
# Scottish Metabolomics Network Virtual Symposium 2021

23 – 24 November 2021

Online via Zoom

## Programme

SCOTTISH METABOLOMICS NETWORK

# Scottish Metabolomics Network Symposium 2021

# PROGRAMME

## Day 1 - Monday 22nd November

***Poster/Sponsor Session***
(Unmanned Posters & Commercial Pages/Links Made Available for Viewing)

## Day 2 - Tuesday 23rd November

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| **09:15 – 09:50** | **Registration** (please log on to the Zoom Link early) |

| ***Scientific Session One – Technology advancement (Chair Natalie Homer)*** | | |
|---|---|---|
| 10:00 – 10:05 | Welcome | Karl Burgess / Will Allwood |
| 10:05 - 10:45 | Keynote: Metabolomics of Sebum provides insights to Parkinson's Disease and an opportunity for non-invasive monitoring | Prof. Perdita Barran *University of Manchester, School of Chemistry* |
| 10:45 - 11:10 | Mass spectrometry Imaging, a novel technique for corticosteroid mapping in mice kidney | Ioannis Stasinopoulos, Shazia Khan, Logan C. MacKay, Roger W. Brown, Matthew A. Bailey, Ruth Andrew |
| 11:10 - 11-35 | On-line metabolomics for bioreactor monitoring | Joan Cortada Garcia, Karl Burgess |
| 11:35 - 12:00 | ***Sponsor presentation: Thermo Fisher*** Unmatched structural analysis: Thermo Scientific Orbitrap IQ-X Tribrid Mass Spectrometer | Elizabeth Crawford |
| 12:00-12:10 | Equality, Diversity and Inclusion (EDI) Introduction | Scott Denham |

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| **12:00 - 13:00** | **Lunch/Poster Session** |

| ***Scientific Session Two – Lipidomics (Chair Ruth Andrew)*** | | |
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| 13:00 - 13:25 | The VitDAL journey: From armadillos to COVID-19 patients and (almost) everything in between | Emma Hurst, Natalie Homer |
| 13:25 - 13:40 | ***Sponsor presentation: Agilent*** The Agilent 6560C IM-QTOF MS: The Latest Developments in Uniform Field Ion Mobility Technology | Hannah Florance |
| 13:40 - 14:05 | The effect of Delta 6 desaturase on PUFAs and lipids synthesis in *Trypanosoma brucei* | Michela Cerone, Terry Smith |

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| **14:05 - 14:20** | **Break** |

| 14:20 - 15:20 | **Poster Session (flash presentations) *Peoples Vote* *(Chair Karl Burgess)*** |
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1. Development and validation of an LC-MS/MS method for the measurement of Pravastatin as a treatment for preterm labour. Joanna P Simpson, Scott G Denham, Eleanor Whitaker, Natalie ZM Homer (UoE).

2. Cardiotoxicity Evaluation of Pioglitazone Exposure in Human Derived Cardiomyocytes Using LC-MS based Metabolomics. Abdullah Al Sultan, Nik Rattray (Strathclyde).

3. Throwing Shapes: Using Ion Mobility-Mass Spectrometry in the Separation and Detection of Oestrogens: James Weatherill, Susan Slade, Shazia Khan, Russell Mortshire-Smith, Jonathan Fox, Nina Denver, Diego F Cobice, Mark Kane, Margaret R MacLean, Ruth Andrew (UoE).

4. Comparing the Metabolic Response of Breast Cancer Cells to Olaparib Treatment. Layla Al Noumas, Nik Rattray (Strathclyde).

5. The MAP UK project. James Christie, Wendy Russell (Rowett - UoA).

6. Illuminating spatial lipidomic profiles of atherosclerotic plaques by mass spectrometry imaging. Sphamandla Ntshangase, Jakub Kaczynski, David E Newby, Patrick WF Hadoke, Ruth Andrew (UoE).

| ***Scientific Session Three – Cancer metabolomics (Chair Phil Whitfield)*** | | |
|---|---|---|
| 15:20 - 15:45 | ***Sponsor presentation: Shimadzu***<br>Exploring non-targeted workflows in high resolution mass spectrometry – how do you find and identify compounds in screening programs with any certainty? | Christine Hinz |
| 15:45 - 16:10 | Determining the metabolic variability of multiple senescent and breast cancer subphenotypes | Domenica Berardi, Nik Rattray |
| 16:10 - 16:35 | Exploring the use of unbiased metabolomics in clinical decision making for cancer | Alejandro Huerta Uribe, Oliver Maddocks |
| 16:35 - 17:00 | Investigating glutaminase dependence in BRAF inhibitor resistant melanoma | Rachel Harris, Saverio Tardito, David Sumpton |

## Day 3 - Wednesday 24th November

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| **09:15 – 09:50** | **Registration** (please log on to the Zoom Link early) |

| ***Scientific Session Four – Natural Products (Chair Will Allwood)*** | | |
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| 10:00 - 10:25 | Multivariate statistical analysis to evaluate the effects of environmental factors on the chemical profile of some plants from the Brazilian Cerrado. | Ana C. Zanatta, Wagner Vilegas, RuAngelie Edrada-Ebel |
| 10:25 - 10:50 | ***Sponsor presentation: Waters***<br>Cycle through the metabolome: Using Cyclic Ion Mobility and Rapid Chromatography to Understand Disease | Adam King |
| 10:50 - 11:15 | Purification and characterisation of seaweed phlorotannins | Will Allwood, Ceri Austin, Julie Sungurtas, Samuel Wright, Huw Evans, Chris Plumber, Gordon McDougall |
| 11:15 - 11-40 | ***Sponsor presentation: SCIEX***<br>Impact of increased MS/MS sensitivity and alternative fragmentation on metabolomic workflows | Iain Mayer |

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| **11:40 - 13:00** | **Lunch/Poster Session** |

| ***Scientific Session Five – Clinical and human studies (Chair Karl Burgess)*** | | |
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| 13:00 - 13:25 | Uncovering a metabolomic signature of treatment responses in patients with rheumatoid arthritis | Cameron Best, Michael Barrett |
| 13:25 - 13:40 | Running on Empty: A Metabolomics Approach to Investigating Changing Energy Metabolism during Fasted Exercise and Rest | Gavin Blackburn |
| 13:40 - 14:05 | ***Sponsor presentation: Bruker***<br>Advancing clinical research using 4D-OMICS | Sven Meyer, Bruker Daltonics GmbH & Co. KG |
| 14:05 - 14:25 | Lipid changes in Alzheimer's disease and cognitive impairment | Areehsa Nazeer, Jules Griffin |

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| **14:25 - 14:40** | **Break** |

| ***Scientific Session Six – Plants, foods and nutrition (Chair Will Allwood)*** | | |
|---|---|---|
| 14:40 - 15:05 | The future of sustainable aqua feeds? Assessment of a genetically modified oil on the phospholipid composition of Atlantic salmon | Richard Broughton, Douglas Tocher, Monica Betancor |
| 15:05 - 15:30 | ***Sponsor presentation: Anatune***<br>The Missing Tile in Metabolic Profiling: How Automation Can Help | Camilla Liscio, Mark Perkins |
| 15:30 - 15:55 | Moringa Metabolites: Nutritional and Economic Value for Malawi | Wendy Russell, Dinka Rees and Madalina Neacsu |
| 15:55 - 16:00 | Save The Date for Aberdeen 2022 | Wendy Russell |
| 16:00 - 16:20 | Close of meetings and Awards | Karl Burgess / Will Allwood |

# Abstracts

## Session 1: *Technology advancement*

**Keynote: Metabolomics of Sebum provides insights to Parkinson's Disease and an opportunity for non-invasive monitoring**

Prof. Perdita Barran (University of Manchester)

Abstract: In this talk I will discuss why and how we have performed metabolomics of sebum in order to diagnose Parkinson's Disease (PD). The methods that we have used include, odour analysis, thermal desorption gas chromatography mass spectrometry (TD-GC-MS), HPLC- MS, direct infusion MS and chemometrics. I will describe how these methods can be used to stratify PD and for early diagnosis.

Bio Sketch: Professor Barran holds a Chair of Mass Spectrometry in the Department of Chemistry, is Associate Dean for Research Facilities and Director of the Michael Barber Centre for Collaborative Mass Spectrometry at the Manchester Institute of Biotechnology, The University of Manchester, UK.
Her research interests include: Biological mass spectrometry; Instrument and technique development; Protein structure and interactions; Dynamic and Disordered Systems; Parkinson's disease Diagnostics; HDX-MS; Proteomics; and Molecular modelling. She is a Fellow of the Royal Society of Chemistry and was awarded the Theophilus Redwood Award from the RSC in 2019, Researcher of the Year 2020 from the University of Manchester and the ACS Measurement Science Lectureship 2021. In 2020 she initiated the COVID-19 Mass Spectrometry Coalition and was appointed as Chief Advisor to the UK Government on Mass Spectrometry as part of their pandemic response. Perdita has had the privilege to mentor 32 graduate students through the successful completion of their PhD's as well as 16 postdoctoral fellows. Perdita has authored over 160 publications in peer reviewed journals which have been cited over 4000 times, by people other than her. In 2021 Perdita founded the company Sebomix Ltd. to exploit sebum as a diagnostic biofluid with a focus on Parkinson's Disease.

**Mass spectrometry Imaging, a novel technique for corticosteroid mapping in mice kidney**

Ioannis Stasinopoulos, Shazia Khan, C. Logan MacKay, Roger W. Brown, Matthew A. Bailey, Ruth Andrew

Abstract: Aldosterone regulates renal sodium reabsorption which is important for blood pressure homeostasis and more recently glucocorticoids have been implicated in this process. Hypertension can be caused by abnormal steroid hormone activity within the kidney. Even though circulatory and urinary steroid concentrations have been measured in the past in hypertensive individuals, steroid concentrations at a cellular level are largely unknown, and the kidney remains a "black box". Mass spectrometry imaging (MSI) permits localisation of steroids in histological zones based on regional markers. This approach has been previously applied to localise steroids in brain and testes, and here is applied to kidney. Our aim was to map and quantify glucocorticoids and aldosterone in different histological zones (cortex, medulla) of kidneys from mice receiving different dietary salt intake using an optimised MSI method, to provide fundamental new information relevant to hormone action in health and in disease.

Cryosections kidney from male C57BL6 mouse (age 12 weeks, n=6: dietary salt low 0.03% vs normal 0.3% vs high 3%) were subject to MSI analysis following Girard T reagent derivatisation and α-cyano-4-hydroxycinnamic acid matrix application. Matrix assisted laser desorption/ionisation (MALDI) was used as a sampling method, coupled to Fourier Transform Ion cyclotron mass spectrometry.

Ions with m/z 458.3010 (Δppm=0.65), 460.3166 (Δppm=0.65), and 474.2957 (Δppm=1.05) were detected, using MALDI, in renal sections for derivatives of 11-dehydrocorticosterone, corticosterone and aldosterone respectively. Untargeted evaluation of ions was conducted to find regional markers that would allow definition of kidney histological zones. Heat maps indicated that corticosterone intensity was higher in the inner cortex than the rest of the kidney. In contrast 11-dehydrocorticosterone was detected in medulla and aldosterone signal was equally strong in medulla and outer cortex. Steroid localisation after treatment with different salt intakes indicated that corticosterone intensity increases in the outer cortex in low salt diet while 11-dehydrocorticosterone and aldosterone remain the same across the kidney sections in different diets This approach provides fundamental new insights into the physiological control of sodium transport by steroids and opens doors to understanding changes in disorders of blood pressure.

**Unmatched structural analysis: Thermo Scientific™ Orbitrap IQ-X™ Tribrid™ Mass Spectrometer**
Elizabeth Crawford (Thermo)

Abstract: Designed for small-molecule analysis, the Thermo Scientific™ Orbitrap IQ-X™ Tribrid™ mass spectrometer redefines small molecule identification and characterization of unknown compounds. The trusted Tribrid architecture provides the richest $MS^n$ data by leveraging the best quadrupole, linear ion trap and Thermo Scientific™ Orbitrap™ mass analyzers combined with intelligent data acquisition. Ease-of-use hardware improvements for automatic, hands-free calibration along with a fit-for-purpose software interface allow you to overcome the traditional bottlenecks in small molecule structure elucidation workflows. An introduction to all the new features will be the focus of this presentation.

## Session 2: *Lipidomics*

**The VitDAL journey: From armadillos to COVID-19 patients and (almost) everything in between**
Emma Hurst, Natalie Homer

Abstract: In this talk I will introduce the Vitamin D Animal Laboratory (VitDAL) and discuss our role in improving patient care with vitamin D analysis. I will describe our DEQAS accredited liquid chromatography tandem mass spectrometry method and discuss its varied application in the analysis of the major circulating vitamin D metabolites, 25-hydroxyvitamin D2/3, across several different species; from armadillos to COVID-19 patients.

**The Agilent 6560C IM-QTOF MS: The Latest Developments in Uniform Field Ion Mobility Technology**
Hannah Florance (Agilent)

Abstract:
Learn about the latest Ion Mobility Spectrometry developments and how data independent fragmentation in conjunction with demultiplexing algorithms delivers considerably enhanced high resolution drift separations. Examples for both small molecule screening studies and for the interrogation of large intact proteins with collision-induced unfolding (CIU) will be given. As the only commercially available IM mass spectrometer able to directly calculate CCS from first principles, the 6560 IM-QTOF is the Industry Standard for Accurate CCS Measurements.

**The effect of Δ6-desaturase on PUFAs and lipids synthesis in Trypanosoma brucei**
Michela Cerone, Terry K Smith

Abstract: Trypanosomatids have been shown to possess an exclusive and finely regulated biosynthetic pathway for de novo synthesis of fatty acids and particularly of polyunsaturated fatty acids (PUFAs).[1] This aspect justifies the high percentage of PUFAs that have been found in the lipid membranes of Trypanosoma brucei.[2] The key enzymes for the process of unsaturation are known as desaturases. The reaction catalysed by this class of enzyme is the insertion of a double bond in a stereospecific and regioselective manner on to the acyl carbon chain of a FA molecule.[3] Thus, the hypothetical catalytic activity for Δ6-desaturase in the native organism T. brucei has been investigated in this study, aiming to confirm the substrate specificity and the activity of the Δ6-desaturases via genetic manipulation to tune the level of expression of Δ-6-desaturases in both procyclic (PCF) and bloodstream (BSF) forms of the native organism T. brucei. We observed distinctive variations in both PUFAs and lipids metabolism via GC-MS and ESI-MS/MS. These variations gave us an insight on the biocatalytic function and essentiality of the Δ6-desaturase. In particular we were able to show an increase or decrease of docosahexaenoic acid (22:6) in BSF and docosapentaenoic acid (22:5) in PCF of T. brucei, accordingly whether Δ6-desaturases gene is overexpressed or knocked-down. Interestingly, we were able to observe via lipidomic analysis an increase in inositol-phosphoceramide (IPC) production, amongst others, as a result of adaptation to different Δ6-desaturases expression levels and fat source content in the media, both in PCF and, surprisingly, in BSF. Thus, we are currently further investigating the potential consequent effect on other proteins expression and any life-stage modifications, in which Δ6-desaturases might be involved.[4]

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## Session 3: *Cancer metabolomics*

**Exploring non-targeted workflows in high resolution mass spectrometry – how do you find and identify compounds in screening programs with any certainty?**
Christine Heinz (Shimadzu)

Abstract: High resolution mass spectrometry based screening is widely used for metabolomics, lipidomics and other disciplines. Although it has been used for many years, compounds identification remains a challenge. Here, we give an overview of the different tools for screening and discuss the pathway from targeted screening (defining the criteria for success) to the approaches that can help non-targeted analysis (NTA) and suspect screening analysis (SSA).

**DETERMINING THE METABOLIC VARIABILITY OF MULTIPLE SENESCENT AND BREAST CANCER SUB-PHENOTYPES**
Domenica Berardi, Nicholas Rattray

Abstract:

INTRODUCTION
Metabolomics is the study of low-molecular-weight metabolites present in biological samples which can be a powerful technology to evaluate drug actions, disease mechanisms, and identify potential biomarkers.

At present, phenotypic sub-types of senescent and breast cancer cells lack comprehensive biomolecular classification strategies[1,2]. Senescence phenotypes have been profiled based on the typology of cellular damage and senescence induction method[3], while breast cancers have been classified based on their molecular features[4]. These methodologies are not sufficient for elucidating the inter- and intra-heterogeneity of the multiple phenotypes of these cell types. Thus, more robust approaches need to be developed to characterise their different phenotypes.

AIM:
This study aimed to assess key metabolic pathways altered following ionization radiation and drug treatment in both senescent and breast cancer cells.

METHODS:
Human fibroblasts were treated with ionizing radiation, hydroxyurea and etoposide to induce cellular senescence. A panel of breast cancer cells with different hormone receptor expression patterns were characterized for their phenotypic and metabolic response to olaparib drug treatment at various doses and durations of treatment. Untargeted liquid chromatography-mass spectrometry (LC-MS) metabolomics analyses in combination with molecular biology based phenotypic assays (cell proliferation and immunofluorescence assays) were performed to determine relative patterns of biochemical pathways altered by drug treatment.

RESULTS:
Senescent and breast cancer cells showed a different expression of molecular and metabolic features that allowed to identify and separate the different sample groups (treated and non-treated cells). A putative analysis of identified metabolic biomarkers sheds light on the major metabolic pathways - energy, amino acid, and lipid metabolism – altered upon ionizing radiation/drug treatment.

CONCLUSIONS:
Untargeted metabolomics is a powerful tool for discerning metabolic differences occurring in response to drug treatment and biological stimuli. This work showed a separation and a putative identification of the major metabolic variables between different senescent and breast cancer phenotypes. Our future work will focus on the targeted analysis of pathways identified from this work.

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[2] Cancer research UK. About breast cancer staging and grades. 2020. p. 1.
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**Exploring the use of unbiased metabolomics in clinical decision making for cancer**

Alejandro Huerta Uribe[1]*, Oliver Maddocks[1]:

[1] Institute of Cancer Sciences, University of Glasgow, Glasgow, UK
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Abstract: One of the main characteristics of cancer cells is their dysregulated metabolism which is mainly a result of their uncontrolled proliferation. This rewired metabolism generates a unique metabolic fingerprint that can be used for earlier cancer diagnosis, to predict treatment response or to aid the selection of patients for clinical trials.

For most of the past decades, the assessment of metabolic changes in cancer was mostly limited to standard clinical chemistry tests. The recent implementation of metabolomics in clinical practice, a powerful tool that systematically measures hundreds of metabolites in cells and body fluid samples, has considerably helped to improve diagnosis, evolution, and management of cancer.

Our current work focuses on the use of untargeted metabolomics for the discovery of potential metabolic biomarkers for the diagnosis of pancreatic ductal adenocarcinoma (PDAC), a highly fatal disease with a 5-year survival rate of approximately 10%. Using human plasma samples from healthy, PDAC and chronic pancreatitis patients (n=300) we have generated four metabolomic datasets using high resolution mass spectrometry coupled to liquid chromatography (LC-HRMS). We then applied a supervised machine learning method for the selection of important features that can serve as potential biomarkers.

## Session 4: *Natural Products*

**Multivariate statistical analysis to evaluate the effects of environmental factors on the chemical profile of some plants from the Brazilian Cerrado**

Ana C. Zanatta[1,2*], Wagner Vilegas[2], RuAngelie Edrada-Ebel[3]:

[1] São Paulo State University (Unesp), Institute of Chemistry, Brazil
[2] São Paulo State University (Unesp), Institute of Biosciences, Brazil
[3] University of Strathclyde, United Kingdom
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Abstract: In medicinal plants, seasonal variation may be one of the effects that influence their production of secondary metabolites[1,2]. The application of the metabolomics approach for the quality control of plant extracts is essentially important because it helps to establish a standard metabolic profile and to analyse factors affecting the efficacy of medicinal plants[3,4]. The Brazilian Cerrado flora is characterized by a rich diversity of native plant species, and several of these plant species have been found to have suitable medicinal properties[5,6]. Some of these plant species include *Byrsonima intermedia A.Juss*. (Malpighiaceae). To better understand the chemical composition of *T. catappa* hydroethanolic extracts on a seasonal basis, a study was conducted using UHPLC-DAD-(ESI)-HRMS and NMR analysis. For the study, leaves of *B. intermedia* were collected bimonthly for two consecutive years[7]. UHPLC-DAD-(ESI)-HRMS data were processed in MZmine2 v.2.538,9 and the output data uploaded into an in-house Excel macro for peak dereplication[10]. MS and NMR were concatenated using the data fusion method[11,12] and submitted to multivariate statistical analysis. In the VIP scores for the NMR data, chemical shifts of methyl, methylene, and aromatic protons were observed. By analyzing the MS data, it was observed that the triterpenes, the quercetin derivatives, and the galloylquinic acid derivates contributed the most to the differentiation of the harvest periods. Samples harvested during the summer had the highest amount of triterpenes, while samples harvested during autumn/winter seasons had the highest amount of phenolic compounds. These compounds play important ecological roles and under stress conditions, these different classes of metabolites can act in different ways in the plant's defence. Triterpenes act as a lipophilic protective layer for leaves, and can block water loss, obstruct UV penetration, protect from high temperatures, among other factors[13,14]; this can be explained by the warm weather and the higher light intensity of the summer season. Water deficiency in drought periods, on the other hand, may have increased the formation of reactive oxygen species and this led the plant to produce antioxidant compounds, such as the phenolic compounds observed[15].

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## Cycle through the metabolome: Using Cyclic Ion Mobility and Rapid Chromatography to Understand Disease

Adam King[1], Nyasha Munjoma[1], Lee Gethings[1], Robert Plumb[2], Ian Wilson[3]

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[3]Department of Metabolism, Digestion and Reproduction, Imperial College London, London, SW7 2BX, UK.

Abstract: Untargeted metabolic profiling of biological matrices has increasingly been employed in helping to understand the mechanism of disease progression and in identification of potential diagnostic markers[1]. High resolution mass spectrometers have been valuable in unravelling the complex and broad variety of compounds that these matrices contain[2]. Coupling these instruments with efficient chromatographic separations and the complimentary information generated from hybrid ion mobility enabled instruments, the understanding of these biological systems is only becoming clearer[3]. Although, many challenges remain due to the diverse coverage of metabolites that require investigating and the impact large cohort studies have on laboratory resources required for the analysis to be statistically robust. Many endogenous metabolites exist in isomeric forms which can result from different biological processes. Being able to fully

characterise statistically significant isomers can help to understand their relationship with disease. This talk will highlight advances in rapid chromatographic methodologies and cyclic ion mobility.

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**Purification and LC-MS$^n$ characterisation of seaweed phlorotannins**

Will Allwood[1], Ceri Austin[1], Julie Sungurtas[1], Samuel Wright[2], Huw Evans[2], Chris Plumber[2], Gordon McDougall[1]:

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Algae-UK Proof of Concept Funding

Abstract: Natural extracts from brown seaweeds native to the UK have been shown to have antiviral activities, including against covid-19, that could be useful in producing biocides that help prevent the spread of these diseases. However, these extracts contain a range of different components which vary in composition between different brown seaweeds and sometimes between location of harvest and season. This talk will describe the project work to identify the most active components and provide an effective means to optimise the extraction of these components into effective natural antiviral agents.
Brown seaweeds produce oligomeric polyphenols called phlorotannins which appear to function as anti-feedants, anti-microbial agents or antioxidants to mitigate against dehydration and UV-B radiation. These may be important for the noted anti-viral activities and various means to enrich and identify the variation in phlorotannin composition using LC-MSn techniques are discussed (1).

1. Allwood JW, Evans H, Austin C, McDougall GJ. Extraction, Enrichment, and LC-MS$^n$-Based Characterization of Phlorotannins and Related Phenolics from the Brown Seaweed, *Ascophyllum nodosum*. Mar. Drugs. 2020 27;18(9):448. Doi: 10.3390/md18090448.

## Session 5: *Clinical and human studies*

**Uncovering a metabolomic signature of treatment responses in patients with rheumatoid arthritis**

Cameron Best, Michael Barrett

Abstract: Rheumatoid arthritis (RA) is a degenerative autoimmune disease involving an inappropriate immune response targeting tissue within the synovial joint. If left untreated/poorly managed, RA can lead to crippling disability and even early death in severe disease. The first-line treatment for patients is methotrexate. However, not all patients respond well/tolerate methotrexate which may lead to a worse outcome since an early and aggressive treatment is usually needed to avoid long-term damage to the joints. Identifying who will respond to methotrexate is therefore of great value, and so there is a dire need to identify biomarkers to predict patient responses to direct the optimal treatment strategy. By utilising existing metabolomic data generated using a LC-MS platform using samples from patients treated with methotrexate, this project aims to develop a metabolomic signature for patient responses to help guide treatment in future cohorts. Various analytical tools, including differential analysis, multivariate analysis, and linear modelling, were used to investigate the possible associations between the metabolomic data

and disease activity changes, along with a supervised machine learning approach to generate a model to predict patient outcomes after a 3-month period using the baseline metabolome.

**Advancing clinical research using 4D-OMICS**

Sven Meyer (Bruker)

Abstract: The field of lipidomics is attracting more and more interest in the research community, due to the essential role of lipids in the emergence and progression of diseases.
The usage of improved acquisition techniques increases the coverage and quality of the data from mass analyzers – and also the total number of compounds that can be annotated.
With this, also the need for a standardized and high-quality reporting of lipid annotations is getting more important.

In this presentation, we will demonstrate our lipid profiling workflow and highlight the benefits of ion mobility separation in 4D profiling studies.

**The role of sphingolipid metabolism in microglia activation and Alzheimer's disease**

Areesha Nazeer[1,2], Abbas Dehghan[2], Rui Pinto[2], Ibrahim Karaman[2], Jian Huang[2], Brenan R Durainayagam[2], Sonia Liggi[2], Luke Whiley[2], Tomonori Aikawa[3], Gonçalo Graça[2], Elena Chekmeneva[2], Stephane Camuzeaux[2], Matthew R Lewis[2], Manuja R Kaluarachchi[2], M Arfan Ikram[2], Elaine Holmes[2], Ioanna Tzoulaki[2], Paul M Matthews[2], Paul Elliott[2], Julian L Griffin[1,2]

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While we have a good mechanistic understanding of sporadic Alzheimer's disease (AD) in terms of the formation of amyloid plaques, much less is known about late onset AD. However, a number of the genes associated with late onset AD are also associated with lipid metabolism. We conducted a metabolome-wide association study (MWAS) of AD-associated loci from GWAS using untargeted metabolic profiling (metabolomics) by ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) which identified an association of lactosylceramides (LacCer) with AD-related single nucleotide polymorphisms (SNPs) in ABCA7. Examining the ABCA7-knockout mouse sphingomyelins, ceramides, and hexosylceramides were increased in brain tissue. In order to further investigate the alterations in sphingolipid metabolism in brain we examined lipid changes in LPS-induced microglial cells. Microglial activation is a prominent feature of neuroinflammation, which is present in almost all neurodegenerative diseases. 1ug/mL of LPS caused an increase in lactosylceramides and sphingomyleins. The increased concentration of these sphinglolipds is associated with an increased expression of sphingosine kinase 1 (SphK1), which controls the de novo biosynthesis of ceramides and sphingolipids. Furthermore, the transcript levels of various inflammatory cytokines were measured by qRT-PCR using mRNA extracted from microglial cells. The activation of microglia significantly increased the levels of transcripts encoding the pro-inflammatory cytokines TNF-α, IL-6, and iNOS. Our work suggests that risk for AD arising from functional variations in ABCA7 are mediated at least in part through ceramides.

## Session 6: *Plants, foods and nutrition*

**The Future of Sustainable Aqua Feeds? Assessment of a Genetically Modified Oil on the Phospholipid Composition of Atlantic Salmon**

Richard Broughton, Douglas Tocher, Monica Betancor (Institute of Aquaculture, University of Stirling)

Abstract: Aquaculture is tasked with fulfilling the demands of a growing population, owing to the constraints on capacity of traditional fisheries. The role of fisheries in the future, as well as the majority of other food systems, will likely be impacted by climate change. Therefore, to meet the demands of a growing population, aquaculture will need to provide the additional capacity by which sustainable and nutritious seafood is produced. Oily fish such as salmon are known to be rich sources of long chain omega-3 fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and are usually biotrophically accumulated within the fish. However, traditional aquaculture diets typically blend vegetable oils, rich in 18 carbon fatty acids, to supplement fish oils, typically reducing the overall feed content of long chain polyunsaturated fatty acids (LCPUFAs). To address the fact that LCPUFAs are a limited resource, genetically modified oilseeds have been developed which are capable of producing these LCPUFAs, with one of their target markets being aquaculture.

Owing to the synthetic approach by which these fatty acids are biosynthesised, the parity of these oils needs to be established. Due to the complexity of lipid synthesis, and the various substrate pools in which the fatty acids reside, the variation in lipid isomers, both acyl and potential SN isomers, was explored in addition to the standard lipid classes. Two diets were used for this study, one typical of industrial feed, rich in vegetable oil, whereas the other contained the EPA and DHA rich oil from the modified oilseed *Camelina sativa*. Trends were discovered both relating to the fatty acid composition, characteristic of both terrestrial and modified oils, as well as tissue specific dietary responses. Preliminary findings also suggest alterations within SN-like isomers, indicating potential lipid fingerprints which remain intact throughout the digestive process.

**The Missing Tile in Metabolic Profiling: How Automation Can Help**

Camilla Liscio, Mark Perkins

**Abstract:** The metabolome has a diverse and sensitive nature as a direct reflection of its capability to capture perturbations in metabolic pathways even after minor stimuli. Hence, execution of metabolomics studies must guarantee analytical consistency, optimal information recovery and minimal *intra group* variability to successfully explore insights on the *inter group*s variation. To achieve that, thorough considerations need to be made to limit the sources of variation and choose the most suitable instrumentation for analysis.

The metabolome's chemical complexity demands access to a diversity of analytical approaches and instrumental platforms to address the physical chemical properties of all the metabolites of interest. Instrumentation choice is often or only focused on the end of the analytical workflow, i.e., the analysis. Hyphenated techniques such as HR-GC-MS and HR-LC-MS are well-established techniques of choice. But this approach leaves out one of the essential parts of any analytical workflow, sample preparation. In fact, despite the excellent performances of the latest available hyphenated techniques, good quality data for complex matrices can only be achieved with robust and reproducible sample preparation. It is within this perspective that the on-line automation of sample preparation finds its perfect scope.

The appeal of automated sample prep does not lie only in very good method robustness and batch-to-batch reproducibility. The extremely accurate flow control (down to 0.1µL/s) in liquid handling and the ability to

control timing accurately (e.g. incubation time for derivatisation purposes) open the doors to what could be considered "high performance" sample preparation. Online automated sample preparation, coupled to either GC-MS or LC-MS, is an instrumental solution which could provide, simultaneously:

1. **Versatility** by offering a wide diversity of analytical techniques to address the chemical complexity of the metabolome
2. **Sensitivity** by giving access to enrichment and de-complexification options which could help increasing analyte concentration factor and reducing matrix load prior to analysis
3. **Uniformity** by allowing the use of the same exact comprehensive platform in all metabolomics research centres

An automated sample preparation platform not only combines - on the same physical piece of instrumentation - a large assortment of techniques suited to address the chemical complexity (e.g., smartSPE®, DiLLME, Mitra®) but it also allows for miniaturization of the process which is beneficial when dealing with limited amounts of precious biological samples.
This talk will give an overview of options for automating metabolomics relevant workflows and will showcase current work done in collaboration with existing customers working with metabolic profiling.

**Moringa Metabolites: Nutritional and Economic Value for Malawi**

Wendy Russell[1], Dinka Rees[1], Madalina Neacsu[1]

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In many parts of sub-Saharan Africa smallholder farmers are dependent on agriculture. Loss of genetic diversity as a result of several challenges, including wide scale monocrop adoption and climate change, is limiting the range of plant genetic resources available to support both a climate and nutritional adaptive base. This transition away from traditional crops has a significant impact on both agricultural and dietary diversity. This results in malnutrition at both ends of the economic spectrum, with both a dependency on World Food Programme (WFP) supplements, as well as increased non-communicable diseases. This project explored the potential of Moringa (Moringa oleifera) to supply Malawi's scaled-up nutrition programmes replacing ready-to-eat therapeutic food as well as providing opportunities for smallholder farmers. This talk will present data on the bioavailability of nutrients and bioactive metabolites in a moringa formulation compared to the WFP supplement; 'Super-Cereal Plus' and the development of scientifically-evaluated fair-trade products to enter the growing international market for nutraceuticals.

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