

Application of Infrared spectroscopy for rapid identification of antimicrobial resistance and study of carbon- deuterium kinetics at the single-cell level

Sahand Shams, Cassio Lima, Yun Xu, Shwan Ahmed, Royston Goodacre, Howbeer Muhamadali

Abstract:

The rise and spread of antimicrobial resistance (AMR) is a critical global concern, threatening both the environment and human health. Traditional AMR identification methods in clinical settings are time-consuming and costly, leading to the initial use of broad-spectrum antibiotics. There is a pressing need for culture-independent and single-cell technologies to enable rapid detection of antimicrobial-resistant bacteria, supporting more targeted and effective antibiotic therapies to prevent further development and spread of AMR. In this study, a non-destructive phenotyping method using optical photothermal infrared (O-PTIR) spectroscopy, combined with deuterium isotope probing (DIP) and multivariate statistical analysis, to detect AMR in Uropathogenic *Escherichia coli* (UPEC) at both single-cell and population levels. Moreover, various combinations of heavy stable isotopes (D, ^{13}C, ^{15}N, and ^{18}O) were employed to evaluate isotopic spectral shifts in the mid-IR region using FT-IR and O-PTIR spectroscopy at both bulk community and single-cell levels. A time-course study explored the kinetics of C-D vibration in *E. coli*, facilitating time-based sampling and assessment of isotopic labelling kinetics. Principal component discriminant function analysis (PC-DFA) of FT-IR and O-PTIR spectral data revealed clear clustering patterns due to C–D signature peaks from deuterium incorporation into bacterial cells, allowing rapid detection and classification of sensitive and resistant isolates at the single-cell level. Additionally, deuterium incorporation into amide groups formed nitrogen-deuterium bonds, shifting amides A and B into the silent region, overlapping with C-D signature peaks. The spectra, second derivative, and PC-DFA scores plot of FT-IR data collectively demonstrated the practicality of monitoring ^{13}C and D incorporation into *E. coli* cells within the first 30 minutes of incubation. The results indicate that O-PTIR is an efficient tool for rapidly detecting AMR at the single-cell level. Furthermore, FT-IR and O-PTIR spectroscopy can effectively monitor the incorporation of heavy isotopes into bacteria at both population and single-cell levels.

Wednesday 27th November

Network science and knowledge mining to make sense of metabolomics data

Fabien Jourdan, INRAE-MetaboHUB, Toulouse, France.

Abstract:

Metabolic modulation is a cornerstone cellular response to genetic or environmental stresses. This plasticity is going beyond central metabolism and may involve complex processes spanning several metabolic pathways. Hence, it is a key challenge to be able to decipher metabolic modulations in a systemic and global perspective.

The aim of the computational methods and tools which will be presented is thus to consider the full complexity of metabolism. To do so, all metabolic reactions the cell is able to achieve are gathered in a single mathematical model call "genome scale metabolic network". Based on this model it is then possible to identify metabolic specificities of different cell lines, predict metabolic behaviours, simulate metabolic responses to single or multiple knock-out and ultimately identify potential drug targets.

Finally, even if metabolic profiles and associated metabolic network modulations are very informative, their biological and physiological interpretation remain a challenge, requiring researchers to gather and connect various pieces of knowledge from a large range of resources. To aid in this task, we introduce FORUM: a Knowledge Graph (KG) providing a semantic representation of relations between chemicals and biomedical concepts, built from a large-scale federation of life science databases and scientific literature repositories. These associations allow to derivate new hypothesis from observed metabolites or anticipate which metabolites could be expected to be measured for a given disease.

Wednesday 27th November

Can attention-based deep learning models help predict molecular structure from MS fragmentation spectra?

Ivayla Roberts, Roy Goodacre, Warwick Dunn, Douglas Kell

Abstract:

Introduction: Many metabolites of interest when observed as features in untargeted liquid chromatography-mass spectrometry studies are of unknown structure. This is due to the fact that only a small percentage of the detected compounds have a reference fragmentation spectrum in public or commercial libraries. Although fragmentation spectra offer the most accurate identification option as they directly reflect the molecule's structural properties including bond strengths, large-scale chemical purchasing is expensive, and MS/MS mass spectra can differ between instruments. De novo approaches to bypass these limitations are therefore required. To facilitate compound identification, numerous computational tools have been developed (e.g. GNPS); however, structural annotation continues to present significant challenges. Consequently, unidentified compounds of interest are often disregarded, and important findings remain unpublished. The work presented here aims to develop a novel machine learning-based tool to support unknown compound annotation by leveraging the information available from sample-acquired fragmentation spectra.

Approach: To this end, a deep learning architecture, specifically a transformer model, was employed to predict fragmentation spectra from a structure. While such models rely on large datasets to extract patterns, reference fragmentation spectra are rare. To circumvent this challenge, we used the transfer learning capabilities of those models, i.e., the model was first trained on a large synthetic dataset of 13 million compounds and subsequently fine-tuned on experimental spectra.

Results and discussion: Preliminary results demonstrated good performance in matching synthetic data with matching experimental spectra, indicating the capacity of this approach to learn the complex relationship between precursor structure and resulting fragments with small volumes of data. In conclusion, we propose a novel deep learning approach to assist in unknown compound annotation and thereby take a step forward in addressing a key limitation of untargeted metabolomics studies.

Wednesday 27th November

SpectruMS: A cost-effective MS/MS foundation model learning the language of mass spectrometry

Daniel Crusius, Tornike Onoprishvili, Jui-Hung Yuan, Vijay Ingalalli, Lila Khederlarian, Aymane Hachcham, Kamen Petrov, Niklas Leuchtenmuller, Sona Chandra, Aurelien Duarte, Andreas Bender, Yoann Gloaguen

Abstract:

Tandem Mass spectrometry (MS) is a key technique for the structural elucidation of molecules from complex biological samples. Despite its importance, interpreting MS2 spectra remains a challenge. Traditional methods rely on reference libraries, which only cover a limited chemical space, leaving many molecular structures unverified when spectra do not match the references. Recent advancements have seen the development of both specialized and foundational deep learning models aimed at enhancing MS2 data interpretation and structure elucidation. However, it is unclear which models perform best and how different model architectures, data quality, dataset size, and spectral representations affect their performance.

Here, we introduce a series of large transformer models for MS2 data analysis designed to aid mass spectra interpretation without extensive labeled datasets. Our models are transformer-based neural networks trained in a semi-supervised manner on > 200 million unlabeled MS2 spectra. We explored various MS2 spectra representations and different feature engineering techniques, such as different spectra tokenization strategies and Fourier features commonly used in computer vision applications, to find the most effective ways to represent MS2 spectra for ML modeling. We fine-tuned our foundation models to predict different endpoints, ranging from simplified tasks such as classification of functional groups to full structure elucidation.

We benchmarked our models to evaluate their accuracy and compared performance to specialized end-to-end trained models. This assessment reveals areas needing improvement, particularly we discuss how feature representations, data quality, and pre-training impact prediction performance. Additionally, we investigate the effects of different pre-training and fine-tuning dataset sizes, which is important for minimizing the computational footprint and training cost of foundational models. Our work aims to help practitioners in the field understand the limitations of dataset size, data quality, and identify the best way to represent MS2 spectra for ML modeling.

Wednesday 27th November

IsoDesign: streamlining experimental design for ^{13}C- metabolic flux analysis

Loïc Le Grégam, Rochelle Kouakou, Jean-Charles Portais, Fabien Jourdan, Floriant Bellvert, Serguei Sokol, Pierre Millard

Abstract:

In systems biology, studying the functioning of the intricate network of biochemical reactions governing cellular metabolism is crucial for elucidating the behaviour of living organisms. Metabolic Flux Analysis (MFA) addresses this need by quantifying fluxes though metabolic pathways, enabling researchers to investigate regulatory mechanisms, identify metabolic bottlenecks, and optimize cellular processes for various applications, ranging from biotechnology to healthcare.

^{13}C-MFA enables the quantification of the rates of biochemical reactions within metabolic networks and uses constraint-based mathematical modelling to calculate metabolic fluxes. In ^{13}C-MFA, the studied microorganism is cultivated in media containing ^{13}C-labelled substrates in isotope labelling experiments (ILE). Mass spectrometry and nuclear magnetic resonance techniques are then employed to measure incorporation of tracer atoms into metabolites within the studied metabolic network. This experimental data is used to calculate fluxes. The precision of these calculated fluxes depends on the quality and on the nature of experimental data and the selection of the most optimal labelled substrates to resolve fluxes across different network topologies. However, choosing the right ^{13}C-labeled forms of the substrates is a time-consuming and labour-intensive task, as multiple configurations must be tested, and the large solution space makes analysing the different combinations challenging.

To accelerate the design of optimal ^{13}C tracers, we present IsoDesign, an open-source python software that streamlines ^{13}C tracer design for ^{13}C-MFA. With its intuitive graphical user interface and by leveraging the influx_si software for flux calculation, IsoDesign provides the necessary tools for efficient design of ILEs, enhancing the precision and reducing the cost of ^{13}C-MFA experiments. We showcase the tool using a study of acetate and glucose metabolism regulation in *E. coli*, showing that IsoDesign significantly facilitates the design of ^{13}C-MFA studies.

Wednesday 27th November

Network-based meta-analysis of metabolomics studies from large-scale public data repositories

Cecilia Wieder, Thomas Payne, Ozgur Yurekten, Callum Martin, Felix Xavier Amaladoss, Noemi Tejera Hernandez, Wanchang Lin, Pieter Dorrestein, Claire O'Donovan, Warwick Dunn, Timothy Ebbels

Abstract:

Large-scale public metabolomics data repositories such as MetaboLights contain terabytes of raw data as well as associated metadata and will continue to grow larger as data deposition becomes essential for publication. A critical issue in metabolomics is compound annotation, and repositories have introduced both manual and automated approaches for harmonising annotations across studies. In the first study of its kind, we exploit harmonised metabolite annotations across hundreds of studies to perform integrative meta-analysis and visualisation, providing unified insights such as multi-study metabolite signatures and global observed metabolite set comparisons. We demonstrate the importance of harmonising metabolite annotations to maximise the impact, reusability, and reproducibility of metabolomics datasets.

A network-based approach was developed to integrate metabolomics data at both the study-level and the metabolite/pathway-level. The metabolite-level meta network is designed to integrate multiple studies with similar context (e.g. same phenotype) and is constructed using differential metabolite co-occurrences represented in bipartite graph form to identify multi-study metabolite signatures. The study-level network demonstrates similarity in assay coverage as well as additional metadata across hundreds of studies, providing an intuitive visual overview of the repository composition.

As an example, the metabolite meta-network approach was applied to six COVID-19 blood plasma studies in MetaboLights. The co-occurrence network showed 10 differential metabolites in at least 3/6 studies, for example pyroglutamic acid was consistently up-regulated in COVID groups in 3 studies, consistent with COVID-19 literature. Such integration can reveal metabolite signatures that are robust across multiple studies and cohorts. Networks can also be explored at the pathway level, unifying metabolite-level signals to provide an overview of active processes across multiple studies.

Harmonising metabolite annotations in public repositories enables large-scale meta analysis, unifying insights across studies and driving the field forward through the re-use of public data, providing new opportunities for the wider community.

Wednesday 27th November

Class level annotation of untargeted MS1 metabolomics data using Gaussian graphical models

Tom Rix, Caroline Sands, Maria Gomez Romero, Elena Chekmeneva, Elizabeth Want, Timothy Ebbels

Abstract:

Annotating metabolites confidently in untargeted liquid chromatography-mass spectrometry is challenging and time-consuming. As a result, efforts are typically focused on a fraction of the detected features. Recent advancements in systematic prediction of chemical class from tandem mass spectrometry (MS/MS) can complement dataset interpretation, providing structural insights for numerous features. However, there is a need for class prediction methods independent of MS/MS acquisition, which is challenging to acquire for low-abundance ions and leaves a substantial portion of features only characterised at the MS1 level. Here, we present a computational workflow to predict chemical class without the need for prior-annotations and without a reliance on acquired MS/MS. Taking as input a pre-processed LC-MS feature table and a structural database, such as HMDB or LipidMaps, our method leverages a guilt-by-association approach using Gaussian graphical models to infer the chemical class for all features in the dataset. For each prediction, our algorithm outputs a confidence score based on a retention time anomaly model fitted to the dataset. Finally, to better understand which classes are differentially regulated between experimental conditions and to facilitate biomarker prioritisation, we implement an over-representation analysis using the predicted classes. To evaluate our method, class predictions were compared against known annotations for two lipid assays from a large Alzheimer's disease study. Our model achieved good prediction accuracy, ranging from 75-95% at the LipidMaps main class level and 65-80% at the subclass level. Additionally, over-representation analysis using predicted class for the set of univariately significant features produced results consistent with published literature. This included an increase in plasma cholesterol and derivatives and a decrease in glycerophosphocholines and sphingomyelins. In summary, the proposed approach shows promise to enable class-level analyses of metabolomic MS1 data, providing biological insights across the entire dataset rather than the small fraction of features typically amenable to MS2 analysis.

Wednesday 27th November

Development of mass spectrometry-based methods to identify novel antigens presented by the unconventional MHC- like protein, MR1

Edward J Mathias, Robert J Simmons, Richard J Suckling, Emma Grant, Mariolina Salio, Gurdyal S Besra, Andrew Creese, Warwick B Dunn

Abstract:

Introduction

MR1 is an MHC-like protein that presents bacterially derived and endogenous metabolite antigens at the cell surface. To date, only a handful of ligands which can bind MR1 have been characterised, mostly intermediates of the vitamin B2 pathway, and while in vitro studies have suggested MR1 can present tumour-specific antigens, they are yet to be identified. Characterising the full ligandome of MR1 can therefore provide a source of new therapeutic targets against cancer and microbial related diseases. As MR1 presents metabolite antigens, a metabolomics-based workflow is most appropriate for the identification of novel ligands.

Methods

Here, we report the first application of both intact and native mass spectrometry techniques to identify antigens presented by MR1. We expressed in mammalian cells WT MR1 molecules, MR1 K43A molecules (that lack the ability to form Schiff base bonds with ligands in the binding groove) and the allomorph MR1 R9H (which does not bind vitamin B2 derivatives) to compare the three ligandomes. As MR1 molecules are glycosylated, to overcome glycan-induced mass shifts, we also employed two deglycosylation methods: mutation-based and enzymatic.

Results and discussion

Analysis of the MR1 metabolome by intact and native mass spectrometry techniques revealed the complementarity of both approaches and feasibility for their application in novel ligand discovery. Here we show ligand-associated mass shifts common across all isoforms suggesting a shared presented antigen. Furthermore, these mass shifts do not correspond to the masses of the known ligands of MR1, implying the detection of a novel ligand. Spectra of the deglycosylated protein further support these findings and demonstrate the success of the two methods employed to remove sugar-induced mass heterogeneity.

The results support the continued use of intact and native mass spectrometry analyses in a metabolomics workflow for MR1 and other MHC-like proteins for antigen discovery and clinical target identification.

Wednesday 27th November

Developing a panel of urinary dietary biomarkers for the intake of foods commonly consumed in the UK

Juliet Vickar, Thomas Wilson, Laura Lyons, Amanda J. Lloyd, Manfred Beckmann, John Draper

Abstract:

Measurement of dietary biomarkers have proven to be more reliable in compensating for the biases associated with traditional methods of dietary assessment. Targeted analysis of urinary biomarkers is an accurate and robust method for quantifying and validating dietary exposure within a population. Multiplexed assays utilising Liquid Chromatography Triple Quadrupole Mass Spectrometry (LC-MS/MS) can provide quantitative levels of dietary biomarkers which enables objective assessment of dietary status at a population scale.

Analysis of commonly consumed foods form the National Diet and Nutrition Survey (NDNS) and recent literature, identified food related metabolites that were candidate biomarkers of UK dietary intake. Following parameter optimisation, using triple quadrupole mass spectrometry, a consensus set of parameters were created. The method validation process involved the reproducibility of the mixture of standard compounds in a calibration standard and master mix of pooled urine samples serving as the quality control (QC) sample. Matrix effect was measured by comparing the analyte response of the standards dissolved in solvent and QC sample at the same concentration level across various experiments

Over sixty compounds were considered based on standard availability and their association with foods mostly consumed in the UK and their optimisation values. These included tea and coffee for beverages, fruits, and vegetables, cereal products, baked or toasted products, egg and diary, and meat and processed meats. The distribution of these metabolites was among several chemical classes including benzene and substituted derivatives, carboxylic acid and its derivatives, flavonoids, phenols, organo-oxygen compounds, organic sulfuric acids, and derivatives and isoflavonoids.

A successful dietary biomarker panel is established following a conventional UK eating pattern, which is appropriate for biomarker validation in free-living individuals.

Wednesday 27th November

The role of metabolomics in screening for malnutrition and frailty risk in UK older adults

Thomas Wilson, Nia Humphry, Laura Lyons, Pilar Martinez Martin, Amanda Lloyd, Manfred Beckmann, Jonathan Hewitt, John Draper

Abstract:

10-15 % of community dwelling older adults are at risk of malnutrition, and approximately 40 % of adults aged over 65, admitted to hospital, have poor appetite. Additionally, pre-operative frailty is a key determinant of post-surgical outcomes and often co-exists with both sarcopenia and malnutrition. Metabolic phenotyping using community collected urine samples, can provide insights into both recent dietary intake & nutritional status as well as mechanistic insights of overall metabolic phenotype, pertaining to nutrient utilisation. Urine samples and frailty screening data was collected from three separate cohorts of UK adults (n = 430) aged over 65, including healthy individuals, elective surgery patients, and individuals identified with an increased risk of malnutrition through primary care.

Non-targeted metabolomics using Liquid Chromatography High Resolution Mass Spectrometry (LC-HRMS) was able to predict malnutrition risk scores, with sufficient accuracy (0.73, $P < 0.01$). Multivariate models for functional capacity of frailty, were strongly predictive with classification accuracies > 0.80 ($P < 0.001$). Metabolites related to amino acid metabolism, were strongly correlated ($r > 0.65$, $P < 0.001$) with measures for muscle strength. Across all cohorts, metabolites indicative of dietary protein consumption (L-anserine, 3-methyl-histidine, taurine) were associated with positive frailty and nutritional outcomes. para-Cresol glucoronide and phenylacetyl-N-glutamine, were weakly associated with lean muscle mass, in both healthy and sarcopenic individuals.

The aetiology of frailty and malnutrition is multifaceted and complex, but a combination of home collected urine samples coupled with high resolution mass spectrometry assays and advanced data analysis can be used to acquire clinically relevant information at high throughput which can aid in the development of routine screening, for preventative care.

Wednesday 27th November

Development of large-scale LC-MS-based metabolomics to investigate dairy cow lameness

Ana S. Cardoso, Martin J. Green, Dong-Hyun Kim and Laura V. Randall

Abstract:

Lameness in dairy cows is a complex, painful condition affecting welfare and productivity. Previous research identified lameness predictors using metabolomics and machine learning (ML) in urine and milk; however, the generalisability of the findings needed to be validated with a larger and more diverse sample. This study employed untargeted liquid chromatography-mass spectrometry (LC-MS) to explore metabolic and lipid profile differences in urine (n=424) and milk (n=425) samples, respectively, between lame and non-lame cows across herds from 10 commercial dairy farms.

The importance of validated and standardised analytical methods for reproducibility and robust data generation in LC-MS experiments was central to this study. Key considerations included sample collection, transport, and storage; minimising instrumental drifts and system instability; addressing run-order trends in large-scale studies; randomisation and use of quality control samples; and protocol harmonisation.

LC-MS analysis was conducted in 4 batches due to the significant number of samples in metabolomics and lipidomics experiments. Batch and all-batch quality control (QC) samples were used to assess data quality. Statistical analysis included multivariate analysis, ML, and stability selection to identify predictors.

Data pre-processing was performed using a tailored untargeted workflow, in which batch-to-batch variation was minimised using Systematic Error Removal using Random Forest.

Results showed good instrumental performance of the LC-MS analysis for untargeted metabolomics and lipidomics, and successful batch-to-batch correction based on the well-designed QC injections. ML mean accuracies varied between farms and were generally slightly higher for the metabolomics results. The stability selection method identified three ions predictive of lameness in urine (one of which phenylsulphate) and 2 in milk (one of which sphingolipid). Further work needs to be done to annotate the other significant ions.

The approach and workflow were found to be powerful for large-scale LC-MS-based untargeted metabolomics and lipidomics, ultimately validating a novel approach to developing lameness detection methods.

Wednesday 27th November

The SEISMIC facility for spatially resolved single and sub-cellular omics

Melanie Bailey, Jake Penny, Emily Fraser, Harpreet Atwal, Kyle Saunders, Abi Cook, Dany Beste, Nicholas Locker, Johanna von Gerichten, Anastasia Kontiza

Abstract:

Single-cell analysis is a new and rapidly growing field that is shedding new insight into the cellular mechanisms behind the pathogenesis and treatment of infectious diseases and cancer. Cells, even within a single tissue, culture or biological fluid are highly heterogenous. Analysis of bulk material therefore only provides an average value, meaning that information is lost. Single cell transcriptomics and proteomics are already making major advances to understand infection, immunity and drug delivery. Single cell lipidomics is highly desirable to understand the endpoint of metabolism, but currently lags behind the other "omics" at single cell level. The SEISMIC facility for spatially resolved single and sub-cellular "omics" is a new multi-user BBSRC facility that is advancing single cell and sub-cellular measurements, for the benefit of bioscientists across the UK.

At SESIMC, we use capillary sampling to sample live cells or sub-cellular compartments under microscope observation. This method of sample capture can be coupled to chromatographic separation, which offers additional advantages in mitigating matrix effects and aiding peak assignment. We will show how live single-cell lipidomics can be achieved using capillary-sampled cells as well as microfluidics. We will also show single cell lipid profiles of cancer cells respond to drug and radiation treatment.

Wednesday 27th November

Targeting brain cancer with precision: Insights from single-cell-based metabolite profiling

Une Kontrimaite, Rui Chen, Sandra Martinez, Ruman Rahman, **Dong-Hyun Kim**

Abstract:

Understanding the metabolic interactions between astrocytes and glioblastoma cells, as well as between invasive and non-invasive cells at the invasive margin of the tumour is crucial for identifying biomarkers of brain tumour recurrence and developing new therapies. This study employed atmospheric pressure-matrix assisted laser desorption/ionisation-mass spectrometry (AP-MALDI-MS) and Orbitrap secondary ion MS (OrbiSIMS), which combine high spatial resolution (1 µm – 10 µm) with high mass resolving power (>140,000 at m/z 200). These imaging techniques offer unique advantages for untargeted metabolite profiling, requiring only a minimal number of cells and reduced sample processing, thus addressing common experimental challenges. The single-cell-based metabolic profiling methodologies developed show promise in enhancing our understanding of cellular metabolism in glioblastoma progression, invasion and recurrence.

The single-cell-based metabolite profiling methods using AP-MALDI-MS and OrbiSIMS were developed by optimising cell culture conditions, cell fixation methods, the type of matrices, primary ion beam settings and depth profiling conditions. These techniques were then applied to investigate metabolic differences between human astrocyte and glioblastoma cells from the tumour invasive margin, as well as between invasive and non-invasive glioblastoma cells.

High-resolution spatial distribution maps of selected metabolites were generated, uncovering regions of metabolic heterogeneity within individual cells. These maps provided insights into the localised metabolic environments, revealing distinct metabolic gradients. A detailed understanding of metabolic heterogeneity within individual glioblastoma cells derived from the invasive margin enabled the identification of various metabolic states that influence cellular functions such as growth, survival and invasion. These insights are crucial for enhancing our understanding of cancer progression and recurrence mechanisms. By leveraging the strengths of AP-MALDI-MS and OrbiSIMS imaging technologies, we can now design experiments to investigate spatial metabolic landscapes, including distinct tumour microenvironments, cell-cell interactions, and drug treatment effects. Ultimately, this advances our knowledge of glioblastoma metabolism and expedites the development of more effective treatments.

Wednesday 27th November

Applying Oxygen Attachment Dissociation (OAD) MS/MS to identify positional isomers in lipid biomarkers associated with ethanol toxicity

Emily Armitage, Alan Barnes, Olga Deda, Thomas Meikopoulos, Christina Virgiliou, Helen Gika, Neil Loftus

Abstract:

Structural characterisation of lipids requires the identification of the head group, length of carbon chains and the number and position of double bonds within them. Collision Induced Dissociation (CID) is useful to identify lipid class and chain length, however identifying carbon-carbon double bond position(s) presents a major challenge in unsaturated lipid characterisation. Revealing double bond position(s) in lipids is vital to understand their biological roles, since minor structural differences between positional isomers can markedly change the biochemical function of a lipid.

Oxygen Attachment Dissociation (OAD) is an innovative fragmentation technology, providing additional structural information to CID on the LCMS-9050 QTOF (Shimadzu Corporation). Gas phase O/OH/H radicals are introduced into the collision cell, providing C=C specific fragmentation. OAD-MS/MS and CID-MS/MS spectra can be acquired simultaneously in positive or negative ion mode to generate sufficient information to identify lipids to the structural level.

In this research, OAD-MS/MS has been applied to enhance the identification of lipids found to be significant when exploring the effects of ethanol toxicity in mouse liver, pancreas and gut. LPC 22:5(n-6,9,12,15,18), PC 16:0_22:5(n-6,9,12,15,18) and LPC O-18:1(n-9) were found to be reduced with ethanol toxicity, while LPC20:5(n-3,6,9,12,15), PC 14:0_18:2(n-6,9) and PC 18:2(n-6,9)/18:2(n-6,9) were increased in different tissues as a response to chronic ethanol exposure.

In the analysis of different tissue extracts, OAD-MS/MS has enabled the structural characterisation of lipid isomers indistinguishable by CID-MS/MS. For example, chromatographically separated isomers of LPC 22:5 in pancreas tissue revealed distinct double bond positions in the fatty acyl chains. One of these (LPC 22:5(n-6,9,12,15,18)) was significantly diminished by chronic ethanol exposure while the other (LPC(22:5(n-3,6,9,12,15)) remained unaffected. This highlights the power of OAD-MS/MS to improve lipid identification required to understand the roles of specific positional isomers, especially when lipid standards can be difficult to obtain.

Wednesday 27th November

Unravelling metabolic dysregulation in head and neck cancer with laser-desorption mass spectrometry imaging

Jasmin Werner, James Higginson, Stefania Maneta-Stavrakaki, Lauren Ford, Dani Simon, Yuchen Xiang, Jagtar Dhanda, Burak Temelkuran, Zoltan Takats

Abstract:

Head and Neck Squamous Cell Carcinoma (HNSCC) ranks as the eighth most common cancer in the UK, associated with alcohol, tobacco, and high-risk HPV strains. Despite its prevalence, HNSCC has poor survival and morbidity outcomes with significant intra- and inter-tumoural heterogeneity and a high mutational burden. However, its metabolic dysregulation remains poorly characterised.

In this study, we utilised Laser-Desorption Rapid Evaporative Ionisation Mass Spectrometry (LD-REIMS) for metabolic imaging on minimally prepared HNSCC samples. Our aim was to demonstrate LD-REIMS as an effective, preparation-free technique for mapping the spatial distribution of metabolic features, thereby revealing the metabolic heterogeneity within and between tumours.

Snap-frozen paired tumour-normal samples were ethically collected from HNSCC patients. LD-REIMS imaging was performed using a 3 µm wavelength OPO laser to generate an aerosol, which was directed to a Waters Xevo G2-XS QToF instrument via a REIMS source. The data was processed using our established mass spectrometry imaging pipeline, followed by unsupervised analysis. A logistic regression model was employed for pixel-by-pixel classification. Metabolic features identified from previous studies were spatially mapped and compared with H&E-stained sections, annotated by a histopathologist.

We imaged 11 HNSCC samples from various anatomical subsites. All tumours were HPV-negative, with most patients having a history of smoking. The OPO laser achieved a resolution of approximately 50 µm, nearing single-cell resolution in larger tumour microenvironment cells. Over 4000 distinct m/z peaks were detected, with more than 1000 peaks showing significant dysregulation. Key dysregulated metabolic species included glycerophospholipids, ceramides, fatty acids, and triglycerides. Initial classification demonstrated excellent diagnostic accuracy in identifying disease presence. Future work will focus on further correlating spatial classification results with histopathological annotations and performing tandem MS/MS analysis to confidently identify important dysregulated metabolic features.

Wednesday 27th November

Insights into the interaction of cells with plastics via label-free optical imaging methods

Cassio Lima

Abstract:

In recent decades, plastics have become indispensable products of daily life. Currently, the majority of plastics are synthetic polymers derived from petroleum-based sources, which takes hundreds of years to decompose due to their physicochemical properties. The mismanagement of plastics has led to a significant environmental burden of growing concern as plastics have been spotted on different places around the globe from the poles to the tropics including seas, oceans, mountains, and urban environments. Recent reports have shown the negative impacts due to the ingestion of nano/microplastics in a wide range of organisms and there is an increasing concern about their effects on human health as recent studies have reported the presence of plastics in daily life consumer products such as bottled water, teabags, baby bottles, food containers, among others. Several label-based techniques have been proposed to study the physico-chemical properties of plastics including their effects to cell metabolism; however, label-free approaches are preferred to avoid interference from labelling molecules with metabolism and to prevent issues such as photobleaching and colour limitations in multiplexing analysis. In this talk, we explore the applications and limitations of infrared and Raman spectroscopy as label-free optical techniques for identifying nano/microplastics commonly found in the environment. We also examine their utility in providing insights into the interaction of plastics with mammalian cells. Finally, we will address how vibrational spectroscopy can serve as a powerful tool for monitoring the production of bioplastics within microbial populations at the single-cell level in a label-free manner.

Wednesday 27th November

Multi-view machine learning for integration of multimodal mass spectrometry data

Lukas Kopecky, Caroline J Sands, Maria Gomez Romero, Shivani Misra, Yuchen Xiang, Lauren Ford, James S McKenzie, Zoltan Takats, Elizabeth J Want, Timothy MD Ebbels

Abstract:

Introduction and Aims: In mass spectrometry (MS) based analysis, the diverse physicochemical properties of metabolites often require the use of several different analytical assays, resulting in largely complementary, but also overlapping, data blocks. Separate analysis of each block fails to capture inter-block relationships, inhibiting multi-assay biomarker discovery and interpretation. Here we propose a workflow using a multi-block–partial least squares (MB-PLS) model to integrate multi-assay metabolomics data, enhancing biomarker discovery and elucidation of unknown metabolites.

Methods: We employed a MB-PLS model coupled with multi-block variable importance in projection and permutation testing to select significant predictor features. We clustered these predictors and compared them to groups defined by structural relationships. The integrative workflow is demonstrated on multi-assay metabolomics liquid chromatography – mass spectrometry (LC-MS) data predicting blood bilirubin levels and Alzheimer's disease status. We are now extending the application to integration of multimodal MS imaging (MSI) datasets.

Results: For the LC-MS data, the integrative MB-PLS models outperformed single-assay models in classification, with a mean increase in accuracy and AUC of +0.082 and +0.087 respectively, indicating that modelling inter-block relationships improved prediction accuracy. MB-PLS models showed that differing LC-MS assays contributed between 24% and 59% to the explanation of the outcome, aiding deeper understanding of the contribution of each assay. Our workflow enabled us to determine a set of potential cross-assay biomarkers in the LC-MS data which, for Alzheimer's disease, included sulfatides, ceramides and phosphatidylinositol whose effect directions agreed with literature. For the MSI example, the modelling approach shows excellent promise to reveal complex but informative patterns present in the different MSI modalities.

Conclusion: Our workflow has the potential to benefit the metabolomics community and beyond as it offers interpretable integrative analysis of multimodal MS data, facilitating elucidation of chemically and spatially diverse predictive signatures.

Thursday 28th November

Assessing the impact of nitrogen supplementation in oats across multiple growth locations and years with targeted phenotyping and high-resolution metabolite profiling approaches

J. William Allwood, Pilar Martinez-Martin, Yun Xu, Alexander Cowan, Simon Pont, Irene Griffiths, Julie Sungurtas, Sarah Clarke, Royston Goodacre, Athole Marshall, Derek Stewart, Catherine Howarth

Abstract:

Introduction: Oats are known as being a health beneficial cereal, high in dietary fiber (e.g. β-glucans), as well as antioxidants, minerals and vitamins. UK agricultural guidelines on nitrogen (N) level are suboptimal. In this study the response of yield, grain quality and metabolites to increased N levels were investigated.

Methods: Four varieties (Mascani, Tardis, Balado, Gerald) were grown in a N response trial (0 control, 50-280 kg N/ha), over two UK locations and growth seasons. Grain yield and milling quality were assessed. β-glucan, total protein and oil content were quantified via NIR. Oat metabolites were assessed by GC-MS and a newly developed and validated UHPLC-MS/MS method optimised towards high chromatographic resolution (Allwood et al. 2021).

Results: N had a significant effect on grain yield. Grain quality traits displayed significant differences between varieties and N application levels. β-glucan content significantly increased with N level. The GC-MS and LC-MS methods captured a wide range of compounds, inclusive of amino acids, organic acids and sugars (GC-MS), vitamins, membrane lipids, and a range of secondary metabolites, including the avenanthramides and their glucosides, avenacins, avenacosides, hordenines and isovitexins (LC-MS/MS). Amino acid metabolism was upregulated by N, as were total protein levels, whilst organic acids were decreased (carbon skeleton scavenging). High N appeared to increase TCA turn over and reduce sugar levels to provide the plant with energy and reductant power to aid N assimilation. N was also directed towards the production of N containing phospholipids (e.g. PC, PE, PI's), likely as a scavenging and storage strategy. High levels of N application negatively impacted the total oat oil levels, and the levels of health-beneficial avenanthramides.

Conclusion: Although N addition significantly increased grain yield and β-glucan content, oat varietal choice as well as negative impacts upon health beneficial secondary metabolites and the environmental burdens associated with N, require further consideration.

Thursday 28th November

Identifying health-promoting chemical attributes of tea after different processing methods using metabolomics and machine learning

Amanda J Lloyd, Alina Warren-Walker, Alison Watson, Jasen Finch, Thomas Wilson, Jo Harper, Katherine Bennett, John Draper, Manfred Beckmann

Abstract:

Leaf samples of Camellia sinensis were collected during the processing of green and black tea at Dartmoor Estate Tea, UK. All samples were adjusted for weight and moisture content to allow for direct comparison using Flow Infusion Electrospray Ionisation Mass Spectrometry (FIE-MS). The FIE-MS analysis was performed using an Exploris 120 mass analyser coupled with a Dionex Vanquish UHPLC system (Thermo-Scientific). For green tea processing, random forest regression identified features associated with the processing steps, resulting in a model with a significant R^2 value of 0.724 ($p < 0.01$, 3000 permutations). A total of 272 m/z features were found to be explanatory (% increase in mean squared error, $p < 0.05$, 3000 permutations).

The analysis of black tea processing was more complex due to the two oxidation branches—4-hour and overnight oxidation prior to roasting. Random forest regression was separately applied to include the 4-hour and overnight oxidation and roasting treatments along with the prior processing stages to identify relevant chemical changes. Models for the 4-hour and overnight oxidation treatments were then compared using t-tests to determine the treatment specific chemical changes. The model incorporating the 4-hour oxidation and roasting treatments yielded a significant R^2 value of 0.92 ($p < 0.01$, 3000 permutations) with 209 explanatory features (% increase in mean squared error, $p < 0.05$, 3000 permutations). The model for the overnight oxidation and roasting treatments yielded a significant R^2 value of 0.883 ($p < 0.01$, 3000 permutations) with 605 explanatory features (% increase in mean squared error, $p < 0.05$, 3000 permutations).

Overnight oxidation of black tea cased a decrease in the abundance of health-promoting catechins (epicatechin, epicatechin-3-gallate, epigallocatechin and epigallocatechin-3-gallate) when compared with green tea. Theanine showed an elevation in black tea processing, followed by a decrease after oxidation.

Thursday 28th November

Metabolomic evaluation of coffee beans and their husks from organic cultivation in the Caparaó region, Brazil

Murilo de Oliveira Souza, Leonardo Marques Paula, Tayná de Oliveira Costa, Vinícius Guimarães Nasser, Geraldo Humberto Silva, Mario Ferreira Conceição Santos

Abstract:

Brazil, the world leader in coffee production and export, also stands out for its high consumption of the beverage, reflecting the strong cultural connection to coffee. In Espírito Santo, the Caparaó region emerges as one of the main hubs for specialty Arabica coffee production, with family farming being the predominant method of cultivation. In organic coffee production, recycling and the use of organic waste are essential. Coffee husks, a nutrient-rich byproduct, have great potential for reuse in the pharmaceutical, food, and cosmetic industries.

This study aimed to evaluate the metabolomic profile of coffee husks from organic cultivation in the Caparaó region, exploring their potential for producing organic beverages with nutritional value. Samples of beans and husks were provided by local producers from the Caparaó region, Brazil. After collection, the beans were subjected to a standardized roasting process at 200°C for 10 minutes and then ground into fine particles.

Metabolomic analysis of the samples was performed using gas chromatography-mass spectrometry (GC-MS). The derivatization of metabolites was carried out simultaneously for all samples, and the data were processed using MS-DIAL software to identify the compounds present.

Chemometric methods were applied to distinguish the metabolomic profiles of the coffee husks and beans. Finally, this study demonstrates the applicability of gas chromatography-mass spectrometry for the analysis of coffee husks and is useful for evaluating the production of pre-workout beverages or other formulations that are sensorially and nutritionally relevant, highlighting their potential as an innovative technological product.

Thursday 28th November

Metabolomic 'global' fingerprinting and data mining on flour

P, Martinez-Martin, A Lloyd, E Jensen, A Winters, C Holister, C Howarth

Abstract:

In a Better Food For All Innovate UK project, Shipton Mill and Aberystwyth University are harnessing data on grains with high nutritional content, concentrating on protein (incl. amino acid profile), vitamins, minerals (incl. copper, iron, manganese, molybdenum, sodium, zinc), soluble (e.g., beta-glucan) and insoluble fibre, oil, and starch content/type, as well as metabolomic 'global' fingerprinting. The aim is to pioneer the development of a naturally, highly-nutritious white wheat flour for bread making, without the use of artificial fortification.

Two sets of flour samples from the milling process at Shipton Mill, streams, were analyzed using high resolution (HR) flow infusion electrospray (FIE) ionisation mass spectrometry (MS), HRMS-FIEMS, Thermo QExactive Plus coupled with Dionex UltiMate 3000 UHPLC, ultra-performance liquid chromatography system metabolomic fingerprinting, to allow the assessment of the chemical composition. After pre-treatment of the data, 6104 m/z and 4927 m/z features respectively, were pulled out for modelling purposes. The chemical composition was visualized by unsupervised Principal Component Analysis (PCA), with a PC1 explaining 38.51% and 34.81% of the variance, respectively, between streams, suggesting significant chemical differences. In both cases, sample distances from similarity values from a regression random forest, were used to plot streams based on similarity to help to formulate different blends with higher nutritional value. Heatmaps from a regression random forest model on the intensity of the features for each steam, allowed us to identify the overlapping chemical pattern with the milling process, identifying the streams low in tentatively annotated biomarkers of importance in a nutritional profile.

In the final months of the project Shipton will perform baking trials using yeast and sourdough cultures and conduct internal organoleptic testing, nutritional analysis of baked products, metabolomic fingerprinting of digestion and nutrient absorption, product evaluation via tasting panel trials and stakeholder engagement sessions.

Thursday 28th November

The MATCHING international ring-trial to evaluate the reliability of metabolomics for "chemical grouping" in regulatory toxicology

M. R. Viant, E. Amstalden, T. Athersuch, M. Bouhifd, T. Ebbels, V. Haake, A. Kende, P. Leonards, G. Lloyd, C. Sands, T. Sobanski, L. Swindale, T. Walk, R. Weber, F. Zickgraf, and H. Kamp

Abstract:

Building on over 20 years of research, metabolomics has reached a critical point in determining its value to regulatory toxicology. One of its principal applications in chemical risk assessment is to support an approach called "chemical grouping and read-across", which utilises metabolomics data to justify "grouping" two or more chemicals together, so that the toxicity data for one chemical can be "read-across" to predict the toxicity of other chemicals. While of considerable interest to both chemical regulators and industry, the reproducibility of metabolomics for grouping has yet to be demonstrated. The aims of the Cefic LRI-funded MATCHING study (MetAbolomics ring-Trial for CHemical groupING) were to determine whether this technology can demonstrate high reproducibility in grouping, via a fully-blinded evaluation, and to propose best-practices. The international consortium comprised six industrial, government and academic partners. An independent team selected eight chemicals (ring-trial partners were blinded to their identities and modes-of-action). Plasma samples were derived from 28-day rat tests, aliquoted, and distributed to partners who applied their preferred LC-MS metabolomics workflows to acquire, process, quality-assess, statistically analyse and report their grouping results to the European Chemicals Agency. Five of six partners, whose datasets passed quality-control, correctly identified the grouping of eight chemicals into three groups, for both male and female rats. Strikingly, this was achieved even though a range of metabolomics approaches were used. Through assessing intrastudy QC samples, the sixth partner observed high technical variation and was unable to group the chemicals. By comparing workflows, we conclude that some heterogeneity in metabolomics methods is not detrimental to consistent grouping, and that assessing data-quality prior to grouping is essential. On-going analyses into the metabolic biomarkers driving the groupings, and their consistency across partners, are underway. This study demonstrates the high reliability of metabolomics for chemical grouping, facilitating its application to regulatory studies.

Thursday 28th November

GC-MS based metabolome profiling of Antarctic marine organisms & lessons learnt in environmental omic

Burke, A., Barrett, N., Peck, L. S., Goodacre, R

Abstract:

The Centre for Metabolomics Research (CMR) is a member of the NERC Environmental Omics Facility (NEOF). A wide variety of environmental projects and sample types are received through NEOF collaborations, posing a tough challenge in developing standardised methods and achieving consistent, quality results. Mass spectrometric, spectroscopic and chemometric techniques are utilised to unveil organism response to changing environments, stressors and nutrition, providing collaborators insight into active biochemical pathways occurring in their systems of interest. Some of the challenges faced in these environmental projects will be discussed during the presentation.

A project with the British Antarctic Survey and University of Cambridge investigated the response of Sterechinus neumayeri (sea urchin) and Odontaster Validus (sea star) to falling salinity of the Antarctic Ocean. Organisms were exposed to a native and two salinity stress conditions, with tissue and extracellular fluid collected at defined time points. The CMR's versatile standard GC-MS method was used in analysis of the extracted samples, in accordance with Metabolomics Quality Assurance & Quality Control Consortium (mQACC) standards. Results reveal intracellular metabolome changes in response to salinity stress, involving organic osmolytes. Osmolytes are low-molecular-mass organic solutes which aid in low temperature protein homeostasis, osmotic regulation and freeze tolerance.

Biological interpretation by collaborators is ongoing, but this work offers insights into adaptations that favour protein synthesis and stability at cold temperatures, and data will support comparisons with non-polar marine animals to identify adaptation to low temperature. These experiments provide a proof of principle study, with the potential to answer fundamental questions about cellular level adaptation and homeostasis on marine species inhabiting the cryosphere that have so far been intractable.

Thursday 28th November

A direct comparison of anion-exchange chromatography-mass spectrometry (AEC-MS) and HILIC-MS for cellular metabolomics

Rachel Williams, John Walsby-Tickle & **James McCullagh**

Abstract:

Primary metabolism is characterised by the interconversion of highly polar and ionic metabolites for example those found in glycolysis, pentose phosphate pathway, nucleic acid metabolism, glycogen metabolism, citric acid cycle and amino acid pathways. HILIC-MS remains the most commonly applied technique for the analysis of highly polar metabolites but recently the development of anion-exchange chromatography-mass spectrometry (AEC-MS) has provided an alternative. These two chromatographic approaches have very different mechanisms of retention and elution with differing advantages and disadvantages in the context of metabolomics applications. Chemical heterogeneity and metabolome complexity remaining a major challenge for both.

Here we examine the advantages and disadvantages using cancer cell metabolism in an exemplar cell-based study. We directly compare the performance of HILIC-MS and IC-MS on the same mass spectrometry platform using the same samples and discuss the advantages and disadvantages for metabolomics applications. We show results for both targeted and untargeted analysis and discuss the results of formal validation. We also comment on our findings regarding sample preparation, a sometimes-neglected consideration but an essential one for metabolites chemically primed for interconversion, such as those found in primary metabolic pathways. Finally, we summarise the implications of our findings for cellular metabolomics more generally and highlight areas for future technical developments.

Thursday 28th November

Getting to know your roommate – NMR metabolomics as a tool to characterise the role of metabolism in symbiotic relationships

Horst Joachim Schirra, Sarah Walsh, Denni Currin-Ross, Luke Husdell, Jessica Broadway, Eric Caragata, Jeremy Brownlie

Abstract:

Model organisms, such as *Drosophila melanogaster*, are useful tools for uncovering fundamental biological processes in systems that are comparatively less complex than higher animals/humans. Here we discuss three examples of using NMR-based metabolomics as a key platform technology to exploring metabolic processes in host-symbiont interactions in insects, including Drosophila:

(1) Infection with the endosymbiont Wolbachia depresses the insulin/insulin-like-growth factor cascade in *D. melanogaster*, whilst inducing the hypoxia signaling pathway. This causes ROS production and ROS adaptations, and other metabolic changes that steer metabolism away from oxygen-intensive pathways and enable metabolite extraction by the symbiont and metabolite provisioning to the host. These responses signify a reprogramming of the host's mitochondrial metabolism rather than an immune response. (2) In contrast, infection with wMelPop in the mosquito Aedes. aegypti triggers host immune responses, including melanogenesis and ROS production. wMelPop is more aggressive, while wMel is more likely to form stable inheritable infections. (3) Wolbachia infection in Drosophila leads to the temporary suppression of viral infections. Understanding the basis for this effect is of great interest in the context of inhibiting the spread of insect-borne viral diseases. NMR-based metabolomics provides evidence for metabolic competition between the endosymbiont and the virus as the underlying basis for the inhibition of viral replication.

These examples show the breadth and depth of insights into host-symbiont interactions that can be gained through model organisms in metabolomics and systems biology.

Thursday 28th November

Biomarkers of Food Intake: how can metabolomics help

Lorraine Brennan

Abstract:

Metabolomics applications in Nutrition Research can grouped into one of the following: (1) Identification of dietary biomarkers for single foods or for dietary patterns (2) Applications to dietary intervention studies to help understand metabolic alterations (3) Study of diet-related diseases and (4) Precision Nutrition. With respect to dietary biomarkers and exposure there has been a proliferation of publications in this field: these biomarkers have the potential to act as objective measures of dietary intake thus overcoming some of the key issues with traditional assessment methods. To date, metabolomic profiling has been successful in identifying several putative biomarkers of food exposure. Similarly, use of combination of biomarkers can be employed to study dietary patterns.

While significant progress has been made to date in the application of metabolomics in nutrition research a number of key challenges remain: addressing these will be key to development of future opportunities.

Poster abstracts

Poster 1

Getting to know your roommate – NMR metabolomics as a tool to characterise the role of metabolism in symbiotic relationships

Sander Johannes Thorbjørnsen Guttorm, Manoj Amrutkar, Anette Vefferstad Finstadsveen, Knut Jørgen Labori, Lars Eide, Helge Rootwelt, Katja Benedikte Prestø Elgstøe, Ivar P. Gladhaug, Caroline S. Verbeke

Abstract:

Neoadjuvant chemotherapy (NAT) has become a standard of care for borderline resectable and locally advanced pancreatic ductal adenocarcinoma (PDAC). However, its clinical benefits are limited by lack of biomarkers to assess treatment response. To address this, global metabolomic profiling on tumor tissue and paired serum samples from treatment-naïve (TN; n=18) and neoadjuvant FOLFIRINOX-treated (NAT; n=17)-treated PDACs was performed using ultimate 3000 HPLC coupled to Q Exactive orbitrap mass spectrometer (MS). Samples were analyzed in both positive and negative ionization modes and full MS and MS/MS were obtained. Metabolites were separated using Pursuit XRs diphenyl column with gradient elution (water/methanol+0.01% formic acid). Data processing and statistical analysis were conducted using Compound Discoverer. Differentially abundant metabolites (DAMs; $p<0.05$) in NAT versus TN group were identified and their correlation with various clinical parameters was assessed. Lastly, diagnostic potential of identified biomarkers was determined by ROC analysis. Total 40 tissue and five serum DAMs were identified, most of these were less abundant in NAT versus TN, were associated with amino acid and nucleotide metabolism. Four DAMs were common to both tissue and serum, and showed a similar pattern of differential abundance: 3-hydroxybutyric acid (BHB: ketone body), 3-carboxy-4-methyl-5-propyl-2-furanpropanoic acid (CMPF: furan fatty acid), glycochenodeoxycholate (bile acid) and citrulline (arginine biosynthesis precursor). Tissue carnitines (C12, C18, C18:2) and N8-acetylspermidine correlated strongly positively with serum carbohydrate 19-9 antigen (CA 19-9) - widely used PDAC biomarker. Serum deoxycholate correlated positively with survival, and negatively with NAT-induced CA 19-9 reduction. These findings revealed NAT-induced changes in PDAC metabolic pathways, mainly amino acid and nucleotide metabolism, and these correlated with reduced CA 19-9 following NAT. Moreover, it revealed that a serum metabolite biomarker panel consisting of deoxycholate, GCDC, BHB, citrulline, and CMPF could potentially be used for early monitoring of treatment response in PDAC.

Poster 2

Valorising waste streams by extracting high value components for the food industry

Cristina Saladas, Nicholas Powles, Howbeer Muhammad Ali, Jonathan Farrimond, Royston Goodacre

Abstract:

Industrial juice manufacture produces ~20-30% by-product known as, pomace, consisting of skins, pulp and seeds. Approximately 160000 tons of blackcurrant pomace (BCp) are produced yearly in Europe.

Suntory's BCp is primarily used in animal feed yet, human consumption is well-documented and has likely health benefits e.g. cardiovascular improvements, glycemic control and anti-inflammatory effects. Blackcurrants are polyphenol-rich, in particular anthocyanins and we have previously examined blackcurrant polyphenol effects on glycemia, satiety and cognitive function. Importantly, while significant previous work has characterized blackcurrant in general, very little work on our BCp has been completed. Subsequently, we cannot directly link our BCp to the health benefits we previously observed. Here we seek to fully understand how to extract the most value from BCp by understanding its composition (particularly with reference to polyphenols and anthocyanins).

Pomace composition analysis will be completed by liquid chromatography-mass spectrometry (LC-MS), gas chromatography-mass spectrometry (GC-MS) and Infrared (IR) spectroscopy.
Presently, we are optimizing polyphenol and anthocyanin extraction. Experiments are ongoing, we have analysed samples obtained from 2022 crop through LC-MS and we are planning to obtain GC-MS and IR data in the next few months.
Future work will validate the optimal extraction method, produce 'optimised' extracts that we could potentially use as an ingredient.

Poster 3

Collaborative research efforts: analysis of ergothioneine and oxylipin lipids

Nigel Gotts, Alois Bonifacio, Stefano Fornasaro, Marina Wright Muelas, Ivayla Roberts, Shree Hegde, Victoria Tyrrell, Valerie O'Donnell, Joseph Turner, Roy Goodacre

Abstract:

Collaborative research both inter- and intra-University presents excellent opportunities to diversify analyses beyond the capabilities of a single lab, plus involve additional personnel for a greatly expanded set of skills. The sum of the group is very much greater than the sum of the individual parts. In this poster we will present findings from two such projects:

1. Quantitation of Ergothioneine in Serum: Results from a cross-technique project in which results from samples analysed by a novel SERS technique were compared to those obtained via the more traditional approach of targeted LC-MS2 and SERS. A collaboration between the University of Trieste and the University of Liverpool.

2. Targeted quantitative analysis of Oxylipin Lipids a story of method transfer: Lessons learnt in the course of successfully transferring analytical protocols between research groups at different institutions. A collaboration between Cardiff University and the University of Liverpool.

Poster 4

MetAssimulo 2: a web app for simulating realistic 1D & 2D metabolomic 1H NMR spectra

Yan Yan, Beatriz Jiminez, Toby Athersuch, Maria De Iorio and Timothy M. D. Ebbels

Abstract:

Metabolomics extensively utilizes Nuclear Magnetic Resonance (NMR) spectroscopy due to its excellent reproducibility and high throughput. Both one-dimensional (1D) and two-dimensional (2D) NMR spectra provide crucial information for metabolite annotation and quantification, yet present complex overlapping patterns which may require sophisticated machine learning algorithms to decipher. Unfortunately, the limited availability of experimental spectra can hamper application of machine learning, especially deep learning algorithms which require large amounts of labelled data. In this context, simulation of spectral data becomes a tractable solution for algorithm development.

Here, we introduce MetAssimulo 2, a comprehensive upgrade of the MetAssimulo 1 metabolomic NMR simulation tool, reimplemented as a Python-based web application. Where MetAssimulo 1 only simulated 1D 1H spectra of human urine, MetAssimulo 2 expands functionality to urine, blood, and cerebral spinal fluid (CSF), enhancing the realism of blood spectra by incorporating a broad protein background. This enhancement enables a closer approximation to real blood spectra, achieving a similarity of approximately 82%. Moreover, this tool now includes simulation capabilities for 2D J-resolved (J-Res) and Correlation Spectroscopy (COSY) spectra, significantly broadening its utility in complex mixture analysis. MetAssimulo 2 simulates both single, and groups, of spectra with both discrete (e.g. case-control) and continuous (e.g. BMI) outcomes, supporting a range of experimental designs and demonstrating associations between metabolite profiles and biomedical responses.

By enhancing both the accessibility and versatility of NMR spectral simulations, MetAssimulo 2 is well positioned to support and enhance research at the intersection of deep learning and metabolomics. This tool not only fosters the development of robust deep learning models but also enhances the interpretability and validation of metabolomic data, thereby accelerating metabolomic science.

Poster 5

Optimising synovial fluid preparation for gas chromatography mass spectrometry metabolomics analysis for orthopaedic research

Yumna Ladha, Adam Burke, Royston Goodacre, Karina Wright, Jade Perry, Martyn Snow, Charlotte Hulme

Abstract:

Synovial fluid (SF) bathes articular joints (e.g. the knee) providing lubrication for movement and nutrients to joint tissues. SF metabolomic profiling can provide a wealth of information about the disease status of joints (e.g. in Osteoarthritis). Careful sample preparation of this highly viscous biological sample is required to ensure maximal metabolite identification. This study aimed to optimise SF sample processing by determining the effects of pre-analytical variables on the number of detectible metabolites and their stability using Gas Chromatography-Mass Spectrometry (GC-MS).

Human SFs from knee replacements were immediately centrifuged and stored at -80°C (n = 5 per test group). Freeze-thaw cycle effect was assessed by exposing samples to either one, two or three freeze-thaws. To assess viscosity effects, samples were enzymatically pre-treated using hyaluronidase. The effect of dilution was assessed by mixing the samples with 0.85% physiological saline solution at either a 1:5, 1:10 or 1:20 ratio. Metabolic profiling was performed using an untargeted GC-MS approach, and the resulting spectra were processed using MSDIAL v4.93 software. Preliminary results revealed a total of 334 detected metabolic features in the control group (undiluted and no-freeze thaws) including 124 metabolites that were reference matched to an external database (Level 2 identification of MSI) and 210 unknown metabolites. The group that underwent two freeze-thaws exhibited a total of 350 metabolites (128 reference matched and 222 unknown) whilst a total of 331 metabolites were detected following three freeze-thaws. Hyaluronidase treatment resulted in increased metabolites (401) compared to the control group. These initial findings suggest that freeze-thawing of SF samples does not greatly affect the number of detectable metabolites. Hyaluronidase pre-treatment may be beneficial in enhancing metabolite detection. Further investigation to determine the effects on individual metabolites is ongoing. The optimisation of SF preparation for GC-MS is essential to ensure accurate and reproducible data is generated.

Poster 6

Metabolome associations with cardiovascular ageing

Shamin Tahasildar, Marc-Emmanuel Dumas, Declan P. O'Regan

Abstract:

Cardiovascular diseases are the leading cause of global mortalities, and ageing is a significant risk factor which is influenced by genetic, molecular, and environmental changes. Cardiovascular ageing is characterised by morphological and functional abnormalities leading to vascular remodelling and diastolic dysfunction, as well as metabolic dysfunction. While previous biological ageing clocks have explored molecular changes with organ-specific ageing, the effects of circulating metabolites on cardiovascular ageing remain unknown. Here, we investigate metabolome associations with cardiovascular ageing.

We analysed data from UK Biobank, a prospective cohort study of 500,000 UK participants aged 40-69 at recruitment. We developed a machine learning model trained on cardiac image-derived phenotypes from 5,396 healthy participants. An age-delta was calculated in 42,438 participants as an estimate of how individual's cardiovascular system has aged relative to a normative population. We overlapped this with the 292,000 UK Biobank participants with NMR metabolomics, identifying 10,208 participants with a cardiovascular age-delta and 168 direct-measure NMR metabolites. Metabolite associations with age-delta were determined through linear regression. Each metabolite was a predictor against age-delta as the dependent variable, adjusted for age, age2 and sex. Multiple testing was accounted for Benjamini-Hochberg correction.

We identified 122 metabolites significantly associated with age-delta. There were 23 metabolites associated with protective cardiovascular ageing, all were high density lipoproteins (HDL). Meanwhile 99 metabolites were associated with accelerated cardiovascular ageing, which included very low- and low-density lipoproteins, triglycerides, saturated fatty acids and glycoprotein acetyls.

The findings are in line with current literature understandings for cardiovascular health. HDLs are attributed with protective cardiovascular effects due to cholesterol regulation functions. However, we find triglycerides in HDLs to be accelerant, suggesting HDL mediation is more complex beyond cholesterol and is reflective of broader cardiovascular health, such as through anti-inflammatory or anti-oxidant mechanisms. Metabolite causality for ageing will be determined through two-sample Mendelian Randomisation.

Poster 7

PCA to reveal non-adherence and non-analytical outliers using global metabolomics and lipidomics in clinical settings

Sander Johannes Thorbjørnsen Guttorm, Barbora Pisklakova, Ales Kvasnicka, Anja Østeby Vassli, Helge Rootwelt, **Katja Benedikte Prestø Elgstøen**

Abstract:

Non-adherence is common in clinical settings, leading to loss of treatment response. In clinical practice this will often prompt institution of new and often inferior treatments with higher risk of complications. In a clinical research setting, the results will be biased and not based on real clinical effects but on the degree of participant adherence. Methods of detecting non-adherence are often insufficient, and since adherence is usually expected and based on a signed agreement, non-adherence is usually not detected. We here aim to reveal the extent of non-adherence in clinical settings, and how the utilization of global metabolomics and lipidomics can detect non-adherence at an early stage. Early detection provides an opportunity to correct and reinstate adherence and effective patient treatment, and to enable exclusion of study participants and samples that would otherwise contribute to loss of detection of significant findings and improper conclusions. We employed multivariate analysis, particularly unsupervised principal component analysis (PCA), to identify non-adherent patient samples. Using PCA, we could easily identify samples that grouped contrary to expectance and logic and follow up to determine if this was caused by non-adherence. By utilizing global metabolomics and lipidomics we also offer a tool for validating medical journals, including monitoring if the patient has received or taken the medication prescribed and/or other medications not ordered. Furthermore, we use these techniques as a quality assurance measure to detect i.e. ethylenediaminetetraacetic acid (EDTA) in plasma samples, ensuring identification of correct sample material. Matrix specific metabolites and lipids is something we investigate closer, in the hope of establishing benchmarks for determining type of sample matrix. Consequently, our findings underscore the unique advantages of global metabolomics and lipidomics for clinical applications, proving capabilities beyond those of targeted methods.

Poster 8

Probing metabolic behaviour of bacteria at surfaces

Daniel Smaje, Blanca Perez Sepulveda, Xiaojun Zhu, Jay Hinton, Rasmita Raval, Howbeer Muhamad Ali

Abstract:

Salmonella bacteria cause severe illness and death worldwide, particularly in low-income countries. As Salmonella outbreaks are often linked to food preparation or agricultural vectors, being able to identify Salmonella strains and serotypes to help track outbreaks is vital; knowing what serotype or strain an individual has contracted is also important for appropriate treatment. The current gold standard for serotyping involves a series of biochemical tests which are time-consuming and require experienced personnel to carry out. As metabolomics-based identification methods could allow for faster serotyping that doesn't require specialised labs and personnel, this study investigated Fourier-transform infrared spectroscopy and rapid evaporative ionisation mass spectrometry for their ability to identify and discriminate Salmonella over a range of 100 strains.

100 strains of Salmonella were collected, cultured and harvested in the laboratory following protocols for metabolomic analyses of bacteria. A range of multivariate statistical methods including PCA, PC-DFA and bootstrapping were applied to the dataset to attempt to classify down to at least the serovar level, allowing comparisons to be made against traditional serotyping or sequencing. Using FTIR to discriminate between serovars yielded up to 99.6% class prediction accuracy. This indicates that FTIR can be used relatively successfully for serotyping, but to improve down to strain level identification further may be required. This work suggests that applying FTIR to Salmonella identification is possible, and may prove to be a useful tool in a range of fields requiring rapid and accurate identification of Salmonella serovars.

Poster 9

A comprehensive and in-depth characterisation of model organism metabolomes

Ralf J. M. Weber, Thomas N. Lawson, Mark R. Viant, Martin R. Jones

Abstract:

Over the past two decades, propelled by technology and the successes of the Human Genome Project, large-scale initiatives to catalogue and functionally annotate model organism genomes, transcriptomes, and proteomes have revolutionised biology. However, our understanding of model organism metabolomes remains comparatively limited, partly due to a lack of metabolome-specific resources. The "Deep Metabolome Annotation" (DMA) strategy, developed at the University of Birmingham, advances this area. Metabolites extracted from biological matrices are systematically separated according to their distinct physicochemical properties using various liquid chromatography mass spectrometry (LCMS) approaches, including fragmentation experiments, and subsequently annotated/identified using bespoke and extensive computational workflows. Using this approach, we have identified and/or annotated over 8500 metabolites in Daphnia magna, a model organism for biomedical, environmental and toxicological research. Propelled by research by the EU H2020-funded PrecisionTox consortium, we have expanded the scope of our innovative DMA strategy to include a broader range of model organisms, specifically Danio (zebrafish), Xenopus (African clawed frog), Drosophila (fruit fly), and Caenorhabditis (roundworm). In doing so, we have also enhanced our analytical methods by incorporating extensive fragmentation experiments, including CID/HCD-based MSn and acquisition of fragmentation breakdown curves. We have also bolstered our computational workflows through integration of cutting-edge metabolite identification engines, including in silico tools. Here, we provide a snapshot of the results from DMA of four model organisms and provide an overview of how we are using this data for cross-species comparative metabolomics. This area of research remains largely unexplored and holds great potential for advancing understanding of biological phenomena. Finally, we provide an overview of the informatics resources we have developed to facilitate access to and use of DMA data by diverse research communities. For the metabolomics research community, this will help to annotate, analyse and integrate metabolomics data more effectively.

Poster 10

A comprehensive computational framework for real-time monitoring and quality control of LC-MS-based metabolomics data acquisition

Ossama Edbali, Andrew D. Southam, Gavin R. Lloyd, Martin R. Jones, Ralf J. M. Weber

Abstract:

Liquid chromatography in combination with mass spectrometry (LC-MS) is a powerful analytical technique in metabolomics, enabling the analysis of thousands of compounds with a diverse range of chemical and biological properties. However, maintaining consistent acquisition of high-quality LC-MS data is challenging due to stochastic and/or gradual shifts in instrument performance over time. This necessitates quality assurance and quality control (QC) methods to monitor and evaluate data quality. Current QC methods are predominantly post-hoc computational processes, meaning that analysis is performed only after data acquisition, which significantly delays the detection of technical anomalies. Shifting towards continuous monitoring and validation of both the instrument and the data acquisition would be preferable. To address this need, we present a computational monitoring framework, developed in Python, that leverages time-series databases and observability platforms to monitor data quality in near real-time. Raw sample data is processed, and instrument-related readings are processed and stored in a time-series database (InfluxDB); instrument performance and data quality are then presented to the user, in near real-time, through observability dashboards (Grafana). Additionally, the user can process data, on the fly, through various workflow systems, including Galaxy, Common Workflow Language (CWL), or via custom R or Python-based workflows packaged in Conda environments. The framework can be installed on Windows, Unix, and macOS through an easy-to-use interface. The framework has been used to analyse the quality of clinical and toxicological studies comprising hundreds of samples; it has also been crucial for retrospective analysis of previously collected datasets. The proposed solution will 1) facilitate decision making for data acquisition and analysis; 2) ensure and improve the reproducibility of LC-MS-based metabolomics studies by bringing the experimenter closer to the instruments and data acquisition process; 3) enhance high-throughput capabilities by allowing rapid automated assessment of instrument performance in near real-time.

Poster 11

Automated extraction of adherent cell lines from 24-well and 96-well plates for multi-omics analysis using the Hielscher UIP400MTP sonicator and Beckman Coulter i7 liquid handling workstation

Lauren E. Cruchley-Fuge, Martin R. Jones, Ossama Edbali, Gavin R. Lloyd, Elena Sostare, Ralf J. M. Weber, Andrew D. Southam, Mark R. Viant

Abstract:

PrecisionTox is a €20M EU Horizon 2020-funded research project that aims to develop new methods for chemical hazard assessment focusing on 'omics technologies applied to non-animal test systems and human cell-lines. This has created an appreciable demand for the automated extraction of polar and lipophilic compounds from adherent-cells cultured in multi-well plates, including HepG2 and HepaRG (hepatocytes), and H295R (adrenal cancer cells). An automated method was sought to detach these cell types from multi-well plates, comparable to the quality of (slow and manual) cell-scraping. Following a review of potential approaches, this study aimed to implement and optimise an automated, high-throughput, reproducible and temperature-controlled sonication-based cell-detachment and extraction method for multi-omics measurements using a Hielscher UIP400MTP sonicator and Beckman Coulter i7 liquid-handling workstation. Initial optimisation was conducted with HepG2 cells to determine the appropriate sonication power required for cell-detachment. Next, a comparative investigation of cell-scraping, sonication using the UIP400MTP (at two power settings), and a traditional sonic water bath was conducted, including the extraction of polar and lipophilic compounds and RNA. This was achieved using an automated, modified Bligh and Dyer biphasic extraction (2:2:1.8 v/v/v chloroform:methanol:water) protocol, producing polar and lipid fractions that were analysed by ultra-high performance liquid chromatography-mass spectrometry (UHPLC-MS) metabolomics. In parallel, the RNA yield and quality were measured. Of the three sonication methods, the protocol using the UIP400MTP sonicator, operated at 60% power, delivered the most consistent results. Considering the polar metabolome, this approach yielded similar sample-to-sample variability as manual cell-scraping and was more consistent based on lipidome analysis. The UIP400MTP (60% power) method was then successfully applied to both H295R cells (24-well plates) and HepaRG cells (96-well plates). Overall, these findings, combined with the benefits of automation, confirm this cell-detachment and extraction protocol can be used in large-scale toxico-metabolomics studies.

Poster 12

Using OrbiSIMS as a label-free approach to deciphering metabolic heterogeneity in glioblastoma

Aimee J. Humphreys, Anna Trzaska, Anna Kotowska, Simon Deacon3 Jonathan Rowlinson, Ruman Rahman, Sandra Martinez Jarquin, Rian L. Griffiths, Dong-Hyun Kim

Abstract:

Glioblastoma (GBM) is an incurable brain cancer with a median survival of around 15 months. There are several barriers to successful treatment, including its extensive invasion into surrounding healthy parenchyma, which makes total surgical resection challenging, resulting in residual disease and later relapse. Additionally, it exhibits both inter- and intra-tumour heterogeneity, which likely accounts for poor therapy response. By utilising surface mass spectrometry techniques, formalin fixed paraffin embedded (FFPE) tissue available in archives can be used as an extensive resource for metabolomic analysis, which can identify and potentially disseminate metabolic pertubations and vulnerabilities in GBM. Following deparaffination with xylene, 2 serial sections of human GBM tissue (4µm thickness), taken from a total of 3 patients, were used to acquire images using the Orbitrap secondary ion mass spectrometry (OrbiSIMS) technique (IONTOF). A 20keV Ar3000+ GCIB analysis beam was used, generating images at a spatial resolution of 50µm. Images were collected in negative polarity at a mass range of m/z 75-1125, with a final pixel size of 50µm, in random raster mode. The mass resolution was set to 120,000 at m/z 200. OrbiSIMS images were combined with a third serial slide, stained with haematoxylin and eosin (H&E), and annotated by a neuropathologist. The results demonstrate the ability to discriminate morphologically distinct regions, including viable and nectrotic regions within the tumour. Distribution of metabolites varied within the same tumour, and between patients, demonstrating the ability to identify both inter- and intra-tumour heterogeneity using this approach. Future work includes combining additional surface mass spectrometry techniques, including liquid extraction surface analysis (LESA) and atmospheric pressure matrix assisted laser desorption/ionization (AP-MALDI) to validate metabolite identity, and to potentially broaden the range of detected metabolites.

Poster 13

Discrimination of periodontal bacteria utilising IR spectroscopy techniques

Jawaher Albahri, Kathryn Whitehead, Heather Alison, and Howbeer Muhamad Ali

Abstract:

Periodontitis is a prevalent condition characterised by progressive destruction of periodontium, resulting from the prolonged interaction between the host immune system and bacterial infection. In this study, we employed Fourier Transform Infrared (FT-IR) spectroscopy to investigate the biochemical composition of nine periodontitis-associated bacteria *Porphyromonas gingivalis*, *Streptococcus anginosus*, *Streptococcus constellatus, Streptococcus gordonii, Streptococcus sanguinis, Streptococcus oralis, Actinomyces israelii, Fusobacterium nucleatum,* and *Parvimonas micra*, at the microbial-community level. Additionally, Optical Photothermal Infrared (O-PTIR) spectroscopy was utilised at the single-cell level, combined with Principal Component Analysis (PCA) and Principal Component – Discriminant Function Analysis (PC-DFA), marking the first application of such an approach for the classification of these bacteria in this study. The results unveiled distinctive biochemical compositions among the selected strains. *P. gingivalis* and *F. nucleatum* exhibited the highest protein and lipid-to-polysaccharide ratios, discerned through the high signal intensities at the amide I and fatty acids regions. Concurrently, variations within the streptococcal strains were observed, particularly in the fingerprint and amide I regions as *S. anginosus* showed the lowest protein-to-polysaccharide ratios. It is worth noting that vibrational techniques offer insights into functional groups, necessitating future investigations utilising Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) for a more comprehensive understanding of protein/peptide molecules. This study not only contributes to comprehending the biochemical diversity of periodontitis-associated bacteria but also represents the first application of FT-IR for microbial community analysis and O-PTIR for single-cell analysis for classifying these bacteria, expanding the analytical techniques for future investigations into their pathogenicity and facilitating the development of targeted therapeutic strategies. Since some of the studied strains are strict anaerobes, the single-cell aspect of O-PTIR highlights its potential for bypassing the long-term culturing requirements of other techniques that require higher biomass.

Poster 14

Metabolomic ageing clock monitors risks of cardiometabolic diseases

Manyi Jia, Kanta Chechi, Abbas Dehghan, Shamin Tahasildar, Declan O'Regan, Janet Lord, Parminder Raina, Mark Lathropd, Marc-Emmanuel Dumas

Abstract:

Age clocks globally describe age-induced alterations on different omic layers at molecular resolution. Metabolomic clocks can capture circulating compounds or enriched biological pathways as ageing signatures. Metabolomic profiling also enables high-throughput data acquisition and makes ageing research readily available in large consortia. Leveraging the Canadian Longitudinal Study of Ageing (CLSA) population (N=~9,500), we constructed a penalised regression model that predicts chronological age from plasma metabolome and tracks risks of cardiometabolic diseases (CMDs). We quantified 1,458 metabolites by untargeted UHPLC-HRMS. Data analysis was performed in Python v3.9 and R v4.2. A LASSO model (with 10-fold nested cross validation, 1000-time bootstrapping) was first developed for age prediction, which selected 375 metabolites in 2815 metabolically healthy individuals unsubmitted to epigenetic profiling. Model was validated in 6617 metabolically unhealthy individuals. Linear regression models were fitted to evaluate associations among different ageing clocks, age gaps, and metabolic outcomes. Adjustment for sex and chronological age and multiple testing correction (Benjamini-Hochberg) were implemented when necessary. Metabolically healthy participants were biologically younger than unhealthy individual. The LASSO model was characterised by 375 metabolites in prediction of chronological age. We further confirmed the correlation between chronological, epigenetic and metabolomic age, and the association between metabolomic and epigenetic age gap. Metabolomic ageing also correlated negatively with HDL, and positively with inflammatory markers, worse glycaemic control, dyslipidaemia, and high blood pressure. Our preliminary findings suggest this metabolomic ageing model associates with clinical markers reflecting CMD risks. Top weighted metabolites selected by the model also denote altered lipid metabolism and chronic inflammation may aggravate biological ageing. The predicted metabolomic age is more closely aligned with the epigenetic aging captured by the Horvath clock (built using 353 CpG sites), instead of the Hannum clock computed from 71 CpGs. This emphasises its potential in prediction of biological aging relevant to adverse metabolic health outcomes.

Poster 15

PrecisionTox: Cross-species, multi-omics analyses as a new approach to chemical hazard assessment

Martin R. Jones, Lauren Cruchley-Fuge, Andrew D. Southam, Gavin R. Lloyd, Matthew J. Smith, John K. Colbourne, Mark R. Viant, Ralf J. M. Weber

Abstract:

Chemicals are crucial to many aspects of the modern world, yet can pose varied and significant risks to human and environmental health. With thousands of new chemicals produced and distributed globally each year, new approaches are urgently required for more rapid, scalable, affordable and ethical assessment of chemical hazards and risks. PrecisionTox, a €20M EU Horizon 2020-funded research project, aims to meet this demand through the development of New Approach Methodologies (NAMs), based on a fusion of: 1) multi-omics analyses, including metabolomics, 2) machine learning, and 3) chemical exposure experiments across phylogenetically-disparate model organisms (Daphnia magna, Danio rerio, Drosophila melanogaster, Caenorhabditis elegans and Xenopus laevis) and the HepG2 human liver cell line. All biosystems will be exposed to (max.) 200 diverse chemicals, yielding > 10000 samples for in-depth molecular characterisation, based on RNA-seq transcriptomics and liquid chromatography-mass spectrometry-based (LC-MS) metabolomics workflows. Resulting data sets will be used to advance understanding of the nature of chemical-induced adverse health effects across diverse model biosystems. They will also provide opportunities to explore the concept of phylogenetic toxicology ("phylotox"), i.e. whether chemical-induced molecular perturbations can be predicted from knowledge of an organism's genetic composition and a chemical's properties. In this work, we present the semi-automated extraction method we have developed for model organisms, based on a Beckman Coulter i7 workstation, to underpin PrecisionTox's multi-omics analyses and facilitate extraction of up to 192 samples per day (five-fold higher than manual equivalent). We also present results from LC-MS metabolomics analyses of resulting extracts, revealing clear and expected shifts in metabolism of non-exposed control samples (e.g. Daphnia magna and Danio rerio), relating to growth and development during the experimental window. For C. elegans and D. melanogaster, we also demonstrate and explore the stark shifts in metabolomes, relative to time-matched control samples, resulting from chemical exposures.

Poster 16

Dietary impact of frog holometabolomes: identification of pumiliotoxin analogues through in-depth metabolomic investigation Oophaga vicentei dermal and gut extracts

Elliot Murphy, Phillip Jervis, Goncalo Rosa, Francesc Puig Castellví, Ines Castro Dionicio, Romina Pacheco Tapia, Brian Gratwicke, Roberto Ibanez, Philippe Froguel, Thomas Bell, Trent Garner, Marc-Emmanuel Dumas

Abstract:

Pumiliotoxins (PTX) are a group of alkaloidal toxins found in the dermal secretions of poison dart frogs (Dendrobatidae) and some species of toads (Bufonidae). These cardiodepressant compounds provide a potent chemical defence against predation and are sequestered from their natural arthropod diet, mainly consisting of mites and ants. Two Panamian Dendrobatidae species of dart frog, Atelopus limosus and Oophaga vicentei, were subject to a chemical investigation of their dermal for PTX content. In this study we applied a targeted and untargeted metabolomic profiling of the skin and gut contents of wild and captive A. limosus and O. vicentei with UHPLC-HRMS. MSn spectra obtained from an IQ-X tribrid orbitrap allowed for the dereplication of compound mixtures in the skin secretions and identification of knowns using the Global Natural Product Social Molecular Networking platform. We document for the first time several known and unknown pumiliotoxin analogues and showed their downregulation after a period in captivity with the absence of a wild diet. We reported bioconversions of pumiliotoxin into frog metabolites, allopumiliotoxins. This study further demonstrates the dietary nature of these compounds and the importance of complex networks of organisms in holobiont and holometabolome studies.

Poster 17

Assessing small polar metabolite detection using mass spectrometry imaging: a comparison of MALDI and LD-REIMS spatial technologies

Georgia Lorentzen, Aleksandra Gruevska, Yu Wang, Daniel Simon, Robert Murray, Zoltan Takats, Marc-Emmanuel Dumas, Zoe Hall

Abstract:

Mass spectrometry imaging (MSI) evaluates the spatial (dis)organisation of metabolites in biological tissue. A variety of technologies can be utilised including among others, matrix-assisted laser desorption/ionisation (MALDI) and laser desorption rapid evaporative ionisation mass spectrometry (LD-REIMS). This study aimed to compare these two technologies for small polar metabolite detection. Metabolite coverage was evaluated on adjacent sections of human liver (10 µm) using LD-REIMS and six common MALDI methods: norharmane, dry-/wet-2,5-dihydroxybenzoic acid (DHB), 9-aminoacridine (9-AA), α-cyano-4-hydroxycinnamic acid (CHCA), and 1,5-diaminoaphthalene (DAN). Untargeted spatial metabolomics was conducted at a pixel size of 25µm for m/z region 100-400 in negative-ion mode by LD-REIMS (REIMS ion source coupled to G2 XS Qtof mass spectrometer) and MALDI (AP-MALDI5 AF ion source coupled to QExactive Plus mass spectrometer). Matrix-associated peaks were filtered from MALDI data through a combination of unsupervised machine learning and spatial distribution checks and remaining peaks were evaluated and compared. A total of 68 features were detected with LD-REIMS. For the MALDI methods, the range for detected *m/z* features was between 29-95 using wet-DHB and DAN, respectively. Tentative annotation was done using the Human Metabolome Database, 11 of features detected with LD-REIMS were annotated, while metabolites annotated from MALDI features varied from 9 (CHCA) to 24 (norharmane). Norharmane offered superior coverage of carbohydrates whilst LD-REIMS was better in detecting nucleotides, lipids, and carboxylic acids. In conclusion, different metabolite classes were detected with different MSI methodologies and utilising a combination of approaches may be required for adequate coverage of small polar metabolites on human liver tissue. Pilot spatial tests demonstrated that fibrotic and parenchymal histological regions of the tissue had different metabolic profiles providing promise for spatial technologies in biomedical applications.

Poster 18

Building informative retention time and MS/MS libraries for reliable metabolite identification in UHPLC-MS metabolomics studies

Hannadi Alamri, Shiva Jalili, Catherine L. Winder and Warwick B. Dunn

Abstract:

Introduction: Liquid Chromatography-Mass Spectrometry (LC-MS) has become the most frequently applied analytical platform in the field of metabolomics and acquires chromatographic retention time (RT), full scan (MS1), and MS/MS data. In discovery (untargeted) studies, identifying metabolites poses a significant challenge due to not having sequenced metabolomes, the non-transferability of RT libraries between assays/laboratories, and the metabolite/lipid coverage available in RT and MS/MS libraries. The Metabolomics Standards Initiative (MSI) categorizes metabolite annotation/identification into four levels, each representing different degrees of confidence and knowledge. Achieving Level 1 identification (highest confidence) can only be facilitated by using retention time and MS/MS libraries (acquired by applying the laboratory-specific assays). To support this within our laboratory, we have developed extensive RT and MS/MS libraries by analysing greater than 1,500 chemical standards of endogenous and exogenous metabolites and lipids.

Method: All metabolite and lipid standards were dissolved in either 4:1 isopropanol/acetonitrile or 3:3:1 methanol/acetonitrile/water and grouped together in mixtures of ten at an approximate concentration of 0.10 – 1.70 Mm. All solutions were analysed applying standard 15-minute assays (aqueous C18 reversed phase, HILIC amide and C18 lipidomics) using a Vanquish H system coupled with a heated electrospray Orbitrap Exploris 240 mass spectrometer (Thermo Fisher Scientific, MA, USA). All data were acquired using three-stepped collision energies (different for positive and negative ion modes). Inclusion lists were applied to ensure MS/MS data were acquired for all detected metabolites.

Results and discussion: Data were processed using XCMS, Compound Discoverer and mzVault to create assay-specific RT libraries and two MS/MS libraries (one for lipidomic assays and one for non-lipidomic assays). Greater than 1500 metabolites and lipids were analysed and incorporated into the libraries from across a wide range of metabolite and lipid classes with a portion not available in other RT and/or MS/MS libraries. The RT and MS/MS libraries will be shared for community use once completed.

Poster 19

Anion-exchange chromatography-mass spectrometry (AEC-MS) for untargeted plant metabolomics

John Sidda, John Walsby-Tickle, Cara Griffiths, Matthew Paul, Ben Davis, James McCullagh

Abstract:

Ion-chromatography-mass spectrometry has played an important role in the analysis of plants for many years due to the highly polar and ionic nature of many plant metabolites. With the advent of online electrolytic ion suppression, IC-MS is increasingly used in untargeted metabolomics studies. Although IC-MS has been deployed in studies for targeted quantitation of specific plant metabolites such as disaccharide monophosphates, relatively few studies have adapted its use for untargeted plant metabolomics. The purpose of this study was to develop a method suitable for untargeted profiling of plant metabolites extracted from both fresh weight and lyophilised plant material using laboratory grown A. thaliana as an example. Recovery of a suite of standard compounds across a range of concentrations was assessed using various extraction solvents with and without additional drying steps to concentrate extracts or additional clean up steps (solid-phase extraction, filtration). All data were obtained by anion-exchange chromatography with online eluent generation using a Dionex ICS-5000+ Ion-chromatography system coupled to a Thermo Q Exactive hybrid quadrupole-Orbitrap mass spectrometer. Up to 8000 compound-features were recorded per run with an IC gradient time of <30 min, with putative identifications of approximately 1500 compounds based on MS1 m/z, isotope pattern matching and product ion spectra. To improve the quality of assignment, the IC gradient length and ionic strength of the hydroxide eluent were altered, allowing compounds previously indistinguishable by MS product ion spectra (for example monosaccharide 6-phosphates) to be identified based upon retention times by comparison with metabolites in our in-house compound library. This method holds promise in plant metabolomics, complementing traditional reverse-phased methods to better understand the role of primary metabolites; for example citric acid cycle, Calvin cycle intermediates and nucleotides which are often challenging to resolve chromatographically. Hypothesis-generating metabolomics studies can facilitate lead compound identification and functional insights.

Poster 20

Applying Fourier-transform Infrared spectroscopy to characterise and differentiate Achromobacter and Pseudomonas Biofilms

Joel Doherty, Sian Pottenger, Jo Fothergill, Roy Goodacre, and Howbeer Muhamad Ali

Abstract:

Antimicrobial resistance (AMR) poses a significant threat to global biosecurity and healthcare. AMRs' pervasiveness and emergence across various settings (natural reservoirs, industrial facilities and clinics) contributes to diminishing productivity and loss of human life. Over 80% of human bacterial infections are associated with biofilms. Failing a paradigm shift, it is forecasted that by 2050 AMR will contribute to more than 10 million avoidable deaths globally. Key AMR mechanisms are described, yet biofilms' significance is poorly understood. Biofilm formation is a universal bacterial feature; current understanding dictates they limit contact with environmental stressors via the action of secreted extracellular polymeric substances (EPS). Bacteria live synergistically in these multispecies communities, compounding the effects of AMR and complicating differentiation. Through the application and development of cryo-sectioning methodologies, molecular imaging techniques and spectroscopy approaches this Biotechnology and Biological Sciences Research Council project aims to address this niche by better describing responses to antimicrobial exposure and adaptive evolution. Furthermore, drug targets, penetration and distribution within and across Pseudomonas biofilms will be probed. The project aims to study the communal and cellular response to antibacterial agents via optimisation, scrutiny and establishment of novel label-free 3D imaging of bacterial cells. Appropriate biofilm embedding, and cryo-sectioning methodologies will facilitate subsequent analysis of bacterial-drug interaction dynamics and drug distribution over time, utilising: stimulated Raman scattering (SRS), coherent anti-stokes Raman scattering (CARS) and optical photothermal infrared (O-PTIR) spectroscopy. Stable isotope probing (SIP), alongside molecular imaging techniques [Raman and Infrared micro-spectroscopy and Desorption Electrospray Ionisation (DESI) mass spectrometry imaging], will be leveraged to provide biofilm depth profiles and indicate metabolic activity and drug distribution across various depths. The current poster explores exploiting Fourier-transform infrared spectroscopy (FTIR) to characterise, quantify and differentiate Achromobacter and Pseudomonas Biofilms. Two genera that modern approaches [such as Matrix-assisted laser desorption/ionisation (MALDI)] has struggled to differentiate.

Poster 21

The workflow for building high-quality spectral libraries using UHPLC - HRMS applied for human metabolomics studies

Romina Pacheco Tapia, Francesc Puig Castellvi, Inés Castro, Chaima Touaibi, Zhaojie Wang, Elliot Murphy, Sachin Dubey, Philippe Froguel, Marc-Emmanuel Dumas

Abstract:

The gold standard for metabolite annotation involves matching experimental MS/MS data against a spectral library and transferring compound names when a high score is achieved (MSI level 2 or 3 annotation). To maximize the number of identified MS/MS spectra with higher confidence and increase sensitivity when measuring complex mixtures of analytes, achieving level 1 annotation is necessary. This work aimed to develop high-quality in-house spectral libraries containing a collection of over 2800 high-purity biochemical molecules. The library spans a broad range of chemical space, including key primary metabolites and intermediates (MSMLSTM – IROA), metabolites from the Human Metabolome Library (MetaSci), and FDA and EMA approved drugs with pharmacological activities (Prestwick Chemical Library®). Chemical standards were prepared and pooled according to their solubility, LogP values, and adduct mass differentiation for compounds eluted within the same chromatographic method. These standards were then analyzed by multiplexed injections using a UHPLC coupled with a high-resolution Orbitrap Exploris 240 mass spectrometer, utilizing both C18 and HILIC columns in negative and positive ESI modes. A semi-automated pipeline was employed for initial data processing, followed by manual data curation to ensure high-quality results. Criteria for inclusion in the library included high-quality MS1 peaks, concordant retention times, and informative MS2 spectra. The outputs of this work include the development of in-house spectral libraries segregated by ionization modes and chromatography techniques. These libraries are available in multiple file formats, including Mascot Generic Format (.MGF), NIST MSP (.MSP), and SQLite database formats (.DB). These in-house libraries enable the characterization of metabolites in samples from different human cohorts analyzed on the Integrative-Metabolome Analytics for Translational and Precision Medicine (IMPACT-PM) platform in Lille. The semi-automated pipeline enhances the utility of the libraries in metabolomic research by segregating them based on ionization modes and chromatography techniques.

Poster 22

Implementation of a semi-automated sample preparation workflow for comprehensive serum metabolomics

Inés Y. Castro-Dionicio, Romina Pacheco-Tapia, Francesc Puig-Castellvi, Sachin Dubey, Elliot Murphy, Philippe Froguel, Marc-Emmanuel Dumas

Abstract:

Metabolomics plays a pivotal role in clinical research, uncovering variations in the human metabolome and its correlation with health-related outcomes. However, the analysis of large-scale studies poses a significant challenge for many untargeted analytical methods. Consequently, the automation of sample processing and analysis has emerged as a crucial advancement to meet the demanding experimental throughput. In this study, we established and evaluated a high-throughput metabolomics workflow for processing commercial human serum samples. Samples underwent extraction for metabolomics analyses using a Janus G3 (Revvity) liquid handling robot, employing two different extraction methods suitable for two separate and complementary chromatographic methods (C18 and HILIC) on 96-well plates. Samples were analyzed using ultra-performance liquid chromatography coupled with HRMS (Vanquish Duo system coupled to a Thermo Scientific Orbitrap Exploris 240 mass spectrometer) in positive and negative ionization modes on the Integrative-Metabolome AnalytiCs for Translational and Precision Medicine (IMPACT-PM) platform in Lille. This workflow was developed and characterized with an analytical batch based upon a 96-well plate containing 72 samples, 11 SQCs, 3 RQCs, 2 blanks, 5 dilution quality control samples. As proof-of-concept, the workflow was tested with a small cohort of serum human samples. We assessed the coefficients of variation (CVs) for both the total number of detected and annotated features across all samples and quality control samples (QCs), and analyzed intensity plots across the injection sequence. To monitor extraction efficiency and data quality, we employed extraction internal standards (eISs) and technical internal standards (tISs), all of which showed CVs < 20% in the QCs. This workflow provides expanded coverage of serum metabolites, rendering the platform suitable for conducting serum untargeted metabolomic analysis, and it will prove beneficial for applications in larger cohort studies.

Poster 23

Aqueous humour metabolome analysis discloses altered arginine metabolism in Parkinson's disease

Joan Serrano-Marín, Alberto Iglesias, Silvia Marin, David Bernal-Casas, Alejandro Lillo, Marc González-Subías, Gemma Navarro, Marta Cascante, Juan Sánchez-Navés, Rafael Franco

Abstract:

Background: The lack of accessible and informative biomarkers results in a delayed diagnosis of Parkinson's disease (PD), whose symptoms appear when a significant number of dopaminergic neurons have already disappeared. The retina, a historically overlooked part of the central nervous system (CNS), has gained recent attention. It has been discovered that the composition of cerebrospinal fluid influences the aqueous humour composition through microfluidic circulation. In addition, alterations found in the brain of patients with PD influence the retina's metabolome. This new paradigm highlights the potential of the aqueous humour as a sample for identifying differentially concentrated metabolites that could, eventually, become biomarkers if also found altered in blood or CSF of patients. In this research we aim at analysing the composition of the aqueous humour from healthy controls and PD patients. Methods: A targeted metabolomics approach with concentration determination by mass spectrometry was used. Statistical methods including principal component analysis, machine learning, and linear discriminant analysis were used to select differentially concentrated metabolites that allow distinguishing patients from controls. Results: In this first metabolomics study in the aqueous humour of PD patients, elevated levels of 16 compounds were found; molecules differentially concentrated grouped into biogenic amines, amino acids, and acylcarnitines. A biogenic amine, putrescine, alone could be a metabolite capable of differentiating between PD and control samples. The altered levels of the metabolites were correlated, suggesting that the elevations stem from a common mechanism involving arginine metabolism. Conclusions: A combination of three metabolites, putrescine, tyrosine, and carnitine was able to correctly classify healthy participants from PD patients. Altered metabolite levels suggest altered arginine metabolism.

Poster 24

Unravelling Fungal-Plant Interactions through Untargeted Metabolomics and Mass Spectrometry Imaging Tools

Marina Ferreira Maximo, Daniel Yuri Akiyama, Renata Moro Baroni, Jonas Henrique Costa, Mario Ramos de Oliveira Barsottini, Jorge Maurício Costa Mondego, Gonçalo Amarante G. Pereira, Taicia Pacheco Fill

Abstract:

The Witches' Broom Disease (WBD) has seriously impaired cocoa farming (chocolate's source) in South America since the 20th century. This fungal disease is caused by *Moniliophthora perniciosa*, a basidiomycete phytopathogen with a hemibiotrophic lifestyle. Although WBD is a widely investigated pathosystem, very little is known about the secondary metabolites involved in the infection. However, uncovering the phytopathogen's chemical mechanisms for overcoming the plant's defence response could be crucial for developing disease control strategies. In this context, we aimed to investigate the chemical relationship between the fungal pathogen and plant host through untargeted in vivo LC-HRMS-based metabolomics, mass spectrometry imaging (MSI), and transcriptomics of healthy and infected fruits in the necrotrophic stage of WBD (collected in the field in Bahia, Brazil), alongside in vitro biocatalysis assays with *M. perniciosa*. Multivariate statistical analyses of our metabolomics data showed a significant metabolic alteration within cocoa tissues due to the infection, with increased phytohormones and polyphenols and the appearance of fungal metabolites in necrotic fruits; DESI-MSI data showed the spatial distribution of plant and fungal metabolites involved in the infection and observed in the metabolomics dataset. We also observed a significant decrease in glycosylated flavones and an increase in their respective aglycones in infected cocoa. In vitro biocatalysis assays with *M. perniciosa* fed with a cocoa glycosylated flavone confirmed the fungus cleaves the sugar unit of the molecule, which could be an important attribute of its pathogenicity in vivo.

Poster 25

MetaboLights - open access metabolomics resource

Callum Martin, Noemi Tejera, Felix Xavier Amaladoss, Mark Williams, Ozgur Yurekten, Thomas Payne, Claire O'Donovan, Juan Antonio Vizcaíno

Abstract:

Data sharing is crucial for the advancement of metabolomics, accelerating scientific discoveries and promoting the development and adoption of standardised protocols, community practices and quality control measures that enhance data interoperability. MetaboLights (https://www.ebi.ac.uk/metabolights/) is a global database for metabolomics studies including the raw experimental data and the associated metadata. The database is cross-species and cross-technique and covers metabolite structures and their reference spectra as well as their biological roles and locations. The adoption of standards (data/metadata) and the usage of ontologies with expert curation adds value and enables the reanalysis of studies by the community as well as compliance to FAIR (findability, accessibility, interoperability and reusability) data principles. Our repository receives individual submissions and enables programmatic submissions for companies, Phenome centres and other large-scale laboratories. The team works closely with international repository partners and collaborates in the development of tools that support discovery of metabolomics and multi-omic research (MetabolomeXchange and OmicsDI) as well as with data standard development efforts (mQACC and OORF). As active members of the metabolomics community, MetaboLights is also involved in initiatives that promote public awareness and community engagement, delivering courses and webinars to introduce metabolomics to the general public and researchers of other disciplines, and hosting workshops at international conferences.

In an effort to facilitate MetaboLights data reuse, the team has developed MetaboLights Labs (https://metabolights-labs.org/), an open source and open access Galaxy Project instance, with publicly available tools and optimised workflows for LC-MS and NMR to use on public data or the users' own data.

MetaboLights strives to enhance reusability of metabolomics data, developing workflows, reinvigorating standards, and promoting collaborations, public awareness and community engagement.

Poster 26

Differentiation between patients with Multiple Sclerosis (MS) and Clinically Isolated Syndrome (CIS) based on UHPLC-QTOF MS untargeted serum lipid profiling

Olga Begou, Petros Pousinis, Natalia Konstantinidou, Christos Bakirtzis, Thomai Mouskeftara, Georgios Theodoridis, Marina Boziki, Nikolaos Grigoriadis, Helen Gika

Abstract:

Multiple sclerosis (MS) is a demyelinating disease of the Central Nervous System (CNS), with Relapsing-Remitting MS (RRMS) being the more frequent form. Clinically isolated syndrome (CIS) often signifies a first demyelinating episode with increased risk of later conversion to RRMS. The identification of biomarkers that may predict the transition from CIS to MS is crucial. The goal of our study was to identify potential lipid biomarkers that could distinguish between these two patient groups, namely, patients with a first demyelinating episode, either in the context of RRMS or CIS, thus elucidating blood-based biomarkers with a potential to depict the pathological process of CIS-to-RRMS conversion. Serum samples from 80 patients in total, including n=18 CIS and n=62 MS, were collected and untargeted lipidomics analysis was performed using a UHPLC-HRMS (TIMS-TOF/MS) platform. Multivariate and univariate statistical analyses revealed distinct differences in the serum lipid profiles between the two groups. Differentially expressed lipid species were identified based on the set criteria of VIP > 1 and q-value < 0.05. Moreover, Spearman correlation analysis was utilized to assess the relationships between the levels of lipid biomarkers and clinical (Expanded Disability Status Scale / EDSS) and radiological (MRI) data. Our analysis identified nine lipid species, including cholesteryl esters (CEs), lysophosphatidylcholines (LPCs), and phosphocholines (PCs), which were significantly different between CIS and MS patients. These lipid biomarkers also showed significant correlations with the number of T2/FLAIR brain and spinal MRI lesions and EDSS scores within the study cohort. Our findings provide proof-of-principle evidence that serum-based lipidomic analysis may elucidate alterations across the CIS-to-RRMS disease continuum, thereby exhibiting potential to elucidate biomarkers that may identify a CIS population at increased risk of conversion to MS. Further large-scale studies are necessary in order to elucidate this field.

Poster 27

Chemical attributes of UK-grown tea- Dartmoor Estate Tea

Alina Warren-Walker, Laura Lyons, Jasen Finch, Thomas Wilson, Jo Harper, Katherine Bennett, John Draper, Manfred Beckmann, Amanda J Lloyd

Abstract:

The Dartmoor Estate Tea plantation in Devon, UK, is renowned for its diverse tea cultivars and commitment to high-quality tea production. The estate's unique microclimate and varied soil conditions contribute to the distinctive flavours and chemical profiles of the teas it produces. The chemical diversity of Camellia sinensis leaf samples were assessed via samples collected from various garden locations within the Dartmoor Estate tea plantation. These samples, which differ by location, cultivar, time of day, and variety, were analysed using Flow Infusion Electrospray Ionisation Mass Spectrometry (FIE-MS). The FIE-MS analysis was performed with an Exploris 120 mass analyser coupled with a Dionex Vanquish UHPLC system (Thermo-Scientific), measuring ion intensities within the m/z range of 55 to 1200. Random forest classification was performed to examine differences between Georgian tea plants located in two gardens. The analysis revealed no significant differences between these garden locations, with a margin value of 0.00443 ($p = 0.572$, 3000 permutations). However, a significant degree of variability was observed within tri-clonal variants ($p < 0.05$, 3000 permutations). Among these variants, TCL-09 exhibited greater similarity to the Georgian varieties compared to TCL-08 and TCL-11, while TCL-11 was found to be most similar to TEV-01. Additionally, random forest regression identified 174 m/z features that were significantly associated with the sampling time point (% increase in mean squared error, $p < 0.05$, 3000 permutations). In addition to these findings, functional and structural enrichment analyses of the chemical clusters revealed that the structural subclass 'Carbohydrates and carbohydrate conjugates' and the metabolic pathway 'Starch and sucrose metabolism' were significant for cluster 1. The abundance of these features increased throughout the day and began decreasing during the night. This research highlights the complex interplay of cultivars, geographical location, and temporal factors on the chemical composition of tea leaves. It provides insightful data on the metabolic pathways influencing tea production and underscores the importance of these variables in determining the final chemical profile of tea products.

Poster 28

LC-MS-based untargeted metabolomics of Bambara groundnut for food security in response to drought – root to seed

Sandra Martinez-Jarquin, Luis Salazar-Licea, Alison Whitby, Christopher Moore, Nichole Yang, Paulina Fleece, Sean Mayes, Dong-Hyun, Kim

Abstract:

Metabolomics has the potential to distinguish between different biological states in various organisms, including plants. In this study, the metabolomic profiles of Bambara groundnut (BG) were analyzed using an untargeted approach in methanolic extracts of leaves and seeds. With the increasing impact of climate change, there is a need to identify plants that can thrive in extreme conditions to sustainably support the growing population. BG, a drought-resistant African legume, shows promise for cultivation in harsh environments. The study involved the assessment of five genotypes of BG under drought and irrigation conditions using LC-MS. The analysis employed an LC Ultimate 3000 with a Sequant zic-pHILIC column and a QExactive mass spectrometer. Additionally, pooled QC samples and a set of 268 standards in 5 mixtures were used for the analysis.

Data analysis was conducted using a modified metabolomic workflow in Compound Discoverer 3.3 and SIMCA P for multivariate analysis, encompassing preprocessing and annotation steps. Furthermore, for the analysis of seed samples, the Random Forest with Boruta algorithm (R 4.3.2) was utilized due to the lack of observable differences with traditional analysis techniques. Compound annotation was performed using online databases and authentic standards.

The identification of key compounds associated with drought in BG represents a significant contribution to enhancing our understanding of this underutilized crop. Moreover, it forms a pivotal part of a larger project that integrates various omics techniques.

Poster 29

Influence of SiNPs in soybean plants: an NMR-based metabolomic approach

Amanda Lemos Quintela, Marco Aurélio Zezzi Arruda, Claudio Francisco Tormena

Abstract:

With the advancement of nanotechnology, nanoparticles (NPs) have emerged with applications in several fields, especially in agricultural production. Among metallic NPs, the production and use of silicon NPs (SiNPs) have been shown to have beneficial effects on plant growth, development, yield and increased resistance to diseases, suggesting their important role under biotic and abiotic stresses. The main aim of this study was to investigate the effect of SiNPs and SiO2 in the production of secondary metabolites in leaves, stems and roots in transgenic soybean plants (RR and Intact) through a NMR-based metabolomic approach. The plants evaluated in this investigation were cultivated in a germination chamber following three different treatments: T0 (control), T1 (with addition of SiNPs) and T2 (with addition of SiO2). The metabolite extraction methodology was performed in deuterated methanol. 1H NMR spectra were acquired on a Bruker AVANCE III NMR spectrometer, 9.4 T, using the zgpr pulse sequence at 298 K. From 1D and 2D NMR spectra of leaves, stems and roots, the presence of lipids, amino acids, organic acids, sugars and polyphenols was observed. Additionally, it was found that there was no qualitative difference in the metabolites of the 1H NMR spectral profiles for the two transgenics in T1 and T2 when compared to the control group, suggesting that the application of Si does not affect the secondary metabolites. From the PCA, it was possible to differentiate the three treatments applied, mainly based on the content of fatty acids, pinitol, genistein, fumaric acid, among others.

Poster 30

Liverpool Annotation of metabolites using Mass sPectrometry Software (LAMPS) - an adaptable software for metabolite annotation using full-scan mass spectrometry data

Wanchang Lin, Callum Martin, Ozgur Yurekten, Noemi Tejera, Thomas Payne, Timothy M.D. Ebbels, Juan A. Vizcaino, Claire O'Donovan and Warwick B. Dunn

Abstract:

Introduction: The annotation or identification of metabolites detected in untargeted metabolomics studies applying liquid chromatography-mass spectrometry can apply full-scan (MS1), retention time (RT) and gas-phase fragmentation (MS/MS) data. Full-scan MS1 data can be applied as a first pass to decrease the number of possible annotations. However, electrospray ionisation is a complex process which generates multiple features (m/z-RT pairs) for each metabolite. The grouping of features and subsequent linkage to a single or multiple molecular formula is required to maximise true positive annotations and minimise false positive annotations. Liverpool Annotation of metabolites using Mass sPectrometry Software (LAMPS): We have constructed a Python package as an easy-to-use software for feature grouping and metabolite annotation using MS1 data only. LAMPS groups features based on RT similarity and positive correlations across multiple biological samples. Genome-scale metabolic models are the source of metabolites applied in reference files though any source of metabolites can be used (e.g. HMDB or LIPID MAPS). The m/z differences related to in-source fragments, adducts, isotopes, oligomers and charge states can be user-defined in the reference file. The software is available as a Python package and will be integrated into Metabolights Labs (https://metabolights-labs.org/). Testing: The open-source software has been tested with a range of datasets collected using different LC-MS systems and sample types where metabolite annotations were known or deduced manually by an expert. The influence of reference file size (number of metabolites present) has also been tested. These results will be presented. Of importance, the software provides putative annotations which require validation using MS/MS and retention time data in libraries for MSI level 1 identifications, where feasible.

Poster 31

Simulated metabolic profiles unveil biases in pathway analysis methods

Juliette Cooke, Cecilia Wieder, Nathalie Poupin, Clément Frainay, Timothy Ebbels and Fabien Jourdan

Abstract:

Initially developed for transcriptomics data, pathway analysis (PA) methods can produce biased interpretations when applied to metabolomics data, especially if input parameters are not chosen with care. This is particularly true for exometabolomics data (e.g. the sample is a biofluid or cell medium), where there can be many metabolic steps between the measured external metabolites and actual internal disruptions within the biological system. However, evaluating PA methods using experimental data is practically impossible when the system's "true" metabolic disruption is unknown. A major advantage of in silico metabolic modelling is the ability to control the metabolic state, allowing for the creation of disruptions in metabolic networks and the simulation of the associated exometabolomic profile. Here, we use constraint-based modelling to simulate metabolic profiles for entire pathway knockouts, in a human genome-scale metabolic network. This approach provides both a known disruption site (knocked-out pathway), as well as a simulated metabolic profile. These simulated metabolic profiles (our simulated ground truth data) can then be used as input for PA methods, which should be able to detect the known disrupted pathway among the significantly enriched pathways for that profile. Through network-level statistics, visualisation, and graph-based metrics, we show that even when a given pathway is completely blocked in the network, it may not be retrieved as significantly enriched when using PA methods with its corresponding simulated metabolic profile. This can be due to various reasons such as the chosen PA method, the initial pathway set definition, or the network's inherent structure. Consequently, this work highlights how the nature of some metabolomics data may not be suited to typical PA methods, provides information on how difficult it may be for each pathway to be enriched based on various properties, and also serves as a benchmark for analysing, improving and potentially developing new PA tools.

Poster 32

Future possibilities for the SEISMIC facility and overview of current research and development projects

Jake Penny, Melanie Bailey, Emily Fraser, Johanna von Gerichten, Kyle Saunders, Abigail Cook, Anastasia Kontiza

Abstract:

SEISMIC (spatially resolved single and sub-cellular omics) is an initiative to develop a national UK facility for single cell omic analyses. SEISMIC allows spatially resolved and sub-cellular sampling from live cell culture. This is achieved through nano-capillary sampling under confocal microscopy utilizing Yokogawa's SS2000. The spatial resolution and live cell sampling capability provides a new and exciting opportunity to investigate spatial aspects of metabolic processes between cells. At SEISMIC, we are exploring downstream analysis of lipids, metabolites and proteins using nano-spray ionization MS (NSI-MS) and LC-MS. The SEISMIC group has developed robust workflow utilising the SS2000 with LC-MS for single cell lipidomics, which will be described in this presentation. We will show how single cell lipid profiles can be reproduced across sampling methodologies and between laboratories. We will also show how this single cell lipidomics approach is sufficient to detect response to biological stimuli such as radiation, peroxide treatment and drug exposure.

Poster 33

Annotation and Dereplication of Oxylipins in Human Urine Using LC- HRMS/MS and Molecular Networking: Insights from the Valencian Community

Ángel Sánchez-Illana, Pablo Miralles, Jaume Béjar-Grimalt, Clara Coscollà, Thierry Durand, Jean-Marie Galano, David Pérez-Guaita

Abstract:

Oxylipins, which include both enzymatic and non-enzymatic oxidized derivatives of polyunsaturated fatty acids (PUFAs), are crucial mediators in oxidative stress, inflammation, and various physiological and pathological processes. However, their untargeted analysis in non-invasive biofluids like human urine is challenging due to their isomeric diversity, complex metabolism, and low concentration levels. To overcome these challenges, we employed an innovative non-targeted LC-HRMS-based epilipidomic strategy. This approach builds upon previous analyses of in vitro PUFAs oxidations and plasma samples, integrating a streamlined oxylipin extraction process for urine samples. This study analyzed 491 urine samples from a demographically representative population within the Valencian Community, as part of the BIOMOVAL study (available at the IBSP-CV Biobank). Sample preparation involved a solid-phase extraction (SPE) protocol using 96-well plates, which was followed by LC-HRMS/MS data-dependent acquisition (DDA) analysis on an Orbitrap ID-X Tribrid Mass Spectrometer. Data processing was conducted using mzmine to extract feature tables and MS2 spectra. For annotation and dereplication, molecular networking workflows through the GNPS ecosystem were conducted, leveraging public MS2 libraries including novel NEO-MSMS library to identify compounds. This study aims to generate a comprehensive dataset for the exploration of oxylipin metabolites in human urine. The findings will enhance our understanding of oxylipin metabolic profiles linked to various environmental exposures and lifestyle factors within a representative adult population.

Poster 34

No Time to Dye - Portable Surface Enhanced Raman Spectroscopy for On-site Detection of Sudan Dye in Palm Oil

Joe Stradling, Wilfred Abia, Howbeer Muhamadali, Roy Goodacre

Abstract:

Palm oil is the most widely used and profitable vegetable oil in the world and is estimated to be in almost half of all packaged products found in supermarkets including pizza, chocolate and toothpaste. However, due to the demand and popularity of palm oil, there is enormous potential for economically motivated food fraud. Recently there is growing evidence concerning the addition of Sudan dyes to intensify the red colour of palm oil to make it appear more nutritionally beneficial, and therefore more economically profitable. However, Sudan dyes are classed as group 3 carcinogens by the International Agency for Research on Cancer (IARC) and have since been banned as food additives. Traditionally, methods used for the detection of these dyes are often time consuming and require extensive sample preparation. Instead, surface-enhanced Raman scattering (SERS) is a powerful spectroscopic technique which harnesses the optical properties of plasmonic nanostructures to amplify Raman scattering of trace analytes and can significantly reduce sample analysis time. Using a portable DeltaNu Advantage Raman spectrometer equipped with a laser wavelength of 785 nm, we show the potential for this technique to detect low concentrations of Sudan dye in palm oil whilst simultaneously allowing an on-site real-time analysis to support ethically produced palm oil and promote transparency within the industry.

Poster 35

Analytical techniques to study the formation and structure of homogentisic acid-derived pigment

Harriet Willett, Cassio A Lima, Juliette H Hughes, Brendan P Norman, Lakshminarayan R Ranganath, James A Gallagher and Royston Goodacre

Abstract:

Alkaptonuria (AKU) is a rare, recessive genetic disorder caused by mutations in the gene encoding for the homogentisate 1,2-dioxygenase (HGD) enzyme. This halts the tyrosine and phenylalanine metabolic pathway leading to an accumulation of homogentisic acid (HGA). Consequently, an alternative metabolism takes place forming a dark brown or black HGA-derived pigment. This pigment is deposited into connective tissues in a process called ochronosis. This causes tissues to become hard and brittle leading to ochronotic osteoarthritis which is painful and debilitating. There is currently no cure although a recently approved drug 'Nitisinone' can halt pigmentation and slow disease progression. As a consequence of being a rare disease, there are many unanswered questions surrounding AKU. Apart from its colour, little is known about the characteristics of HGA-derived pigment. It has always been thought that oxidation of HGA to benzoquinone acetic acid (BQA) and subsequent reaction of BQA forms pigment. However, this originates from structural similarity between HGA and hydroquinone and is supported by little evidence. In contrast, there is increasing evidence that pigment formation occurs via a radical reaction. Additionally, pigment structure is unknown. Similarity to melanin has led to polymeric conclusions although this is neither proven nor disproven. Increasing understanding of pigment could help in working towards a cure. Increased understanding could help to elucidate the mechanism by which tissues and pigment interact in ochronosis. This could identify therapeutic targets with the potential of removing or preventing pigmentation vastly improving patient quality of life. This presentation highlights the progress made in studying HGA-derived pigment. Spectroscopic techniques including Raman and Fourier Transform infrared have been combined with other analytical techniques such as gel permeation chromatography and mass spectrometry. This has enabled us to increase understanding of pigment structure and the reaction leading to its formation.

Poster 36

Comparison of different reversed-phase liquid chromatography stationary phases for untargeted and semi-targeted metabolomics assays

Catherine L. Winder, Wanchang Lin, Hannadi Alamri, Shiva Jalili and Warwick B. Dunn

Abstract:

Introduction: Reversed-phase is the most frequently applied stationary phase in liquid chromatography-mass spectrometry assays used in metabolomics studies to investigate water-soluble metabolites. C18 stationary phases dominate in reported metabolomic studies. However, other potentially complementary reversed phase stationary phases are available (e.g. phenyl, biphenyl and PFP) and could provide complementary or improved chromatographic resolution and separation. The research presented compares different reversed-phase stationary phases and assess similarities and differences between reversed-phase and HILIC stationary phases for water-soluble metabolites.

Methods: Ten-component mixtures of metabolite standards (>800 metabolites) as well as mammalian biological samples were analysed applying four different stationary phases (C18, biphenyl, PFP and HILIC-amide) using a Vanquish UHPLC system coupled to an electrospray Exploris 240 mass spectrometer operating in positive and negative ion modes. Raw data processing was performed using Compound Discoverer v3.3. Chromatographic resolution and selectivity were assessed to compare the four stationary phases.

Results and conclusion: All four stationary phases provided some complementarity in either/and elution series, retention times or chromatographic resolution. No single stationary phase retained all metabolites. The choice of stationary phase should be based on the classes of metabolites to be investigated.

Poster 37

Investigating the metabolomic profile of a novel bacterial isolate grown on terephthalic acid (TPA) and ethylene glycol (EG) for the sustainable production of valuable chemicals

Hiya Deshpande, Dong Hyun Kim, Samantha Bryan, Rachel Lousie Gomes

Abstract:

Polyethylene terephthalate (PET) is produced using two key raw materials – terephthalic acid and ethylene glycol. Even though PET is more recyclable than other polymers, it ends up in landfills and creates a significant ecological problem. In recent years, biological methods of PET degradation have been identified, which utilise microbial enzymes such as hydrolases and cutinases which degrade the ester bonds of PET. The breakdown products, TPA and EG can be utilised by some microorganisms and could act as a feedstock to obtain a fully biologically process for recycling PET into alternate useful chemicals. This project investigates the metabolic capability of a novel bacterial isolate, Bacteria X*, capable of degrading TPA using untargeted metabolomics to identify the metabolic pathways affected, and gain insight into its ability to produce valuable compounds.

Bacteria X was cultured in M9 (minimal media) with 10 mM TPA, 10 mM glucose and 10 mM TPA+EG as the carbon sources in different growth experiments. The growth was determined using dry cell weight and the consumption of TPA from media was measured using HPLC. The spent media and cell pellets were analysed using liquid chromatography mass spectrometry (LC-MS). The metabolomic analysis of the spent media revealed potential value-added products, including fatty acids and metabolites which highlight the biotechnological potential of the Bacteria X. Statistical analysis shows that the overall metabolomic profile of the bacteria grown in TPA differs from that grown in glucose or TPA + EG. Further analysis is required to identify the pathways upregulated in Bacteria when grown on TPA compared to glucose. This project highlights the potential of using the metabolism of microbes for industrial and environmental applications ranging bio-manufacturing to bioremediation.

*unnamed due to IP restrictions

Poster 38

Profiling the dynamic metabolome of colorectal cancer cells following irradiation

Aaron Kler, Matthew Fok, Gabrielle Grundy, Marco Sciacovelli, Catherine L. Winder, Warwick Dunn, Dale Vimalachandran

Abstract:

Background: Locally advanced rectal cancer (LARC), treated with preoperative chemoradiotherapy (NCRT), displays varying treatment responses. Despite extensive research in the "omics" fields, clinical practice lacks predictive biomarkers to guide the response NCRT in LARC. Metabolomics has successfully identified biomarkers for cancer diagnosis, therapy, and prognosis, including in colorectal cancer. However, no predictive biomarkers of response/non-response to NCRT have been reported. Therefore, the aim of this study was to identify the metabolic alterations in in-vitro models associated with the irradiation of colorectal cancer cell lines to identify potential biomarkers to enhance the predictive capacity of NCRT

Methods: Three colorectal cancer cell lines (HCT-116, HT29 and MDST8) were irradiated with a single rapid dose of 4 Gy. Cells were quenched and extracted at 1, 4 and 24 hours post-irradiation. Biphasic cell metabolome extraction was performed followed by complementary HILIC and reversed-phase lipidomic untargeted UHPLC-MS assays.

Results: No differentially accumulated individual metabolites were shared amongst the three cell lines. However, several common amino acid pathways were found to be statistically significant after pathway analysis between the cell lines. These included branched chain amino acids (valine, leucine, isoleucine); glutamate and aspartate metabolism; cysteine and methionine metabolism as well as glycine, serine and threonine metabolism

Conclusion: The shared amino acid pathways may contribute to radio-resistance by enhancing energy generation, reactive oxygen species neutralisation, and double-stranded DNA repair. Further research into these pathways is warranted to combat these survival mechanisms against radiation.

Poster 39

Development of a semi-targeted metabolomics assay for analysing mammalian urine and faeces by GC-MS

Shiva Jalili, Hannadi Alamri, Wanchang Lin, Catherine L. Winder and Warwick B. Dunn

Abstract:

Introduction: Metabolomics, the comprehensive study of metabolites within biological systems, is crucial in understanding fluctuations in metabolite levels due to genetic or environmental changes. Semi-targeted assays, which combine the advantages of untargeted and targeted assays, offers significant rewards such as simpler data processing and biological interpretation. LC-MS is the most frequently applied analytical platform in metabolomics; however, gas chromatography-mass spectrometry (GC-MS) offers many advantages including high sensitivity, higher chromatographic resolution, and higher rates of metabolite identifications. However, to provide highly confident biological interpretations, sample-specific metabolome annotation lists are required. This research aims to define sample-specific metabolome annotations and develop a semi-targeted gas chromatography-mass spectrometry assay to routinely detect and identify metabolites in urine and faeces samples.

Method: All publicly available GC-MS, LC-MS, and NMR datasets available in MetaboLights and Metabolomics Workbench containing data for mammalian urine and faeces which were deposited before July 2022 were applied. Metabolite annotations/identifications were downloaded, integrated into a single comprehensive file, and assigned InChIKeys, using the Chemical Translation Service and manual searches of PubChem. The frequency of metabolites reported in both data repositories were calculated and the metabolites detected in greater than four studies were considered routinely detected.

Results and Discussion: Ther research revealed that MetaboLights and Metabolomics Workbench contain 109 urine and 155 faeces studies (54 GC-MS, 170 LC-MS, and 40 NMR studies). A total of 2,037 metabolites (1717 for faeces and 320 for urine) were reported in more than four studies. This comprehensive list of metabolites provides a valuable resource for defining target lists for semi-targeted assays. Many of these metabolites are available commercially as chemical standards and are currently being analysed to develop sample-specific semi-targeted metabolomics assays for urine and faeces.

Poster 40

Metabolomic analysis of primary head and neck squamous cell carcinoma (HNSCC) tissue reveals changes to one-carbon metabolism that associate with the disease phenotype

Nikolaos Batis, James Higginson, Gavin R. Lloyd, Matthew J. Smith, Lauren Cruchley-Fuge, Andris Jankevics, Rachel Spruce, Hisham Mehanna, Ralf J. M. Weber, **Andrew D. Southam**

Abstract:

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer worldwide. Relapse rate is high, and incidence is rising. To develop effective therapies, a greater understanding of disease mechanism is required. It is known that metabolism is altered in cancer (including HNSCC) and that this may play a role in driving the disease. This study aims to characterise metabolite profiles of primary patient HNSCC tissue to inform on disease mechanism. Metabolites were extracted from solid tissues using a biphasic extraction (methanol/water/chloroform). To simultaneously measure the relative abundance of multiple polar metabolites within the samples, we applied HILIC ultra-high performance liquid chromatography-mass spectrometry (UHPLC-MS) metabolomics. This workflow was applied to paired tumour and surrounding non-tumour tissue (each from the same patient) from patients with Human Papillomavirus (HPV) negative HNSCC. Univariate, multivariate, correlation and pathway enrichment analyses were applied. Retention time and/or fragmentation matches to chemical standards and/or on-line libraries was used for metabolite identifications. HNSCC induced changes to metabolites related to one-carbon metabolism included tumour-associated increases of S-adenosylmethionine (SAM) and SAM metabolites (dimethylarginine, 1-methylnicotinamide, trimethylysine, S-adenosylhomocysteine, polyamines, and methylated nucleosides). This suggests that one-carbon metabolism may play a role in the HNSCC disease phenotype.

Poster 41

Metabolomic profiles to explore biomarkers of adverse body composition parameters in non-small cell lung cancer patients

Ziyi Zhong, Howbeer Muhamadali, Masoud Isanejad

Abstract:

Background: Cancer-associated cachexia (CAC) and poor muscle quality negatively impact the prognosis of non-small cell lung cancer (NSCLC). However, effective treatment strategies remain elusive due to the poorly understood pathophysiology of body composition phenotypes in cancer. Metabolomics is a promising approach to elucidate the mechanisms underlying the exacerbation of CAC in NSCLC.

Study objectives:

• To determine plasma metabolic profile in NSCLC with normal body composition vs. adverse body composition.

• To screen and identify differential metabolites, and to perform pathways analysis to elucidate related pathways using online KEGG and HMDB databases.

Experimental approach

Population: This research project collaborates with Liverpool Heart and Chest Hospital, Liverpool, UK. Plasma samples, computed tomography (CT) images and patient information (sex, age, ethnicity, smoking and drinking status, disease stages and subtypes, history of disease and medications and laboratory data) were obtained from 46 NSCLC patients and 28 age-matched healthy controls.

Body Composition assessments: We are analysing CT images of T4 level to calculate the cross-sectional area of skeletal muscle, visceral adipose tissue, subcutaneous adipose tissue, intermuscular adipose tissue and skeletal muscle density.

Metabolomic assessments: Fourier transform infrared spectroscopy (FTIR) has been completed on plasma samples to show that lung cancer has distinct features compared to control, which we are analysing for further investigation. We also plan to complete untargeted gas chromatography-mass spectrometry (GC-MS) to explore differences in metabolomic profiles according to body composition in NSCLC.

Research significance: This is the first study to characterize the metabolomic profile according to different CT-based body composition parameters in NSCLC patients, which will enhance our understanding of the underlying biology. Findings may serve as potential diagnostic, intervention, or prognostic targets for personalized management of CAC in NSCLC patients.

Poster 42

Dog and cat dried blood spot samples stored at -80°C for 3 years: consistency in biological insights and approaches to sample bridging

Kelli Goodman, Joshua Wilson, Matthew Mitchell, Alison Colyer, Brian Keppler, Annie Evans, **David Allaway**

Abstract:

Dried blood spot (DBS) sampling is a rapid, cheap, sustainable method with minimal processing and can be achieved using remnant blood samples in clinics, or from pinpricks in-home. The DBS metabolome has a similar capacity to the plasma metabolome to discriminate phenotypes, making it suitable for biomarker discovery. However, for biobanking, DBS samples should provide reliable/comparable data after years of storage. This study (ethics approval 5492) investigated the suitability of DBS for biomarker discovery after long term storage. Objective 1 investigated if two profiling analyses (3 years apart) provided similar discrimination between phenotypes independently. Objective 2 evaluated the suitability of combining DBS data sets using a human DBS Quality Control (QC). DBS cards (n=54) stored at -80^{0}C were profiled twice across 3-years, using the same platform QC samples. Analysed independently, the two runs showed similar ability to separate samples by phenotype. Statistically significant metabolites detected in pairwise comparisons between cats and dogs, and each of 4 dog breed classes, identified that 77% (range 59-86%) were identified in both analyses. (Others trended ($p<0.1$) in the second analysis or had a small fold-change (<25%) between pairwise comparisons in the first analysis.) Random Forest analyses correctly identified the breed class of 2 blinded samples in both datasets, with similar predicted probability accuracy. Bridging the two datasets through the QC sample provided a merged dataset (561 metabolites). Analyses showed that biology drove most of the variance, and although a run-day effect was visible in the PCA, Hierarchical Clustering Analysis showed perfect or reciprocal pairing of 53 of 54 samples with one sample not pairing but remaining within the breed cluster. The data are consistent with DBS being a suitable sample for biobanking/biomarker discovery. Using a bridging matrix of the same species and running anchor pools were recommendations for metabolomic analysis of biobanked material.

Poster 43

Using Raman spectroscopy to monitor biological processes by simulated addition of glucose & lactate

Clark Gray, Royston Goodacre

Abstract:

The ability to monitor dynamic biochemical changes in real-time is pivotal for advancing our understanding of cellular metabolism and optimizing biotechnological processes. Raman spectroscopy, a non-invasive and label-free analytical technique, offers a promising solution. This study investigates the application of Raman spectroscopy to monitor biological processes through the simulated addition of glucose and lactate, two critical metabolites in cellular respiration.

We employed a series of controlled experiments to simulate the metabolic conditions experienced by cells, by systematically varying concentrations of glucose and lactate in aqueous solutions. Our results demonstrate that Raman spectroscopy can detect changes in glucose and lactate levels with high specificity and sensitivity, even in complex biological matrices. The spectral fingerprints of glucose and lactate were discernible across a range of concentrations, allowing for the real-time monitoring of their dynamic interplay. Furthermore, this method proved effective in distinguishing between different metabolic states induced by varying metabolite levels.

Future work will aim to extend this approach to live cell cultures, while integrating it with off-line monitoring of bioprocessing media, leading to on-line monitoring of biopharmaceutical processes.

Poster 44

Developing analytical methods for the detection of thiols associated to axillary malodour

Amy Colleran, Cassio Lima, Nigel Gotts, Yun Xu, Steve Paterson, Allen Millichope, Stephanie Murray, Royston Goodacre

Abstract:

The generation of odour is widely known to be due to the microbial conversion of odourless precursor molecules secreted by the apocrine glands into volatile odorous compounds by the skin microbiome. One type of VOC generated by the skin microbiome are thiols. These thiols strongly contribute to the odour but are only found at low detectable quantities. Additionally, these molecules are highly volatile and small, making them difficult to sample and analyse. This leads to the loss of information, including the lack of quantification of thiols produced, which is needed for understanding the formation of odour and the associations with the bacteria present within the skin microbiome that produce these thiols. The information provided by these thiols can lead to improvements in efficacy testing of cosmetic products such as deodorants and anti-perspirants, designed to reduce thiol generation.

Due to the chemical and physical properties of sulfurous compounds, we can optimise analytical techniques to try and detect the thiols of interest. In this presentation, I will be discussing the work carried out to improve the detection of thiols using spectroscopic and spectrometric techniques.

Poster 45

IN-U-Bar: Improving Nitrogen - Utilisation in Barley for Scotland's green recovery – environmentally friendly grain production for the brewing and distillery industries

Raul Huertas, George Epaku, Lawrie Brown, Simon Pont, Martina Picmanova, Tim George, Rob Hancock, **Will Allwood**

Abstract:

Introduction: In Barley, high nitrogen (N) levels are key for grain distilling quality, however N is a considerable environmental burden. Improving N utilisation efficiency (NUE) is essential to protect against eutrophication and to reduce carbon footprint of N fertiliser use. The effect of N application on barley development, grain yield and quality, especially at the molecular level, are poorly understood. Improved understanding will assist development of new high quality malt barley lines that better protect the environment.

Methods: Six barley varieties, Golden Promise (industry standard), Bere Unst (landrace, predicted high NUE), Wicket and Sassy (predicted high NUE), Power and Class (predicted low NUE) (n6) were subjected to N supplementation or deprivation under long day greenhouse conditions. Plant height, tiller number, chlorophyll level and fluorescence were collected weekly. At 6 weeks post germination, photosynthetic rate was determined via gas exchange measurements and the primary tiller leaves sampled. Leaves were sampled again at 10 weeks post germination prior to senescence. At harvest maturity, grains were dried, 1000 grain weight (TGW), ear dry weight per plant, and grain weight per plant were recorded. Amino acids, sugars (sucrose, fructose, glucose), and starch, were quantified by targeted HPLC methods. Total protein quantification was via the BioRad DC Protein Assay. C, H and N were determined in grains with a CHNS/O elemental analyser. Leaf and grain samples were subjected to metabolite profiling by UHPLC-LTQ-Orbitrap XL MS and UHPLC-Orbitrap IQX MS (Allwood et al. (2021). Data were processed within XCMS online and metabolite annotation performed with PutMedID. PCA and univariate analysis were performed within MatLab R2023a.

Results: Grain yield was significantly influenced by N level, however, TGW minimally changed between N treatments, indicating that whilst yield is reduced, the size and weight per grain (i.e. grain quality) is not clearly affected. Ears per plant, ear weight, and tiller number were higher under N supplementation, tallying with higher yield. Within leaves, the glucose levels increased from 6 to 10 weeks post germination regardless of N treatment, with sugar levels being highest under N supplementation. For grains conversely, glucose levels were higher under N deprivation. Amino acid and protein content were higher under N supplementation for both grain and leaves. Total grain starch levels revealed negligible differences. The UHPLC-MS metabolomics data revealed similar PCA clustering of varieties as observed in genome-based clustering in PCoA, highlighting the genetic impact and control of metabolism. The metabolomics data are at the stage of identification of key metabolic differences between barley varieties and N treatments.

Conclusions: N supplementation leads to greater yields, protein and amino acid levels, however measures of malting quality such as TGW show very limited difference. Measures of malting quality such as grain starch and sugar content were not negatively affected by N deprivation, in fact in the case of glucose, N deprivation resulted in higher grain levels. This potentially highlights the energy demanding nature of N uptake, amino acid and protein synthesis, diverting sugars to provide both energy (ATP) and C skeletons for amino acid synthesis, or potentially reflecting that the plants are investing more greatly per a unit grain where N is restricted (survival strategy). Whilst protein levels are elevated under N supplementation indicating enhanced grain quality, it is yet to be seen the effect on specific proteins such as amylase, which is key to starch breakdown during the malting process.

Poster 46

Rapid microbore metabolic profiling of human liver diseases plasma samples using a multi-reflecting time-of-flight mass spectrometer

Ana Sanchez Lorenzo, Adam M King, Emma Marsden-Edwards, Elizabeth Want

Abstract:

Acute-on-chronic liver failure (ACLF) and acute liver failure (ALF) are severe conditions characterized by acute decompensation (AD) of liver function and systemic inflammation, often leading to multi-organ failure and high mortality rates. However, ACLF and ALF have distinct pre-clinical backgrounds, impacting their pathogenesis and treatment strategies. Analysing plasma metabolites and lipids facilitates detection of biomarkers associated with disease progression. To increase cost-efficiency of untargeted analysis and decrease batch effects in large-scale studies, high-throughput methods have been explored to reduce analysis time. Here, these methods were combined with high-resolution mass spectrometry (>100 FWHM) with fast acquisition speeds (~3mins) and vacuum-jacketed technology (VJC), increasing resolution and reducing peak dispersion, to analyse blood samples from four patient groups: healthy control (n=20), Acute-on-chronic liver failure (ACLF) (n=25), cirrhosis (n=19), and acute liver failure (ALF) (n=54).

Untargeted hydrophilic interaction liquid chromatography (HILIC) and reversed-phase (RP) lipid methods were conducted using a HILIC ACQUITYTM BEH amide column (1.0 x 50 mm) and ACQUITYTM BEH C8 (1.0 x 50 mm) column respectively, and their corresponding prototypes with VJC technology. The SELECT SERIES MRT system (>100 K FWHM) was used to acquire MSe data using data independent acquisition (DIA) mode in positive and negative ionisation modes. Data were processed using Mzmine and Xcms.

Our data demonstrate that rapid profiling methods provide good metabolome coverage, with VJC technology generating sharper peaks, improving peak fidelity and separation of coeluting compounds, e.g. PC36:1 and SM42:3,O2 with mass error of 0.1ppm and 170-200K FWHM mass resolution. Multivariate analysis of RP data showed overlap between ALF and ACLF groups, but separation from controls, with dysregulation of lysophosphatidylcholines, phosphatidylcholines and sphingomyelins in liver disease patients. HILIC analysis showed significant differences in amino acids and biogenic amines in disease groups. These rapid profiling methods hold promise for large-scale clinical studies and biomarker discovery.

Poster 47

Harnessing metabolomic techniques for wildlife populations: Characterising the faecal metabolome of the okapi (*Okapia johnstoni*) through the assessment of variability and identifiability during global extraction optimization.

Rebecca Mogey, Katie Edwards, Roy Goodacre

Abstract:

According to the International Union for Conservation of Nature's Red List of threatened species, the okapi (*Okapia johnstoni*), is endangered, with wild populations decreasing by 50% over the past two decades. Like many species of conservation concern with dwindling populations, it is important to determine the conditions they need to thrive in a changing environment. One approach to this, is to make use of the complex biological samples they leave behind. Although challenging, non-invasive sampling allows researchers to assess aspects of physiology without interference, which could lead to experimental bias when considering an individual's homeostasis or state of well-being.

Ex situ individuals provide valuable insurance populations for endangered species. As these individuals can be studied more closely than their in situ counterparts, they can provide valuable insight into species-specific behaviour and physiology, with physiological well-being trending as a topic of interest in recent years. To understand overall animal well-being and provide the conditions necessary for individuals and populations to thrive, conservation physiology research must harness metabolomics as a lens through which to view the multifaceted nature of how physiology, biochemistry and external conditions impact species survival.

In the present study, an integrated metabolomics approach using both LC-MS and GC-MS global metabolomics is being developed to assess the variability of the okapi faecal metabolome. Metabolites are being putatively identified using current in-house libraries as well as external resources. This work represents a first step in furthering the understanding of the faecal metabolome in the okapi, with the hope that this can be employed to assess the health and success of this species in more depth, through the future identification of novel faecal biomarkers. Novel information gained in these species could help to improve not only conservation efforts for these species but also add insight into other ruminants, benefitting agriculture (food security).

Poster 48

Fast scanning MRM quantitative lipidomics analysis

Rebekah Sayers, Paul RS Baker, David Colquhoun, and Ian Moore

Abstract:

Introduction: Herein, a method is presented and tested to sensitively detect and quantify lipids using a comprehensive lipidomics panel using the multiple reaction monitoring (MRM) scan mode with an ultrafast mass spectrometer. The panel consists of ~2000 lipid molecular species identified at the fatty acid level of structural specificity and depends on chromatography to separate lipids by class to mitigate isobaric interference. Due to lipids eluting as a class, the speed at which individual MRM transitions are measured is crucial to ensure good coverage and quantitative accuracy. A balance must be struck between the extent of the target list and the resulting data quality, especially during periods of high MRM concurrency. However, the SCIEX 7500+ system with fast MRM technology addresses this problem. It is ideal for large panel quantitative screening to provide more data points across individual peaks to improve peak shapes and the calculated %CVs for quantitative measurements.

Methods: Experiments were designed to compare the quantitative performance of lipid analysis using different dwell and pause times. Our findings reveal that a combined scan time of 3 ms (1.5 ms dwell and 1.5 ms pause times) on the SCIEX 7500+ system resulted in data with superior quantitative precision compared to the classic SCIEX 7500 system running at its fastest recommended combined scan rate of 5 ms. This advantage was most evident during periods of high MRM concurrency, with 59% more molecular species quantified with a %CV < 20 compared to previously established methods. The faster data acquisition yielded more data points across analyte peaks, which generated better peak shapes and improved lipid isomer resolution compared to the SCIEX 7500 system.

Preliminary Data: There appears to be a movement within small molecule omics from the classic untargeted experimental approach to biomarker discovery to a targeted method that utilizes a broad panel of metabolites. This is partly due to the challenges associated with untargeted data analysis and the growing need to measure biomolecules quantitatively. Consequently, metabolomic analyte panels are expanding from single-class panels to more extensive lists incorporating multiple classes. A faster scanning speed is required to maintain quantitative performance to accommodate the increasing lists of biomolecule targets. The SCIEX 7500+ system can measure analytes at a combined speed of 1.2 ms per MRM transition (0.5 ms dwell and 0.7 ms pause times), enabling an 800 MRM/s scan speed. This fast scan rate was used to analyze samples in an unscheduled fashion to establish retention times, which resulted in 7-9 data points acquired across the analyte peak, facilitating RT determination. Faster data acquisition also improved the analyte peak shape, which resulted in better resolution of closely eluting isomers and a significant improvement in quantitative precision overall for the assay. During a period of the experiment with the highest MRM concurrency (~925 concurrent MRMs), there was a 60% increase in the number of molecular species measured with a %CV <20% using the SCIEX 7500+ system at a scan speed of 3 ms. Overall, the faster scan speed improved quantitative precision across all lipid classes in a global, broadly targeted panel of lipids. These results establish this platform as an ideal instrument for any large panel of analytes.

Poster 49

Quantitative analysis and structural characterization of bile acids using the ZenoTOF 7600 system

Ozbalci Cagakan, Colquhoun David, Baker Paul, Proos Robert, Seferovic Maxim, and Horvath, Thomas

Abstract:

Introduction: Primary bile acids are essential for emulsifying and absorbing dietary fats and can be converted to secondary bile acids by the gut microbiota. Bile acid analytical methods often use triple quadrupole mass spectrometers—the quantitative performance of which can suffer from chemical interference. High-resolution mass spectrometers with sufficient sensitivity can reduce background interferences and improve the quality of quantitative data. Bile acids are comprised of multiple groups of isomers that cannot be distinguished using CID-based fragmentation alone. However, electron-based fragmentation such as EAD, provides diagnostic fragments that distinguish these isomers. Here, the speed and sensitivity of the ZenoTOF 7600 system was leveraged to quantify and structurally characterize bile acids in human plasma samples.

Method: Plasma was isolated from human blood by centrifugation, extracted, mixed with an internal standard solution, and split for injection on the QTRAP 7500 and ZenoTOF 7600 systems. Chromatographic separations were performed using a Phenomenex Kinetex XB-C18 column for the ZenoTOF 7600 system and a Restek Raptor C18 column for the QTRAP 7500 system using mobile phases of 10 mM ammonium formate in water (A) and pure acetonitrile (B). Samples were analyzed on both systems using a scheduled multiple reaction monitoring (sMRM) scan mode. EAD-based product ion spectra were acquired in positive ion mode for structural characterization. LOD and LOQ were calculated from calibration curves, and Pearson correlation calculations were performed using Prism software.

Preliminary Data: A bile acid standard curve was generated and analyzed on both the QTRAP 7500 and ZenoTOF 7600 systems. Optimized parameter settings for each instrument allowed for the separation of isomeric bile acids with baseline resolution. The internal standard curve ranged from 0.01 to 1000 nM, but the QTRAP 7500 experienced detector saturation at 1000 nM, unlike the ZenoTOF 7600. To match conditions, the dynamic range was truncated to 0.1 to 100 nM for final calculations, sufficient for measuring bile acids in human plasma. Rat plasma extracts were analyzed, and data were acquired using a high-resolution multiple reaction monitoring (sMRMHR) scan mode. The ZenoTOF 7600 uniquely identified specific lipid molecular species using both CID- and EAD-based fragmentation. The system's high-resolution capabilities excluded contaminating peaks, increasing measurement accuracy. Standard curves were created for each analyte to calculate LOD and LOQ. Both instruments showed comparable sensitivity, with the QTRAP 7500 being slightly more sensitive for some compounds. Both instruments effectively measured bile acids in human plasma, showing linear correlation in concentration data with r values >0.99 and p values <0.0001. The ZenoTOF 7600's EAD-based fragmentation provided detailed structural information, distinguishing bile acid isomers and potentially identifying novel conjugated bile acids. This capability is valuable for bile acid metabolism research, highlighting the system's quantitative sensitivity and qualitative capabilities. (214/300)

Poster 50

Quantitation and structural characterization of lipid mediators by high-resolution mass spectrometry

Tom Ruane, Sayers Rebekah, Proos Robert, Baker Paul, Norris Paul, Zheng Yi, Leyen Klaus van, and Holm Ted

Abstract:

Lipid mediators are crucial for the initiation, propagation, and resolution of inflammation, making them key targets for anti-inflammatory drug development. These molecules, present in pM-nM concentrations, are challenging to analyze and typically require sensitive triple quadrupole mass spectrometers. However, a new ultra-sensitive high-resolution mass spectrometer now provides precise *in vivo* measurements of lipid mediators while simultaneously providing for their structural characterization.

The ZenoTOF 7600 system, a high resolution mass spectrometer, measures endogenous lipid mediators with the sensitivity comparable to that of a high-end triple quadrupole instrument. It uses traditional collision-induced dissociation (CID) and electron-activated dissociation (EAD) for detailed structural characterization. The system's tunable electron beam produces EAD-based fragments to distinguish lipid mediator isomers. (103/120)

Rats underwent ischemic stroke (MCAO model), and plasma samples were taken after 24 hours. Rat plasma serum was extracted using a solid phase extraction (SPE) column, eluted with methanol (MeOH), reconstituted in 100 µL MeOH, and stored at -20°C. For HPLC MS/MS analysis, 10 µL of the sample was injected on a ZenoTOF 7600 system with an OptiFlow Turbo V ion source and an Exion UHPLC. The mobile phases were (A) 0.1% acetic acid in water and (B) 0.1% acetic acid in 84:16 ACN/MeOH, at 0.4 mL/min over 21 minutes. Data were acquired using a high-resolution multiple reaction monitoring (sMRMHR) scan mode and processed with SCIEX OS software. (108/120)

The data presented demonstrate the use of the ZenoTOF 7600 system to quantitate and fully characterize lipid mediators using both CID- and EAD-based fragmentation. The results presented will demonstrate that this level of molecular characterization can be achieved on a liquid chromatography (LC) time scale, which enables high throughput data acquisition in samples of diverse origins. The data presented will show that the ZenoTOF 7600 system with EAD is uniquely capable of the specific structural identification of lipid molecular species in simple and complex matrices. (86/300)