SCOTTISH METABOLOMICS NETWORK

Scottish Metabolomics Network Symposium 2017

City of Glasgow College: Riverside Campus
2nd and 3rd November 2017

Delegate Programme Booklet

Welcome and Introduction

We extend a very warm welcome to all delegates to the City of Glasgow College: Riverside Campus for the Scottish Metabolomics Network Symposium 2017. The network was established with the aim of encouraging the exchange of experiences and ideas between researchers based at Scottish Universities who are working in the field of metabolomics (and in some cases lipidomics).

The two-day meeting will discuss advances and challenges in metabolomic analysis and its applications. There are a range of presentations and posters from seasoned academics as well as early stage career researchers and PhD students based at institutions throughout Scotland. Colleagues from industry will also be highlighting some of their latest developments.

We would like to take this opportunity to thank all our sponsors whose support has been crucial in allowing us to organise the meeting. We very much hope that you will find the meeting enjoyable and that there are opportunities to make new contacts and build collaborative links.

If you require a certificate of attendance please request one via e-mail (wtcrf.education@ed.ac.uk).

Karl Burgess, Gavin Blackburn, Gilliam Mackay and Naomi Rankin
University of Glasgow

The Scottish Metabolomics Network would like to thank all our sponsors for making this symposium possible

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Day One – PROGRAMME

10.20 – 10.50	**Registration, Refreshments**
10.50 – 11.05	Karl Burgess Welcome and update on Scottish Metabolomics Network
11.10 – 12.20	***Scientific Session 1 – Metabolomics and Cancer*** **CHAIR: Gillian Mackay**
11.10 – 11.30	**Andrew Finch (Institute of Genetics and Molecular Medicine, University of Edinburgh)** Marvellous Mitochondria: rewiring of mitochondrial metabolism under energetic stress
11.35 – 11.55	**Jurre Kamphorst (Beatson Institute for Cancer Research, University of Glasgow)** The fats about cancer: using lipidomics approaches to study how lipid metabolism and signalling contribute to tumour progression
12.00 – 12.20	**Tong (Alex) Zhang (Institute for Cancer Sciences, University of Glasgow)** Metabolism of non-essential amino acids in Cancer cells: Cysteine essentiality Early career researcher eligible for the Royal Society of Chemistry: Analytical Division prize
12.20 – 13.20	**Lunch, sponsor exhibition and Networking**
13.20 – 14.30	***Scientific Session 2 - Metabolomics and Nutrition*** **CHAIR: Jeffrey Huang**
13.20 – 13.40	**Madalina Neacsu (Rowett Institute of Nutrition and Health, University of Aberdeen)** Impact of eating breakfast on your metabolome
13.45 – 14.05	**Adel Alghamdi (Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde)** Untargeted Metabolomics Screening for Crohn's Disease Biomarkers in Paediatric Patients Using Liquid Chromatography/Mass Spectrometry Method Early career researcher eligible for the Royal Society of Chemistry: Analytical Division prize
14.10 – 14.30	**Joanne Connolly (Waters)** A non-targeted small scale metabolomic study of retail pomegranate juice products to investigate the nutritional and quality characteristics using a novel data independent acquisition mode and ion-mobility on a QTof MS instrument Sponsor Presentation

14.30 – 15.00	**Coffee/tea, sponsor exhibition and Networking**
15.00 – 16.10	***Scientific Session 3 – Lipidomics and Steroids*** **CHAIR: Ruth Andrew**
15.00 – 15.20	**Natalie Homer (Edinburgh Clinical Research Facility, University of Edinburgh)** The challenges of developing quantitative assays of metabolites metabolic pathways
15.25 – 15.45	**Phil Whitfield (Department of Diabetes and Cardiovascular Science, University of the Highlands and Islands)** Lipidomic Strategies to Investigate Inflammatory Processes
15.50 – 16.10	**Stuart Snowden (King's College London on behalf of Shimadzu)** Association of unsaturated fatty acid metabolism and severity of Alzheimer's disease pathology Sponsor Presentation
16.10 – 17.00	**Poster Session**

Civic Reception

Time: 19:00-19:45

Address: City Chambers, George Square, Glasgow, G2 1DU

Dinner at Drygate Brewry

Start Time: 20:00

Address: 85 Drygate, Glasgow G4 0UT

See below for more information on both venues.

Day Two – PROGRAMME

09.30 – 10.40 ***Scientific Session 4 – Metabolomics and Parasitology***
CHAIR: Gavin Blackburn

09.30 – 09.50 **Fiona Achcar (Institute of Infection, Immunity and Inflammation, University of Glasgow)**
Exploring parasite metabolism using both metabolomics and mathematical modelling

09.55 – 10.15 **Terry Smith (School of Biology, University of St Andrews)**
Identification and Characterization of AEP-Containing Metabolites in Trypanosoma cruzi

10.20 – 10.40 **Neil Walsh (Sciex)**
Benefits of SWATH® Acquisition, a DIA Technique over Traditional Data Dependent Analysis for High Resolution Untargeted Metabolomics Applications
Sponsor Presentation

10.40 – 11.10 **Coffee/tea, sponsor exhibition and Networking**

11.10 – 12.20 ***Scientific Session 5 - Metabolomics and new technologies I***
CHAIR: Will Allwood

11.10 – 11.30 **Hannah Florance (SynthSys, University of Edinburgh)**
Personal Experiences of Metabolite Profiling using GC/QTOF-MS

11.35 – 11.55 **Emily Abraham (School of Chemistry, University of St Andrews)**
Combined Metabolomic and Genomic Approaches to Antibiotic Discovery
Early career researcher eligible for the Royal Society of Chemistry: Analytical Division prize

12.00 – 12.20 **Sufyan Pandor** (**Agilent Technologies)**
My journey into utilizing various techniques to investigate Manuka honey authenticity
Sponsor Presentation

12.20 – 13.20 **Lunch, sponsor exhibition and Networking**

13.20 – 14.30 ***Scientific Session 6 - Metabolomics and new technologies II***
CHAIR: Naomi Rankin

13.20 – 13.40 **Rónán Daly (Glasgow Polyomics, University of Glasgow)**
Statistical ideas in the analysis of metabolomics data

13.45 – 14.05 **Jeni Haggarty (Glasgow Polyomics, University of Glasgow)**
Biofilm Metabolomics: the development of chromatographic methodology for the analysis of dual-species pathogenic biofilms
Early career researcher eligible for the Royal Society of Chemistry: Analytical Division prize

14.10 – 14.30 **Ken Cook** (**Thermo Fisher Scientific)**
New Separations and High-Resolution MS/MS Identification of Lipids
Sponsor Presentation

14.30 – 15.00 **Coffee/tea, sponsor exhibition and Networking**

15.00 – 15.45 **Keynote Address, pizes and close**
CHAIR: Karl Burgess

Professor Susan Rosser (SynthSys, University of Edinburgh) The Synthetic Biology Landscape: Challenges and Opportunities for Metabolomics
Keynote Address

15.45 – 16.15 Closing Remarks, Poster Prizes and Announcement of 2017 Meeting (Will Allwood and Jeffery Huang)

Speaker Biographies and Abstracts

Keynote speaker
Professor Susan Rosser

Professor of Synthetic Biology, Biological Sciences, SynthSys, University of Edinburgh

Susan is Professor of Synthetic Biology at the University of Edinburgh. She is Director of the Edinburgh Mammalian Synthetic Biology Research Centre, Co-director of the Edinburgh Genome Foundry for synthetic DNA synthesis and assembly. She is also holds a prestigious EPSRC Leadership Fellowship in Synthetic Biology. Her research focuses on developing tools for synthetic biology approaches for pathway and genome engineering in bacteria, yeast and mammalian cell systems. The applications of her work include rapid strain engineering for production of high value secondary metabolites, cell lines for protein production, engineering bacteria to generate electricity and developing genetic tools for bio-computation: engineering cells to sense, process and memorise information.

Previously Susan was a lecturer in the Institute of Molecular, Cell and Systems Biology at the University of Glasgow before being promoted to Professor in 2012. Susan studied microbiology and genetics at the University of Dundee before doing a PhD on the mechanisms of multiple antibiotic resistance. She then moved to the Institute of Biotechnology at the University of Cambridge to work on the biotransformation of cocaine and high explosives.

The Synthetic Biology Landscape: Challenges and Opportunities for Metabolomics

In this presentation, I will give an overview of the current synthetic biology landscape with examples from academic and industrial research. I will aim to highlight challenges and opportunities for metabolomics.

Dr Fiona Achcar
Leverhulme Trust Fellow, Institute of Infection Immunity and Inflammation, University of Glasgow

Fiona Achcar is Leverhulme Trust funded fellow at the University of Glasgow. Her background is in computational modelling of metabolism and in the last few years has focused particularly on Parasite metabolism and Metabolomics. With skills and knowledge in both Computer science and biology, She is able to derive inference from biological datasets using computational methods with efficiency. Her modelling work started with iron metabolism in yeast and extended to glucose and then other metabolic pathways in parasitic protozoa. In her recent work, she uses metabolomics data to reconstruct metabolic network. She also uses mathematical modelling to explain some unexpected patterns observed in these metabolomics datasets.

Exploring parasite metabolism using both metabolomics and mathematical modelling

Mathematical modelling and metabolomics are two sets of tools used to explore cellular metabolism. In this talk, I will show how I combine the two to explore metabolism using *Trypanosoma brucei* as an example. *Trypanosoma brucei* is a parasite that causes Human African Trypanosomiasis, a potentially lethal disease. The metabolism of the bloodstream form of the parasite has several unique features that have been investigated, in the search for potential drug targets. Mathematical modelling has been used as a valuable tool to decipher glycolysis, the parasite's only energy source. I will show how we use both modelling and experimental work to decipher the parasite pentose phosphate pathway, another essential pathway that generates the NADPH used in the cells' protection against oxidative stress.

Dr Rónán Daly
Head of Data Analysis , Glasgow Polyomics, University of Glasgow

Rónán obtained an MSc in Artificial Intelligence and a PhD in Machine Learning from the University of Edinburgh. After this he performed post-doctoral research in the statistical modelling of cell reporter systems and mass spectrometry data. He has also worked as a lecturer in mathematics and statistics, and as a software engineer. His interests are in the use of machine learning and Bayesian statistics applied to complex biological data sets. He has performed research in the use of LC-MS data for the detection of metabolites. He is particularly interested in the application of sophisticated statistical techniques to model data obtained from these machines in order to quantify and control the inherent uncertainty and provide robust identification and quantification. Rónán has interests in the application of Bayesian techniques in order to obtain principled statistical analyses of complexbiological data. This also includes the use of encoded contextual knowledge in order to obtain more powerful conclusions of significance and provide more robust control of false positives.

Statistical ideas in the analysis of metabolomics data

After spending long periods of time processing LC-MS data, researchers might be forgiven for resorting to a PCA plot or a list of significantly changing peaks as the output of their analysis. However, in doing so, they might be ignoring other possible analyses that could answer questions such as “Is it possible to predict the effect of a treatment on a given individual’s metabolome” or “can I associate metabolite levels with a phenotypic measurement”. This presentation examines the in-depth analysis of example data sets and the thought processes that led to the statistical techniques used. I focus on non-prescribed analyses and what might be possible given a data set with which you had no up-front involvement. Throughout the presentation, I emphasize the dangers of pattern hunting and the need to carefully think about over-fitting models. Concepts talked about will include classification, regression, covariates, mixed models and graphical output.

Dr Andy Finch

Chancellor's Fellow, Institute of Genetics and Molecular Medicine, University of Edinburgh

After his PhD, Andy Finch went to work with Gerard Evan at the UCSF Cancer Center (in San Francisco) on mechanisms of oncogene-induced tumour suppression. During this period, He developed his interest in apoptosis, tumour suppression and homeostasis. He returned to the UK to work with Alan Warren at the MRC Laboratory of Molecular Biology (LMB) in Cambridge where he studied a disorder of ribosome biogenesis called Shwachmann Diamond Syndrome. Since setting up his lab at the Cancer Research UK Edinburgh Centre (part of the Institute of Genetics and Molecular Medicine, IGMM), he has focussed upon metabolic stresses elicited by oncogene activation. He set up the mass spectrometry capability at the IGMM, with particularemphasis upon metabolomics, and continues to develop the metabolomic and lipidomic expertise there. Supported by an excellent group of PhD students and his great colleagues in IGMM Mass Spectrometry (IGMM-MS), Andy is enjoying his busy life in the IGMM.

Marvellous Mitochondria: rewiring of mitochondrial metabolism under energetic stress

Mammalian cells with defects in mitochondrial respiration (rho0 cells) exhibit a profound mitochondrial redox defect and therefore lack the ability to convert glutamine into aspartate via the TCA cycle and this leads to inhibition of nucleotide biosynthesis and lack of viability. Exogenous pyruvate has long been known to support viability of rho0 cells and the mechanism for this has recently been described through direct production of aspartate via pyruvate carboxylase. Indeed, aspartate itself can support viability of rho0 cells. We studied the metabolism of aspartate in rho0 cells through stable isotope tracing and found metabolic flux through malate and fumarate and, surprisingly, onto succinate. This indicates that aspartate reverses the activity of several dehydrogenases and that it can drive reversal of the mitochondrial redox defect through reversal of the TCA cycle. Inhibition of this pathway (using aminooxyacetate to inhibit transamination of aspartate to oxaloacetate) prevented the rescue of viability by aspartate, indicating that the reversal of dehydrogenase reactions was essential for rescue of viability. Our results indicate that maintenance of redox balance is a key factor in the viability of respiration-deficient cells and that this is as important as aspartate production in the rescue of rho0 cell viability by pyruvate.

Dr Hannah Florance
Metabolomics Facility Manager (Edinomics) , SynthSys, School of Biological Sciences, University of Edinburgh

A graduate from Sheffield Hallam University, Hannah had spent a year in industry where she learned an appreciation for identifying and characterising novel drug metabolites using LCMS. She then pursued a path of protein chemistry, firstly in the biotech industry at PPL Therapeutics before returning to academia at the University of Edinburgh where she eventually specialised in peptide mass fingerprinting. After a PhD with Prof Perdita Barran at the University of Edinburgh looking at protein conformation in solutions and the gas phase she moved down to Exeter where she set up the metabolomics mass spectrometry facility, looking at, amongst a myriad of projects, the effect of plant pathogens on their metabolomes, and screening urine for bladder cancer. She returned to Edinburgh to the UK Mammalian Centre for Synthetic Biology in 2015 to set up a metabolomics core facility, principally to support the Centre's research.

Personal Experiences of Metabolite Profiling using GC/QTOF-MS

GCMS is an analytical technique which provides excellent chromatographic resolution with peak widths averaging 6 seconds. With an ability to readily resolve isomers, along with the existence of well populated databases, it has historically been the analytical method of choice for many metabolomics studies.

GCMS is, by its very nature a selective process, with all compounds needing to be volatile. The means by which we derivatise our extracts to achieve that level of volatility becomes self-selecting. Therefore, is this truly untargeted, or in fact targeted analysis? Can we elicit more information about the compounds by running different ionisation techniques such as electron ionisation (EI) and chemical ionisation (CI)? Can the gas used in the collision cell affect spectra, impacting on id scoring? By running different derivatisation protocols and ionisation methods, this talk discusses the pros and cons of using GCMS as a tool for metabolite profiling.

Dr Natalie Homer
Mass Spectrometry Core Manager, Edinburgh Clinical Research Facility,
University of Edinburgh

Dr Natalie Homer obtained her undergraduate degree in Chemistry at the University of Strathclyde. She continued her studies there with a PhD in Dr John Reglinski's laboratory, using both NMR and mass spectrometry as analytical tools to investigate oxidative stress in postmenopausal women, which was a collaborative project between Dr Corinne Spickett of the Immunology Department and Dr Rhoda Wilson of the Department of Medicine, Glasgow University. Since completing her PhD in 2002 she has managed the Mass Spectrometry Core of the Edinburgh Clinical Research Facility. The core specialises in targeted analysis of small molecules, in particular steroid hormones and drugs such as paracetamol, using LC-MS/MS and GC-MS instruments.

The challenges of developing quantitative assays of metabolites metabolic pathways

Once metabolites or a particular metabolic pathway have been identified as of interest then quantitative information is needed to further understand the biology.

The method must be accurate, precise and furthermore exploit the capabilities of the instrument to give the best quantitative assay. Bioanalytical method validation using is a well-described approach to assay set up, which includes a number of points of consideration. Translating the research question into a valid assay is a challenge. Introducing appropriate labelled standards, identifying the biological range of the calibration curves and being aware of potential interfering analytes in the chromatographic development are all important. Determining limits of detection of each analyte and planning the calibration curve accordingly, defining the dynamic range of the assay, optimising the extraction procedure and assessing sample stability once extracted are all necessary considerations.

Two examples will be presented. An assay developed for Tryptophan metabolites and its application to biological samples. The second example is a metabolomics screen, which identified 20 compounds in a cohort of Congenital Adrenal Hyperplasia patients on long-term glucocorticoid treatment. Six of these metabolites were chosen to be used as a screening assay for glucocorticoid sensitivity. Their chemical nature differed and the method development was a particular challenge. The challenge was to incorporate as many of the six into one simple extraction and analysis as possible.

Dr Jurre Kamphorst
Group Leader, CRUK Career Development Fellow, Cancer Metabolism Research Unit, Cancer Research UK Beatson Institute , University of Glasgow

Jurre performed his PhD research with Thomas Hankemeier, in the department of Analytical Biosciences at Leiden university, the Netherlands. After completing his PhD in 2009, he Joined the lab of Josh Rabinowitz at Princeton University as a postdoctoral fellow. Here he worked on cancer metabolism. Jurre started his lab at the Beatson in 2014, focusing on the role of lipid metabolism and signalling in cancer. Also in that year, he obtained a prestigious Cancer Research UK Career Development Fellowship.

The fats about cancer: using lipidomics approaches to study how lipid metabolism and signalling contribute to tumour progression

In our laboratory at the Beatson Institute, we study the role of lipid metabolism and signalling in cancer. For this, we exploit state-of-the-art lipidomics and stable isotope tracing approaches, which we combine with other biochemical and molecular biological assays. Thus far, a particular interest has been to elucidate how conditions of the tumour microenvironment affect lipid metabolism in cancer cells. This led to the identification of a fatty acid saturation buffering role of triglycerides in hypoxic cancer cells, and in the discovery that stromal cells release pro-oncogenic LPA lipids in pancreatic tumours. In this presentation I will give an overview of our analytical strategies and will give examples of how those were applied to increase our understanding of cancer lipid metabolism.

Dr Madalina Neacsu
Research Fellow, The Rowett Institute, University of Aberdeen

Dr Madalina Neacsu is a biochemical engineer specialised in natural products food formulation, metabolism and bioactivity. During her career Madi worked both in academia and industry, being in charge of the development of several plant-based bioactive-formulations and has contributed to the compilation of dossiers for successful ESFA-approved health claims. Currently working as research fellow at The Rowett Institute, University of Aberdeen, UK.

In the last five years her research focussed on assessing the effects of supplementing diets with plant based foods on human nutrition and health through running series of acute and chronic human dietary intervention studies. Currently, Madi is exploring the potential of high protein crops for developing bioactive-formulations and food ingredients for specialised sustainable functional foods. She is actively collaborating with industry reformulating healthier foods using sustainable food ingredients.

Impact of eating breakfast on your metabolome

Whole-grains are linked to reduced risk of several chronic diseases and are an important source of dietary fibre. When compared with an equivalent serving of fruits or vegetables, a recommended portion of whole-grain cereals deliver substantially higher amounts of bound phytophenols available for colon metabolism. Moreover, their dietary fibre escape digestion and is fermented by host bacteria to short chain fatty acids (SCFA). Eight healthy volunteers 18-55 years old, BMI (18-30 kg/m^2) consumed a test meal containing a recommended dose (40 g) and high dose (120 g) of ready-to-eat wheat bran cereals and the systemic and colonic metabolites determined quantitatively by LC-MS and GC-MS. A wide range of phytophenols (43 metabolites) were absorbed/excreted within 5 h of consumption, including most of the major parent compounds identified in the test meal (16 of the 21), several metabolites also found to be significantly increased in the colon. Butyric acid demonstrated the largest increase in colon (108 %) from baseline to 24 hours following consumption of the recommended serving ($p<0.05$). Acetate, propionate and butyrate showed significant anti-inflammatory activity ($p<0.001$ for acetate and butyrate and $p<0.01$ for propionate) for their *in vivo* concentrations. Not all of the metabolites were increased with the higher dose, suggesting some limitation in absorption due to intrinsic factors and/or the food matrix. No significant difference in the plasma, urine and faecal concentrations of SCFA between servings was observed, with the exception of butyric acid in urine after 24 hours consumption ($p<0.05$). The important amount of systemic and colonic bioavailable phenolic metabolites and SCFA may, in part, explain the evidence for the protective effects of whole-grain cereals.

Professor Terry Smith
Biomedical Sciences Research Complex, School of Biology, University of St Andrews

Smith has over 24 years experience working with protozoan parasites. Since establishing my research group in Dundee (2003), I have gained a standing within the parasitology field for undertaking lipidomic analysis to aid phenotyping of genetically or chemically manipulated parasites. Since moving (Oct. 2007) and establishing the first ever Cat 3 facility in St Andrews, I was able to consolidate and expand my studies into other areas of parasite lipid metabolism, using the world-class facilities and collaborations in Chemistry, Biology and Medicine available at St Andrews. The University completed building of a new £13 million purpose built Biomedical Science Research Complex which we moved into in Jan 2012. My group occupies biochemistry and chemistry labs as well as a larger Cat 3 suite within this new this new complex. The building also has a purpose-built floor for analytical instrumentation, including lipidoomic dedicated 4000 Q-trap, orbitrap exactive and several other LC- and GC-mass spectrometer. Smith has numerous on-going collaborative lipidomic and focused metabolomics analyses. These are not just of protozoa, but include numerous other pathogens, bacteria, yeast and viruses as well as model cell-lines of human disease and organisms and other diverse samples as salmon, fruit flies and plant tissues.

Identification and Characterization of AEP-Containing Metabolites in *Trypanosoma cruzi*

The neglected tropical disease, American Trypanosomiasis (also known as Chagas' disease) is the most important parasitic infection in Latin America, and the most lethal endemic infectious disease in the Western Hemisphere. At the time of writing 8-10 million individuals are currently infected with the disease and a further 25 million are at risk. Currently available treatments against Chagas' disease are ageing, ineffective, and often exhibit severe side effects, thus new treatments are urgently required. One possible drug target against *Trypanosoma cruzi* - the etiological agent of Chagas' disease - is the biosynthesis of the parasitic glycosylphosphatidylinositol (GPI) anchors. All *T. cruzi* GPIs and glycosylinositolphospholipids share a common Man_4(AEP)GlcN-Ins-PO_4 core, where AEP is 2-aminoethy)phosphonate. AEP has been demonstrated as a virulence factor in several human pathogens, although the role of AEP on GPI-anchors in *T. cruzi* is unknown. In this study we have identified, previously unreported, high-energy donors of AEP - a cytidine nucleoside termed CDP-AE (analogous to CDP-EtN), and lipid donors of which were characterised: through fragmentation of the NL126 These structures exhibit a remarkable similarity to ethanolamine phosphate and its downstream derivatives of the Kennedy pathway. Although the transferase of AEP to GPI-anchors is still unknown, these discoveries may inform future studies.

Professor Philip Whitfield
Head of Lipidomics Research , Department of Diabetes and Cardiovascular Disease, University of the Highlands and Islands

Phil completed his PhD in lipid biochemistry at Great Ormond Street Institute of Child Health, University College London and then went on to work as a Hunter's Hope Foundation Postdoctoral Research Fellow at Adelaide Children's Hospital. He continued his research at Addenbrooke's Hospital in Cambridge before taking up a lectureship at the University of Liverpool. He is currently Head of Lipidomics Research at the University of the Highlands and Islands where his research involves the study of lipid metabolism in diverse areas of biology and medicine.

Lipidomic Strategies to Investigate Inflammatory Processes

Inflammation is a physiological response to infection or injury that is regulated through complex signalling pathways in a wide range of inflammatory cells. Defects in these processes can result in failure to resolve inflammation causing tissue damage and contributing to the pathogenesis of many prevalent diseases including asthma, chronic obstructive pulmonary disease (COPD) and atherosclerosis. The onset of inflammation is driven by bioactive lipid mediators known as eicosanoids. These lipids are formed from the polyunsaturated fatty acid, arachidonic acid (20:4; n-6) through the cyclooxygenase, lipoxygenase and cytochrome P450 pathways and include prostaglandins, leukotrienes and hydroxyeicosatraenoic acids (HETEs). However, the analysis of these lipids represents a considerable challenge. The presence of multiple isomeric species combined with their low concentrations in biological samples means that highly selective and sensitive assays are required. Targeted lipidomic strategies are being employed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) for the characterisation and quantification of eicosanoids and related lipids. This presentation will provide an overview of these methods and their application to studies focused on understanding inflammatory processes in disease states.

Early Career Researcher Biographies and Abstracts

We have four early career eligible for the Royal Society of Chemistry: Analytical Division Prize

Ms Emily Abraham

PhD Student, School of Chemistry, University of St. Andrews

Emily Abraham completed her undergraduate degree at the University of Bristol where her final project involved reconstructing the biosynthetic pathways of statins in filamentous fungi. She then moved to St Andrews to start her PhD in Dr Rebecca Goss' group. In her PhD she is using a combination of molecular biology and genome mining approaches to identify novel antibiotics. Emily is also developing Genochemetic approaches to access analogues of antibiotics to improve their bioactivities.

Combined Metabolomic and Genomic Approaches to Antibiotic Discovery

Natural products provide an unparalleled starting point for drug discovery, with over 60% of anticancer agents and over 70% of antibiotics entering clinical trials in the last three decades being based on such compounds. The majority of such compounds have been derived from microbial sources. However, as the same highly potent compound can be produced by many different microbes, there is always a risk of rediscovering the same antibiotic using a traditional bacterial screening approach. Recently however, advances in genome sequencing mean that a vast number of bacterial genomes are now available. This sequence data has revealed that only a small proportion of microbial biosynthetic capability has been tapped and excitingly there are many more natural products waiting to be discovered. We are employing a state of the art approach for antibiotic discovery where we are using a combination of genomics and metabolomics to identify novel antibiotics. We are reading the genomes of a series of actinomycete bacteria to try and identify signature genes for natural products such as hybrid non-ribosomal peptide synthetases, polyketide synthases, terpenoids and lantibiotics. We can then target biosynthetic geneclusters that are very different from known gene clusters, and therefore likely to produce novel antibiotics, for heterologous expression. By identifying and discounting previously known and isolated compounds from further analysis, we can focus efforts on pursuing the compounds that are likely to show the highest novelty.

Mr Adel Alghamdi
PhD Student, Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde

Adel Alghamdi is a 3rd year PhD student working with Dr David Watson in the field of metabolomics. Adel earned his bachelor degree in Saudi Arabia from the College of Pharmacy, King Saud University. After spending nearly 3 years working as pharmacist in King Faisal specialist hospital- Jeddah, he moved to Taif University as an assistant teacher in the college of Pharmacy. Adel received his MSc degree from the University of Glasgow in clinical pharmacology. Most recently, he was posted as a lecturer in the College of Clinical Pharmacy, Al-Baha University. His PhD project is focused on understanding metabolic phenotypes in inflammatory bowel disease using metabolomics.

Untargeted Metabolomics Screening for Crohn's Disease Biomarkers in Paediatric Patients Using Liquid Chromatography/Mass Spectrometry Method

Introduction: Crohn's disease (CD) is considered to result from the metabolic changes between host and microbiota. These metabolic could be returned after using Exclusive enteral nutrition. In this study, as untargeted metabolomic study, we examine the differences in the metabolome between control and diseased subjects after exclusive enteral nutrition (EEN) was applied as a treatment. Methodology: Five faecal samples were collected from 11 CD children (n=55) and compared to 11 healthy controls. All samples were freeze dried then extracted by Chloroform/Methanol/Water at a volume ratio of 1:3:1. Extracts were analysed on a ZIC-pHILIC column coupled to an Orbitrap Exactive Instrument. Data extraction was carried out by using mzMatch. All data processing, was implemented using SIMCA software v.14.1 for multivariate and Metaboanalyst for univariate analyses. Results: In a comparison between CD children with healthy controls, based on 683 putative metabolites, the metabolomic profile appeared with high discrimination. For example, tryptophan pathway was significantly downregulated in children with CD. In addition to that, tyrosine metabolism discriminated CD from healthy children. Amino acids such as L-proline, valine and alanine have a clear difference between CD and healthy children.

Dr Jennifer Haggarty
Post doctoral research assistant, Glasgow Polyomics, University of Glasgow

Completed a PhD developing chromatographic methodology for the analysis of dual species pathogenic biofilms at the University of Glasgow in September 2017. Currently working with Glasgow Polyomics and Ingenza Biosciences developing continual sampling methods for real-time metabolic analysis of fermentation cultures as part of the IBioIC Accelerator Programme.

Biofilm Metabolomics: the development of chromatographic methodology for the analysis of dual-species pathogenic biofilms.

Polymicrobial diseases arise when multiple microorganisms colonize a host and form multi-species biofilms. The composition and the interactions between organisms within polymicrobial biofilms govern disease severity and patient outcomes. Polymicrobial infections are of significant interest because of the escalating development of antimicrobial resistance and the increasing involvement polymicrobial biofilms in chronic and systemic infections. The Gram-positive bacteria *Staphylococcus aureus* and dimorphic fungi *Candida albicans* have been shown to coexist within the human host in polymicrobial biofilm communities which often result in increased disease severity and mortality. Metabolomics offers a powerful analytical tool to gain a better understanding of the interactions between this bacteria and fungus. The overall aim of the research was to develop novel metabolomics methods and to apply these methods to the analysis of a *S. aureus/C. albicans* dual species biofilm to aid in the understanding of the relationship between this bacteria and fungi. An untargeted liquid chromatography-mass spectrometry separation method was developed that effectively retained both polar and nonpolar compounds by serially coupling a reversed-phase liquid chromatography (RPLC) column to a hydrophilic interaction liquid chromatography (HILIC) column via a T-piece. Microscopic and molecular characterisation enabled visualisation of the dual-species biofilm, while metabolomics analysis highlighted significant changes in a number of pathways including purine, pyrimidine, methionine and cysteine metabolism between the *S. aureus* and *C. albicans* mono- species and the dual species biofilms. The results obtained indicated that the relationship between *S. aureus* & *C. albicans* may not be completely synergistic, as previously suggested.

Dr Tong (Alex) Zhang
Research Technician, Institute of Cancer Sciences, University of Glasgow

As an undergraduate I studied Pharmaceutical Sciences at China Pharmaceutical University, graduating in 2002. In 2008 I obtained a PhD in Analytical Chemistry working with Dave Watson at Strathclyde University. Subsequently as part of Dave's lab I focused my research on metabolomics studies including development and application of hyphenation techniques (LC-MS, LC-MS/MS, GC-MS, LC-UV-ELSD and NMR) and the relevant data processing/ interpretation using chemometrics methods. In 2015 I joined Dr Oliver Maddocks group at the University of Glasgow investigating non-essential amino acid metabolism in cancer cells.

Metabolism of non-essential amino acids in Cancer cells: Cysteine essentiality

Reprogrammed metabolism has been defined as one of the fundamental hallmarks of cancer cells. While metabolic alterations can evolve to support rapid growth and proliferation, they can also diminish metabolic flexibility. In this study, we found that cancer cells generally cannot proliferate without a supply of exogenous cysteine/cystine and interestingly some cells showed acute cell death. Stable isotope tracing showed that methionine, the precursor for production of cysteine, is diverted into the polyamine synthesis pathway. This diversion decreases the flow of methionine into the transulfuration pathway for cysteine synthesis, leading to poor cysteine production in cancer cells. Furthermore, cells showing deletion of the gene for methylthioadenosine phosphorylase (MTAP) were highly sensitive to cysteine starvation. MTAP is an important enzyme in methionine recycling and without this pathway cancer cells fail to convert MTA back to methionine and consequently efflux MTA. MTAP loss therefore decreases the efficiency of methionine metabolism and further prevents cysteine synthesis explaining the high sensitivity to cysteine starvation for these MTAP-deleted cells.

Industry Speaker Biographies and Abstracts

Dr Joanne Connolly

Business Development Specialist for OMICS, Waters

Joanne completed a PhD in Mass Spectrometry at UMIST in Manchester in 2002 supervised by Simon Gaskell, followed by a post-doctoral position at the National Institute of Health in Bethesda, USA. She was affiliated to both the Diabetes and Mental Health Institutes. Her first few years following this were spent with Shimadzu Biotech as an MS applications specialist. Joanne then joined Waters in 2007 where she was a member of the application team in Manchester responsible for the complex proteome applications area. In 2009, she became team manager in the application Laboratory before moving to her current role as Business Development Specialist for OMICS for the Northern Europe region in 2011. Joanne is responsible for helping customers be successful by introducing novel technology and workflows into the laboratories through collaboration. She has co-authored 12 peer reviewed publications with collaborators through this initiative covering many varied application areas.

A non-targeted small scale metabolomic study of retail pomegranate juice products to investigate the nutritional and quality characteristics using a novel data independent acquisition mode and ion-mobility on a QTof MS instrument

In this study, we report the potential of a new data independent acquisition (DIA) mode on a QTof instrument in combination with a scanning quadrupole mass filter and ultra-fast detection system. This methodology alongside ion mobility enabled QTof-MS (IM-QTof-MS) were used as tools to improve analytical selectivity and facilitate the process of marker identification in complex juice samples following a simple sample preparation step. The resulting information was further subject to database searching which indicates the presence of several significant polyphenolic compounds and processing additives in a selection of commercially available processed juice products in the U.K.

Mr Sufyan Pandor
LCMS Field Application Specialist, Agilent Technologies

Agilent is my first proper job after graduating so I am quite new to metabolomics, joining as a customer engineer 5 years ago I have recently joined the application specialist team for LCMS based in Cheadle UK. I started my PhD at the University of Huddersfield part time whilst working at Agilent now just a couple of years to go.

My journey into utilizing various techniques to investigate Manuka honey authenticity

A look at my journey through the minefield that is metabolomics. Being quite new to LCMS I will share my experiences from a few perspectives; an engineer, a PhD student and an application specialist. Manuka honey fraud is a hot topic at the moment and as the crooks get smarter how can we develop quick sensitive robust models to detect fraud.

Dr Ken Cook
EU Bio-Separations Expert CMD, Thermo Fisher Scientific

Background
Started as a University lecturer in Biochemistry, University of Newcastle upon Tyne
29 years' experience with Dionex then Thermofisher Scientific in Chromatography solutions.
Current job title is the European Bio-Separations expert for Thermofisher Scientific where Bioseparations for Proteins, peptides and metabolomics are primary application fields.

Instrumentation experience
Most forms of liquid chromatography including HPLC, FPLC, IC, SFE, Capillary electrophoresis. This includes most detection systems and interfacing with MS.
Strong links to the consumable division where the sample preparation and multiple column technologies are essential for bio-separations, also the HPLC and Mass Spectrometry divisions to form the best total solutions for characterisation.

Application experience
Bio-Separations including; Metabolomics, Glycomics, Lipidomics, Proteomics and Bio-Therapeutics
HPLC applications including UHPLC and LCMS
Ion Chromatography including conductivity, electrochemistry and ICMS

New Separations and High-Resolution MS/MS Identification of Lipids

Lipids create a problem in analysis due to their chemical diversity and many isoforms which can be isobaric. In order to understand Lipid structure-functional relationships, specific and sensitive analytical tools allowing separation and identification of structural isomers are required. Liquid chromatography coupled on-line to mass spectrometry (LC-MS) allows high-throughput characterization of Lipids in biological samples. However, separation must be achieved for isobaric compounds and MS/MS can play an important role in identification.

Dr Stuart Snowden

Senior Postdoctoral Researcher, King's College London, on behalf of Shimadzu UK Limited

- Senior Postdoctoral Researcher, Nov 2017 – present: University of Cambridge, Metabolic Research Laboratories.
- Senior Postdoctoral Researcher, Nov 2015 – Nov 2017: Kings College London, Institute of Pharmaceutical Sciences. I have been responsible for performing routine metabolomics analysis as well as developing novel LC-MS and GC-MS metabolomics techniques and applying these to both plasma and CSF samples of patients with Alzheimer's disease, and subsequently analysing the generated data and preparing results for publication and oral presentation at conferences.
- Postdoctoral Researcher, Nov 2013 – Oct 2015: Kings College London, Institute of Psychiatry, Psychology and Neuroscience. I worked with collaborators to establish methods for metabolite profiling of brain tissue then analysing a cohort of 124 human brain samples, and subsequently analysing the generated data and preparing results for presentation at
- conferences and publication. I was also responsible for designing and selecting the study cohort for a large project looking for metabolite biomarkers of Alzheimer's disease.
- Postdoctoral Researcher, Feb 2012 – Oct 2013: Karolinska Institutet, Department of Medical Biochemistry and Biophysics. My main responsibilities were to develop from scratch a range of analytical LC-MS platforms for both non-targeted and targeted methods for measuring small molecule metabolites in a range of bio-fluids and applying these methods to samples from a range of respiratory diseases. I also processed and analysed the generated data and either prepared reports to detail the results for collaborators or wrote them up for publication.
- University of Wales Aberystwyth (Sept 2007 – Oct 2011) Ph.D Metabolomics and Molecular Plant Pathology
- University of Wales Aberystwyth (Sept 2004–July 2007) BSc – Marine and Freshwater Biology

Association of unsaturated fatty acid metabolism and severity of Alzheimer's disease pathology

In the presentation, I am going to talk about a study in which I measured the metabolite composition of human brain samples to determine the role of metabolism in Alzheimer's disease. The metabolism of the unsaturated fatty acids (UFA) arachidonic, oleic, eicosapentaenoic, docosahexanoic, linolenic and linoleic acids were shown to be strongly associated with the severity of disease pathology. We have hypothesised that these UFAs are contributing to pathology as precursors of a range of highly bioactive lipid mediators including prostaglandins, leukotrienes and resolvins.

Dr Neil Walsh
Business Development Specialist – Advanced Workflows, Sciex

Neil spent his laboratory working life in contract research organisations for several years in the field of bioanalysis and metabolite ID to where he finds himself now as business development specialist for biopharma and Omics as SCIEX.

Benefits of SWATH® Acquisition, a DIA Technique over Traditional Data Dependent Analysis for High Resolution Untargeted Metabolomics Applications

As interrogation of the metabolome moves from a discovery into verification into the A data independent acquisition (DIA) technique known as SWATH® acquisition enables the identification and quantification of a higher number of metabolites in known unknown metabolomics workflows compared to standard data dependent acquisition approaches thus enabling a deeper profile of the metabolome. In addition, SWATH® acquisition allows the collection of MS and MSMS data in a single injection and builds a digitized map of detectable metabolites in your sample. Which enables retrospective data mining meaning as your hypothesis changes there is no need to go back and re-run your sample but just to re-mine the data.

Poster Titles and Abstracts

All poster presentations eligible for the Royal Society of Chemistry: Analytical Division Prizes: first prize, two runner-up prizes and the People's Choice prize. You can cast your vote for the people's prize on Survey Monkey (details to be provided on the day).

1. Abdulwahab Awadh Alamri and David Watson
Strathclyde school of pharmacy and biomedical science, University of Strathclyde

Effect of High glucose (25 mM) as oxidative stress inducer on the pulmonary artery SMCs

Oxidative stress is a prevalent moderator in pathogenicity of recognized cardiovascular risk factors. In addition, it possibly arbitrates effects to arise no well-defined variables that participate in residual risk not elucidated by traditional factors. Functional oxidative alterations of cellular components are a fundamental step in cellular dysfunction and recent studies implicate oxidative stress as a mediator of pulmonary arterial hypertension. Detecting oxidative stress markers has been the interest of many scientists as they have the potential to perform as an "integrator" of an abundance of processes that mediate cardiovascular pathobiology. In order to profile the affected metabolites that may be dysregulated when PASMCs are exposed to oxidative stress, high glucose media (25 mM) was used as stressor in smooth muscle cells in comparison to low glucose media (5 mM) and metabolic profiling was carried out using hydrophilic interaction liquid chromatography high resolution mass spectrometer. As a result, 38 putative biomarkers were obtained. Among these metabolites, Glutathione disulphide (GSSG) as an oxidative marker induced by about 1.5-fold (P = 0.008). The pentose phosphate pathway and oxidative phosphorylation metabolites were depleted under oxidative stress conditions. In conclusion, metabolic profiling demonstrates oxidative effect using high glucose media on pulmonary artery smooth muscle cells. In addition to the metabolic profiling result, viable cell counts showed a higher number of cells in the stressed cell samples with high glucose media than low glucose media cell samples. The latter observation fits with the aetiology of pulmonary arterial hypertension which involves proliferation and remodelling vascular tissue.

2. Ibrahim Alanazi Catherine Henderson, David Watson, M. Helen Grant.
Strathclyde school of pharmacy and biomedical science, University of Strathclyde

Investigation of Cobalt Neurotoxicity in Vitro Using Metabolomics

Human exposure to cobalt arises from many sources, and recently it was shown to be released from the wear debris of cobalt-chrome hip replacement implants. This gives rise to high circulating Co blood concentrations and potentially multiple systemic adverse effects which may include CNS toxicity, in patients. The mechanism of CNS toxicity is unknown. To investigate this, human neuroblastoma (SH-SY5Y) and astrocytoma (U373) cells were treated with Co (0-200 μM) for 72 hour. The cells metabolomics profile was analysed using high resolution liquid chromatography mass spectrometry and the data was processed by Xcalibur, Mzmine, and Simca-P software packages. Co appeared to induce DNA deamination and methylation in both cell types, resulting in cell-death due to thymine depletion. Uracil and 5-methylcytosine levels increased (at 200 μM Co, up to 28 times more than the relevant control) in a direct correlation with Co concentrations. In contrast, thymine levels were higher (20 times higher than the relevant control cells at 50 μM of Co) at lower doses of Co and declined (to become10 times higher than relevant control cells, at 200 μM of Co) as more deamination occurred as a result of increasing Co exposure. SH-SY5Y cells were more affected by Co than U373 cells. They showed greater ability to prevent the DNA deamination and produce more thymine to compensate for Co induced thymine loss. These findings indicate, for the first time, that cobalt induces DNA deamination and methylation and that cells have the ability to reverse this deamination.

3. Samyah Alanazi, Dr David G. Watson and William Harnett.
Strathclyde school of pharmacy and biomedical science, University of Strathclyde

Metabolomic profiling of the effects of synthetic analogues of the parasitic worm product ES-62 on LPS stimulated macrophages

It has been reported that stimulating macrophages with bacterial products such as lipopolysaccharide cause them to produce the proinflammatory cytokines IL-12 (a Th1 directing cytokine), IL-6 and tumor necrosis factor- alpha (TNF-a). Pre-treatment of these macrophages with ES-62, a glycoprotein secreted by the parasitic filarial nematode Acanthocheilonema viteae that possess anti-inflammatory effects, was found to supress the production of these cytokines (Al-Riyami and Harnett 2012). However, ES-62 is not suitable for drug therapy due to its potential immunogenicity and therefore a library of small molecule analogues (SMAs) was designed and tested for the previously mentioned effects (Al-Riyami, Pineda et al. 2013). SMAs 11a and 12b among library members were found to mimic ES-62's anti-inflammatory effects (Al-Riyami, Pineda et al. 2013). These interesting findings justify further testing to find out more about the SMAs' potential as drugs or even to elucidate their mechanism of action. Thus, the major purpose of this project is to enhance knowledge on the process of immunomodulation by ES-62 and associated SMAs in the context of the metabolome.

4. Adel Alghamdi, Vaios Svolos, Konstantinos Gerasimidis and David Watson
Strathclyde school of pharmacy and biomedical science, University of Strathclyde

Untargeted Metabolomics Screening for Crohn's Disease Biomarkers in Paediatric Patients Using Liquid Chromatography/Mass Spectrometry Method

Introduction: Crohn's disease (CD) is considered to result from the metabolic changes between host and microbiota. These metabolic could be returned after using Exclusive enteral nutrition. In this study, as untargeted metabolomic study, we examine the differences in the metabolome between control and diseased subjects after exclusive enteral nutrition (EEN) was applied as a treatment. Methodology: Five faecal samples were collected from 11 CD children (n=55) and compared to 11 healthy controls. All samples were freeze dried then extracted by Chloroform/Methanol/Water at a volume ratio of 1:3:1. Extracts were analysed on a ZIC-pHILIC column coupled to an Orbitrap Exactive Instrument. Data extraction was carried out by using mzMatch. All data processing, was implemented using SIMCA software v.14.1 for multivariate and Metaboanalyst for univariate analyses. Results: In a comparison between CD children with healthy controls, based on 683 putative metabolites, the metabolomic profile appeared with high discrimination. For example, tryptophan pathway was significantly downregulated in children with CD. In addition to that, tyrosine metabolism discriminated CD from healthy children. Amino acids such as L-proline, valine and alanine have a clear difference between CD and healthy children

5. Aliyah Alhawiti and David G. Watson
Strathclyde school of pharmacy and biomedical science, University of Strathclyde

Application of deuterated aniline and p-(trimethylamino) aniline as tagging agents in the metabolomics analysis of sugars by LC-MS

Metabolomics analysis of sugars is challenging because of the presence of various isomers in α- and β-pyranose and furanose forms in equilibrium. These isomers lead to poor peak shapes in LC-MS and the sugars cannot be distinguished simply based on MS/MS or accurate masses, thus necessitating chromatographic resolution. Reductive amination at low pH offers the best approach for chromatographic separation since it causes all the four conformations of the sugars to form one product. This approach uses a weak base and a reducing agent which is stable at the low pH required to keep the sugars in their ring open aldehydic forms to convert the sugars into amines. The current study evaluated two aniline-based tagging agents D_5-aniline and p-(trimethylamino)aniline for the derivatisation of hexoses, pentoses, disaccharides and glycoproteins in biological fluids. The analysis employed ZIC-HILIC columns with 0.01% formic acid in water (A) and 0.01% formic acid in acetonitrile (B) as mobile phases under isocratic conditions. The derivatives of common hexoses such as glucose, galactose, fructose and mannose, and those of common pentoses including xylose, ribose, 2-deoxy-D-ribose, and arabinose were resolved with greatly improved peak shapes. The yield of the tagging reaction was high and D_5-aniline has so far proved to be the more effective at achieving separation of the isomeric derivatives. The method has been successfully applied in the detection and quantification of sugars in urine using ^{13}C-labbeled internal standards in order to determine any link to metabolic disease states.

6. Will Allwood[1], Yun Xu[2], Raphaelle Palau[1], Tom Shepherd[1], Catherine Howarth[3], Pilar Martinez Martin[3], Athole Marshall[3], Sarah Clarke[4], Roy Goodacre[2], Derek Stewart[1]
[1]James Hutton Institute, [2]Manchester Institute of Biotechnology, The University of Manchester, [3]Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, [4]ADAS Gleadthorpe

Application of metabolomics approaches to study the effect of nitrogen elevation on winter oat metabolite composition and quality traits

Developing high quality oats is constrained by a lack of information on the impact of genetic and environmental/management factors. Current knowledge on optimal nitrogen levels is insufficient and both greater oat yields and higher quality oats could be achieved. In this study, four winter oat varieties were grown in two locations in replicated nitrogen response trials. The oats were analysed with a rapid 15 minute C18 UHPLC gradient in conjunction with full MS and data dependent analysis (DDA) MSn. The data were chromatographically aligned and deconvolved within XCMS online and peaks putatively identified based upon accurate mass derived molecular formula matching to multiple databases. Data visualisation/reduction approaches were applied with PCA and a novel method, t-distributed stochastic neighbourhood embedding (t-SNE). t-SNE showed a greater capability in detecting sample class clusters compared to variance capture oriented methods such as PCA, however since tSNE is based on a distance matrix, the significance loadings are not directly available. Therefore, a significance test known as ANOVA-simultaneous component analysis (ASCA) was applied with multiple factors, oat variety, location and nitrogen level, being investigated. The identifications of the most significant ions have been obtained from the accurate mass data, as well as MS2 and MS3 spectra obtained from DDA-MSn. Considerations of the agronomic significance of the metabolite profiling results a long with correlations to the oats physical traits is currently ongoing.

7. Abdulmalik Alqarni and David Watson,
Strathclyde school of pharmacy and biomedical science, University of Strathclyde

Metabolomic Profiling of the Effects of Melittin in combination with LPS on U937 Cancer Cells Using Mass Spectrometry

Bee venom is a major defence tool of the honey bee. It consists mainly of melittin, phospholipase, hyaluronidase and amino acids and is used widely in research. Bee venom and its constituents have been found to have therapeutic activities and applications for certain diseases. They are reported to have anti-inflammatory and anti-cancer effects. Lipopolysaccharide (LPS), a component isolated from gram-negative bacteria induces the pro-inflammatory cytokines in macrophage cultures. The combination of LPS and melittin was found to amplify this effect. In the present study, metabolomic profiling of U937 cells after their transformation to macrophages was carried out in order to observe their response to melittin, LPS, and a combination of both. The potential of melittin as an immune adjuvant therapy was assessed in term of metabolomics. The levels of 80 metabolites were significantly different ($p < 0.05$) between LPS treated and LPS+ melittin treated cells. Cytotoxicity studies were carried out to obtain IC50 values for melittin (5.94 µg/ml) and LPS (116.8 µg/ml), respectively. Effective chromatographic separation of metabolites was obtained with liquid chromatography-mass spectrometry (LC-MS) using ZIC-pHILIC column. Overall, this study suggested that melittin might have some potential in immunotherapy.

8. Mohammad Alrofaidi, David Watson, Nigel Pyne and Susan Pyne
Strathclyde school of pharmacy and biomedical science, University of Strathclyde

Comparison of metabolite coverage in different cell lines and use of relative retention times

Methods have been developed for ZIC-HILIC and ZIC-pHILIC columns in combination with mass spectrometery in order detect greatest number of metabolites which are important in untargeted profiling study and detection of polar compounds. Standard metabolite mixtures are often run along-side sample batches to establish retention times. However, some metabolites are unstable in solution requiring frequent re-preparation. To compensate for retention time shifting on zwitterionic columns from batch to batch or from time to time, 13C-glycine was used as a time reference standard. In the current study 250 metabolite standards were examined on ZIC-HILIC and ZIC-pHILIC columns using relative retention time in comparison with 13C-glycine. In addition, metabolites in extracts from five different cell lines were investigated in order to identify which metabolites could be detected in each cell line. In conclusion, relative retention times and abundance for the metabolites from standard mixtures and cell extract of five different cell lines, which were run on ZIC-HILIC and ZIC-pHILIC columns were established in order to make methods less dependent of re-running of standard metabolite mixtures with each batch of samples.

9. Mansour Alzahrani and David Watson,
Strathclyde school of pharmacy and biomedical science, University of Strathclyde

Development of derivatisation method for investigation testosterone and dehydroepiandrosterone using mass spectrometry

A sensitive liquid chromatography-electrospray ionization-tandem mass spectrometric (LC–ESI-MS/MS) method for the simultaneous quantification of testosterone (T) and its precursor, dehydroepiandrosterone (DHEA), in human saliva was developed and validated. Despite its previously reported presence epi-testosterone was absent from saliva. The saliva was deproteinized with acetonitrile, purified using a Strata-X cartridge, derivatized with 2-hydrazino-1- methylpyridine, and subjected to LC-MS/MS. The recovery rates of the steroids during the pre-treatment were about 90%. Quantification was based on selected reaction monitoring using characteristic transitions, and deuterated T was used as an internal standard. This method certified the reproducible (inter- and intra-assay precision, and accuracy), quantification of the salivary androgens using a 0.5ml sample and the limits of quantification for both androgens were at 15pg/ml. As an initial step in the practical application of the developed method in the sports field, the salivary androgens, pre- and post- training, will be examined in football players.

10. Holly Brunton, Ricky Cunningham, Rosanna Upstill-Goddard, Giuseppina Caligiuri, Eirini Maria-Lampraki, Stephan B Dreyer, Viola Paulus-Hock, Ulla-Maja Bailey, Derek Wright, Pablo Baquero, Craig Nourse, David Chang, Peter Bailey & Andrew Biankin.
University of Glasgow

Metabolic stratification of Pancreatic Ductal Adenocarcinoma identifies two major therapeutically targetable subtypes

The characterization of Pancreatic Ductal Adenocarcinoma (PDAC) by integrated genomics analysis revealed four major subtypes, namely Pancreatic progenitor, Immunogenic, aberrantly differentiated endocrine exocrine (ADEX) and Squamous (Bailey et al., 2016). Understanding what drives a particular subtype and what genes are essential for subtype progression and maintenance is essential to identify key subtype-specific therapeutic targets. To address these questions, we performed transcriptional analysis on 52 PDAC patient derived cell lines (PDCLs) and identified deregulation of multiple metabolic pathways including fatty acid degradation, arginine and proline metabolism, AMPK signalling and genes involved in maturity onset diabetes of the young (MODY). Metabolic characterization subtyped the squamous group as being highly catabolic and mainly utilizing glycolysis, whereas the progenitor subtype behaved anabolically and utilized fatty acid oxidation as its primary energy source. At the heart of these metabolic phenotypes we identified Hepatic Nuclear Factor 1 Alpha (HNF1A) and Hepatic Nuclear Factor 4 Alpha (HNF4A) as master regulators of metabolic switching between the progenitor and poorly prognostic squamous subtype. Finally, we carried out an siRNA screen to identify subtype specific therapeutic targets directed towards glycolysis or lipid synthesis.

11. Nina.Denver[1], S.Khan[1], R.Andrew[1], NZM.Homer[1], MR.MacLean[2].
[1]University of Edinburgh, Queen's Medical Research Facility, Mass Spectrometry Core
[2]Institute of Cardiovascular and Medical Sciences, College of Medical, Veterinary and Life Sciences, University of Glasgow

Development of an LC-MS/MS approach for analysis of estrogen and its metabolites in human plasma for investigation of pulmonary arterial hypertension

Pulmonary arterial hypertension (PAH) is a life-limiting disease characterised by an increase in pulmonary artery pressure of over 25mmHg at rest leading to eventual right ventricular failure. There is a much higher incidence of PAH in females suggesting estrogens and their bioactive metabolites (hydroxyestrogens and methoxyestrogens) may play a role in both development of and protection against PAH. Comparison of estrogen metabolite levels in PAH patients and healthy subjects may elucidate the role of estrogens in PAH. Our aim was to quantify estrogens simultaneously by liquid chromatography tandem mass spectrometry (LC-MS/MS) Low circulating levels, structural similarity and poor propensity to ionise presented analytical challenges. Derivatisation to permanently charged molecules by formation of methylpiperazine (MPPZ) derivatives enhanced ionisation of all estrogen metabolites and thus sensitivity. The reaction progressed using sodium bicarbonate as a base catalyst for most analytes: formation of hydroxyestrogen-MPPZ derivatives was more efficient using an *N*-diethylanaline base catalyst. Chromatographic separation of derivatives was achieved using a C18- -PFP (150 x 2.1 mm; 2 um) column. Typical limits of detection of derivatives were in the range 0.043 – 4.34 pg on column. Extraction efficiencies of 93 – 111% were obtained for most metabolites from plasma using Oasis MCX® cartridges with elution and washes being optimised to reduce matrix induced ion suppression. Initial screening of relevant plasma samples showed 16hydroxy E2 and 2methoxy E1 to be the most abundant metabolites in PAH patients. Therefore, we have demonstrated that MPPZ-derivatisation with LC-MS/MS analysis allows detection of estrogen in this disease cohort and healthy controls.

12. Tom Shepherd, Gary Dobson, Diane McRae, Julie Sungurtus, Simon Pont, Raphaelle Palau, Colin Alexander, Susan Verrall, Derek Stewart & Louise Shepherd.
The James Hutton Institute

A Comparison of the Metabolite Compositions of Cereals Grown under Sustainable and Conventional Cultivation Practices using GC-MS

The Centre for Sustainable Cropping (CSC) is a sustainable cropping system at Balruddery Farm in Angus to quantify the environmental and economic costs and benefits of sustainable crop production. Over the course of a six-year rotation the aim was to optimise inputs, yield, biodiversity and ecosystem processes. Six different crops (field beans, potato, spring and winter barley, winter wheat and winter oilseed rape), commonly grown in Scotland, were used. For each crop, five varieties were grown under conventional and sustainable cultivation practices. The impact of cultivation practice on crop quality and quantity was assessed, and this report is concerned with the impact on the metabolite compositions of the three cereals. Freeze-dried powdered samples were extracted using a water-methanol-chloroform biphasic system to give separate polar and non-polar extracts that were suitably derivatized for analysis by gas chromatography-mass spectrometry (GC-MS). Metabolite abundances were calculated relative to an internal standard. 147 polar metabolites were detected, 65 with known identities consisting primarily of amino acids, carbohydrates and organic acids, and 82 unknowns most of which were carbohydrates. 81 non-polar metabolites were detected, 65 of known identity consisting mainly of saturated, unsaturated, hydroxy and poly-oxygenated fatty acids, fatty alcohols and 5-alkylresorcinols. Broad-scale variation was assessed using principal component analysis (PCA) and individual metabolites were tested for significant effects of year, variety and cultivation practice. Analysis of the first five years data suggests that inter-varietal variation in composition and year to year variation may be more significant than the effects of cultivation practice.

13. Alexandre Foito, S. Freitag, J. Sungurtas, C. Santos, I. Costa, C. Jardimb, , G. Garcia, R. Ramos, R. Menezes, D. Mendez-Sevillano, M. Ottens, G. Dobson, G. McDougall, C. Hackett, J. Graham, D. Stewart
The James Hutton Institute

BacHberry: Bio-prospecting strategies identify a novel bioactive phenolic compound from a Rubus germplasm collection

Fruits from the genus Rubus, such as raspberries and blackberries, are not only important horticultural crops but their consumption has been associated with health benefits. Indeed, these fruits have substantial levels of (poly)phenolic compounds which have been linked with bioactivities for a range of human diseases, such as cancers, cardiovascular and neurodegenerative diseases. Traditionally, the discovery of bioactive natural products from biological material involved the collection and screening of plant material using biological assays. From this step, there are two main approaches used for the discovery of bioactive compounds; bioassay-guided fractionation and pure compound screening. The bioassay-guided fractionation approach reduces the complexity of the extracts down to its single bioactive components by a repeated series of fractionation and bioassay testing until the successful isolation of an active compound. The alternative approach aims to isolate and elucidate the structure of the majority of the secondary metabolites present in the crude extract and subsequently test pure compounds (i.e. standards) for bioactivity. In this study, the two approaches were combined resulting in the identification of 5 compounds with bioactivity for Huntington's disease.

14. Marco Fernandes[1] and Holger Husi[1,2]
[1]BHF-Centre of Cardiovascular and Medical Sciences (ICAMS), University of Glasgow, Glasgow [2]Lipidomics Research Facility, Department of Diabetes and Cardiovascular Science, University of the Highlands and Islands, Inverness

Multi-omics Data Integration of Primary Glomerulonephritis

Primary glomerulonephritis is the main cause of chronic kidney disease (CKD) and end-stage renal disease (ESRD) worldwide. Here we integrated and analysed multi-omics datasets from transcriptomics, proteomics and metabolomics studies derived from independent investigations adressing primary glomerulonephritis, namely Focal segmental glomerulosclerosis (FSGS), IgA nephropathy (IgAN) and membranous glomerulonephritis (MGN), in order to better understand the involved pathways associated with pathogenesis. Using a combination of statistical and clustering and molecular modelling tools we were able to confirm the involvement of several biological processes and pathways already known to be associated with primary glomerulonephritis and specifically with FSGS, such as transforming growth factor-β/Smad, integrin-linked kinase, canonical Wnt/β-catenin and PI3K/Akt signalling pathway. Overall the functions of the majority of gene products significantly differentially expressed were associated with signalling pathways, protein trafficking and with the regulation of glomerular capillary permeability. Based on miRNA-mRNA pathway relationships we found a significant involvement of the glycosaminoglycan degradation and ECM-receptor interaction pathways. Moreover, integrative analysis of gene and metabolite data using a combined approach of pathway enrichment with pathway topology confirmed the involvement of the glycosaminoglycan degradation pathway in glomerulonephritis and also revealed a significant association with the D-glutamine and D-glutamate metabolism pathway. Additionally, over-representation of transcription factors associated with developmental processes in FSGS poses the potential relevance of reactivation of developmental pathways. This study contributes to a better understanding of the intricate molecular features and pathways in primary glomerulonephritis pathogenesis and thus paves the way for the discovery of novel biomarkers and potential drug targets.

15. Shazia Khan[1], Diego F Cobice[1], Dawn EW Livingstone[1], Andrew McBride[1], C. Logan Mackay[2], Brian R Walker[1] and Ruth Andrew[1]
[1]Centre for Cardiovascular Science, Queen's Medical Research Institute, University of Edinburgh, [2]SIRCAMS, School of Chemistry, University of Edinburgh

Time-dependent Cortisol Turnover in Tissues using Stable Isotope Tracers

Glucocorticoid hormones critically regulate diverse biological process in health and disease. 11β-Hydroxysteroid dehydrogenase 1 (11βHSD1) catalyses generation of active glucocorticoid hormones in many tissues and is a potential drug target. Here a tracer contained four deuteriums, 9,11,12,12-d4-cortisol (d4F) was infused for 7 days into male mice (n=3/group) to observe tracer kinetics in plasma and metabolic tissues. Tracer (d4F) turnover to d3-cortisone (d3E) by loss of the 11-deuterium and regeneration of d3F by 11βHSD1-mediated reduction was assessed in plasma by LC-MS/MS and in tissue homogenates and sections using matrix assisted laser desorption ionization coupled to Fourier transform cyclotron resonance mass spectrometry. After 6h infusion, d4F, d3E and d3F were detected with low signal intensity (S/N<8) in liver and brain but signal intensity increased (S/N>100) until 24h and reached steady state, while in adipose the process continued to equilibrate until 48h. An 11βHSD1 inhibitor (UE2316) was administered (25 mg/kg oral gavage) and mice culled immediately or 1, 2 and 4 hours post-dosing. UE2316 impaired d3F generation in whole body (53.1%), liver (54.2%) and to a lesser extent (~33.3%) in brain. Differential inhibition in brain regions was observed; active glucocorticoids were suppressed to a greater extent in hippocampus and cortex than in amygdala. These data confirm that the contribution of 11βHSD1 to the tissue glucocorticoid pool, and show that the consequences of enzyme inhibition on active glucocorticoid concentrations are substantial Tissue sampling by mass spectrometry imaging is valuable in pharmacokinetic and pharmacodynamic studies during drug development.

16. Grace McGregor, Sergey Tumanov, Jurre Kamphorst, Owen Sansom
Beatson institute for Cancer Research, Garscube Estate, Glasgow

Measuring cholesterol metabolism in pancreatic cancer

Pancreatic ductal adenocarcinoma has a 5-year survival rate of 2%; research is urgently necessary to develop treatments. In pancreatic cancer, lipid and cholesterol metabolism genes are altered. Key enzymes involved with cholesterol synthesis via the mevalonate pathway and cholesterol uptake are amplified in pancreatic cancer. How these alterations change local and systemic cholesterol metabolism, and contribute to PDAC progression, remains virtually unknown. To address this, we developed methodology to measure cholesterol metabolism. This involves cholesterol derivatization, and gas chromatography – mass spectrometry analysis. Combined with the use of stable isotope tracers, we can follow cholesterol biosynthesis over time. Research at the Beatson Institute revealed pancreatic cancer KrasLSL.G12D/+; p53R172H/+; PdxCretg/+, KPC mouse cells are sensitive to Simvastatin. This drugs targets HMG-CoA reductase, the rate limiting enzyme in the mevalonate pathway. Our lab has shown KPC cells preferentially take up cholesterol, but under low serum conditions, they synthesise it. Simvastatin blocks the synthesis, but cells are able to compensate by taking up cholesterol, even in low serum. Despite this adaptation, cell growth is compromised under statin treatment, even in high serum. This suggests another arm of the mevalonate pathway may be critical for pancreatic cell growth. *In vivo* we have measured cholesterol and fatty acid de novo synthesis using deuterated water. We will use this to investigate lipid metabolism and the action of statins in PDAC mouse tumours. We aim to characterise the potential role of cholesterol metabolism in PDAC, and unveil the therapeutic potential of inhibiting it.

17. Kevin Mendez[1,2], Stacey N. Reinke[2], Hayley Abbiss[2], Ingrid A. Laing[3,4], Laura Coleman[3,4], Siew-Kim Khoo[3,4], Anthony Bosco[3], Peter LeSouef[5], John Moncur[6], Robert D. Trengove[2]

[1]School of Veterinary and Life Sciences, Murdoch University, [2]Separation Science and Metabolomics Laboratory, Murdoch University, [3]Telethon Kids' Institute, Australia, [4]School of Biological Sciences, University of Western Australia, [5]School of Paediatrics and Child Health, University of Western Australia, [6]SpectralWorks Limited, UK.

Trends of TMS-derivatised serum metabolites analysed by GCqTOF MS: observations from a metabolomics-based childhood asthma study

Asthma is a common, chronic inflammatory disease of the airways which varies widely in cause and severity. Asthma attacks are characterised by chest tightness, shortness of breath, coughing and wheezing and the underlying pathophysiology for which remains unknown. The Mechanisms of Acute Viral Respiratory Infections in Children (MAVRIC) study is a cohort study which recruited children upon presentation to the emergency department at Princess Margaret Hospital for Children with acute lower respiratory illness including wheeze and/or asthma. A comprehensive panel of clinical data and bio-specimens, including serum, were collected. Within the present study, 228 serum samples were obtained from the MAVRIC cohort with the aim to determine a metabolite biomarker of asthma. One hundred and seventy-eight samples were from MAVRIC children, while 50 samples were from controls. Serum metabolites were extracted and analysed as their TMS derivatives using the Agilent 7200 quadrupole time-of-flight GC/MS system. Samples were extracted and derivatised in 7 batches and analysed continuously without inlet, column or source maintenance. TOF-MS data were imported into SpectralWorks' AnalyzerPro® for deconvolution and untargeted processing of the metabolite data. Pooled analytical quality control samples were initially processed, with 183 metabolites found in >80% of the samples. All samples were subsequently processed against the generated library. After applying a quality control-robust spline correction (QC-RSC) algorithm for batch correction, 52 features had an RSD within the range 5.4 – 29.7%. Thirty of these features were putatively identified using the National Institute of Standards and Technology (NIST v2.0) mass spectral library.

18. Fiona C. Moreton[1], Naomi Rankin[2], Gavin Blackburn[2], Angela Welch[3], Karl Burgess[2], Keith Muir[1], Christian Delles[4]

[1]Institute of Neuroscience and Psychology, University of Glasgow , [2]Glasgow Polyomics Facility, University of Glasgow, [3]Stroke Research Team, NHS Greater Glasgow and Clyde, [4]Institute of Cardiovascular and Medical Sciences, University of Glasgow

Metabolomic studies in the inherited small vessel disease CADASIL

CADASIL is an inherited small vessel disease causing stroke and dementia. Vascular dysfunction results from accumulation of abnormal extracellular NOTCH3 protein in CADASIL, but the full extent of pathophysiology remains unclear. Altered cerebral metabolism has been identified. Direct examination of brain tissue is difficult. Metabolomics allows the analysis of samples from patients to explore the interactions occurring in biochemical networks, potentially providing insight into disease mechanisms. Adult patients with a genetic diagnosis of CADASIL were compared to age-matched controls without a history of stroke, dementia, vascular or renal disease. Medical and drug history, demographics and cardiovascular risk factors were documented. Metabolomics was performed using HILIC chromatography on a ZIC-pHILIC column connected to an Exactive mass spectrometer. 22 CADASIL patients (median age 53 years, range 26-67) and 22 controls (52years, 29-63) were recruited. Using principal component analysis, we observed clear separation between CADASIL cases and controls. Putatively identifiable differences between the groups were mainly due to changes in histidine, purine and glycerophospholipid metabolism. Concentration of glutamate was lower whereas concentration of adenosine, which may be protective against ischaemia in white matter, was increased in patients with CADASIL. Differences in medication use was not responsible for differences. Some clustering was seen in patients with fewer lacunes than those with more. The metabolome in CADASIL patients is different to that of healthy controls and differences may offer pathophysiological insights, and the opportunity to identify markers of disease severity and progression.

19. Ali Muhsen Ali and David G. Watson
Strathclyde school of pharmacy and biomedical science, University of Strathclyde

An Enhanced LC-MS Approach to Detection Carboxylic Acid and Its Oxidised in Biological Samples by Derivatisation with Choline Coupling

Fatty acids and other metabolites containing a carboxyl group is of high interest in biomedicine because of their major role in metabolic pathways. Tag carboxylic acid compounds with a permanent positive charge such as quaternary ammonium compounds will increase the LC-MS detection sensitivity and selectivity. This study will describe a new and novel strategy for analysing carboxyl-containing compounds in biological samples by soft ionization (ESI) LC-MS after being coupled to choline. Carboxylic acids were coupled to choline using 2-Fluoro-methyl–Pyridinium p- toluene sulfonate (FMP) coupling reagent. The analysis of the derivatised fatty acids was performed by using ACE-HPLC (C18) column coupled with an Orbitrap Exactive mass spectrometer. Thirty-six plasma samples were collected from nine participants before and after ingestion of beetroot juice were studied. Extracted data underwent principal components analysis (PCA) and Orthogonal Partial least squares- discriminant analysis (OPLS-DA). PCA results show clustering of different fatty acids according to their plasma sample and OPLS-DA was applied to identify the fatty acids that are responsible for the variation. Plasma test results showed that choline coupling reactions were successful in detecting fatty acids metabolites in biological samples and could also identify the important oxidised fatty acids responsible for the observed variation. In conclusion, a new and easy method was developed to detect carboxylic acid derivatives in beetroot plasma samples. The method proved to be precise and reproducible and can quantify oxidised fatty acids compounds to 5 nanograms.

20. Naomi Rankin[1,2], Stefan Weidt[2], Erin Manson[2], Naveed Sattar[1], Karl Burgess[2] and Paul Welsh[1]
[1]Institute of Cardiovascular and Medical Sciences, University of Glasgow, [2]Glasgow Polyomics Facility, University of Glasgow

Quantification of carboxymethyl lysine in plasma using isotope dilution mass spectrometry

Introduction: Carboxymethyl lysine (CML) is an advanced glycation end-product (AGE) formed when protein reacts with reducing sugars. Circulating CML is a potential biomarker for cardiovascular disease but is also increased in those with diabetes, renal impairment or a poor diet. Method: Before quantification of CML using mass spectrometry the plasma was reduced in borohydride/borate buffer; denatured in trichloroacetic acid; hydrolysed at 110 C for 24 hours using hydrochloric acid and dried to completion. It was then reconstituted in 5 mM Nonafluoropentanoic acid (NFPA) containing deuterated CML (CML-d_4) and carbon-13 (^{13}C) lysine as internal standards. We used a 96 well plate method for sample preparation resulting in a 5-fold reduction in hands-on sample preparation time compared to published methods. CML, CML-d_4, lysine and ^{13}C-lysine were separated using a Waters BEH C18 column (with an NFPA:acetonitrile gradient) and detected using a Thermo Orbitrap Exactive. This reversed phase ion pair method resulted in a 9-minute injection to injection analysis time. Results: CML was separated from Lysine using this method, as well as from a near-by peak with the same mass:charge (m/z) ratio as CML (210.12), thought to be valyserine. The CML concentrations detected were similar to published reference ranges. The CV of CML detection was 18%, however when this was normalised to the lysine concentration, to account to variation in sample processing, the CV dropped to 11%. Conclusion: The high-throughput sample preparation, good CVs and good linearity allow this method to be used for quantification of CML in large clinical research cohorts.

21. Dr Simon Thain, TL Science LTD

Developing a commercial Metabolomic Fingerprinting test in Atlantic Salmon developmental (Smolt) staging

The farming of Atlantic Salmon is one of Scotland premier industries. A key stage of the husbandry process is knowing when the young fish (Parr) kept in fresh water farm are at a developmental stage ready (Smolted) to be transferred to sea water farm sites. Various morphological traits used to stage fish are not always clear or reliable and tradition biochemical tests based on osmoregulation capabilities in gill tissue have proved to be highly variable. TL Science is investigating various new approaches to determine the global physiological maturity of the fish with the objective of providing more accurate and forward predictive testing for the industry. One of these approaches is to use Metabolomic analysis. Data will be presented on Fingerprinting and Targeted approaches to discover biomarkers that are of diagnostic use to the Salmon Farming industry.

22. Isabel Vincent, Rónán Daly, Bertrand Courtioux, Amy Cattanach, Sylvain Biéler, Joseph M. Ndung'u, Sylvie Bisser, Michael P. Barrett
Glasgow Polyomics Facility, University of Glasgow

Metabolic Markers to Stage Human African Trypanosomiasis

Treatment for human African trypanosomiasis is dependent on the species of trypanosome causing the disease and the stage of the disease. Currently, staging relies upon detecting the very low number of parasites or elevated white blood cell numbers in CSF. Improved staging is desirable, as is the elimination of the need for lumbar puncture. Here we use metabolomics to probe samples of CSF, plasma and urine from 40 Angolan patients infected with *Trypanosoma brucei gambiense*, at different disease stages. Urine samples provided no robust markers indicative of infection or stage of infection due to inherent variability in urine concentrations. Biomarkers in CSF were able to distinguish patients at stage 1 or advanced stage 2 with absolute specificity. Eleven metabolites clearly distinguished the stage in most patients and two of these (neopterin and 5-hydroxytryptophan) showed 100% specificity and sensitivity between our stage 1 and advanced stage 2 samples. Neopterin is an inflammatory biomarker previously shown in CSF of stage 2 but not stage 1 patients. 5-hydroxytryptophan is an important metabolite in the serotonin synthetic pathway, the key pathway in determining somnolence, thus offering a possible link to the eponymous symptoms of "sleeping sickness". Plasma also yielded several biomarkers clearly indicative of the presence (87% sensitivity and 95% specificity) and stage of disease (92% sensitivity and 81% specificity). A logistic regression model including these metabolites showed clear separation of patients being either at stage 1 or advanced stage 2 or indeed diseased (both stages) versus control.

23. Sze Ying, Andrew C Gill and Paula J Brunton
Division of Neurobiology, The Roslin Institute, University of Edinburgh

Quantifying neuroactive steroids following acute forced swim stress using HPLC-MS/MS

The steroidal profile of the rat brain is known to undergo rapid and dramatic changes after acute stress. This study aimed to develop a method of quantifying neurosteroids using LC-MS/MS, which shows higher specificity as compared to traditional quantification methods such as radioimmunoassay. The detection of neurosteroids by LC-MS/MS can be challenging due to their low physiological concentrations, poor ionisation efficiency and susceptibility to ion suppression. Optimisation of the method, which included the comparison of two sample clean-up procedures and the screening of five chemical derivatisation agents, was carried out in order to improve assay sensitivity and robustness. Sample clean-up with a C18 solid phase extraction cartridge, followed by chemical derivatisation with Girard's T reagent was selected as the method of choice for the detection of neurosteroids. With this method, nine neurosteroids including allopregnanolone, pregnenolone, progesterone, testosterone, dihydroprogesterone (DHP), corticosterone, deoxycorticosterone (DOC), dihydrodeoxycorticosterone (DHDOC) and tetrahydrodeoxycorticosterone (THDOC) could reliably be detected in brain tissues. This method was used to determine neurosteroid concentrations in two regions (frontal cortex and hypothalamus) of the male and female rat brain 30 minutes after the onset of swimming stress. Acute swim stress increased levels of glucocorticoids and progestogens, but not testosterone in the frontal cortex and hypothalamus of both male and female rats. Sex differences were also observed in progestogen levels under basal conditions and following stress. This method can eventually be extended to study the role of neurosteroids in other forms of chronic or early life stress, where impaired neurosteroidogenesis has been implied.

24. Kerstin Ziegler, Vincenzo Alessandro Laudicella, Adam Hughes, Stefano Carboni, Mary K. Doherty, Phillip D Whitfield
University of the Highlands and Islands

Development of Lipid Profiling Strategies for Shellfish

The profiling of complex lipids such as phospholipids, glycosphingolipids and triacylglycerols in biological samples by liquid chromatography-tandem mass spectrometry (LC-MS/MS) can be achieved using either high resolution accurate mass (HRAM) or triple quadrupole instruments. Triple quadrupole systems have selective scan modes that readily lend themselves to lipidomic analyses. In particular, precursor ion and neutral loss scans have the capability to inform not only the class of lipids but also provide information on the fatty acid chain length and degree of unsaturation. Further, triple quadrupole instruments, when used in the multiple reaction monitoring mode (MRM), permit accurate quantification of lipid species over a large dynamic range. We have established a two-step approach for lipid profiling in shellfish species by LC-MS/MS using a triple quadrupole system. First, lipids are identified through a combination of class-specific fragmentation and lipid detection software. In a second step, quantitative analysis of lipid species is performed in MRM mode using appropriate internal standards. We are applying this methodology to characterise and quantify lipids in blue mussels (*Mytilus edulus*), a commercially important species for the Scottish aquaculture industry.

Industry Poster Titles and Abstracts

25. Nathan Hawkins and Camilla Liscio, Anatune Ltd

Development of a Fully Automated Platform for Routine, High Throughput Metabolic Phenotyping of Amino Acids

Amino acids are key primary metabolites, structural building blocks of proteins and biosynthetic precursors of pharmacologically active and biochemically/industrially important secondary metabolites. Routine amino acids is important in a range of sectors including: Diagnosis & management of metabolic diseases; Biomedical research and epidemiological studies in health and Nutrition; Nutritional testing of foods; Large scale field/breeding trials for plant quality of nutritional traits (nutritional quality, disease resistance); Food and agriculture science; Metabolic flux analysis (fluxomics) in biochemical studies; Fermentation reaction monitoring (biopharma, industrial biotechnology, synthetic biology; Peptide/protein characterisation and QC. Current approaches to amino acid analysis (HPLC or ion chromatography with pre- or post- column derivatization) have a range of limitations including variable recoveries and poor precision (matrix effects); long analysis times, limited throughput and slow turnaround times. Furthermore, many of the methods have performance limitations in one of more areas (sample stability, resolution, dynamic range, precision and detection limits). Building on initial proof-of-concept work, we have developed a fully automated end-to-end solution for amino acid analysis by GC-FID/GC-MS, using SmartSPE™. The solution provides accurate and precise results and high recoveries of up to 50 amino acids and related compounds. It can be used for a wide range of sample matrices (soils, plant & food extracts, beverages, bio-fluids and fermentation broths). Sample throughput is up to 72 samples per day, delivering increased capacity and faster turnaround times compared to other amino acid analysis platforms.

26. Nathan Hawkins and Camilla Liscio, Anatune Ltd

High Throughput Metabolic Phenotyping Platforms for Routine Fatty Acid Methyl Ester Analysis

Robust and reproducible sample preparation methods are fundamental to the delivery of high quality metabolomics and lipidomics data. Metabolic phenotyping using Fatty acid methyl esters (FAME) is routinely done for studies in: Metabolic Engineering & Synthetic Biology; Aquaculture (increasing nutritional quality in farmed fish; Plant Breeding (increasing oilseed yield); Human & Animal Health & Nutrition (Plasma PLFA); Lipidomics (complementary to LC/MS/MS); Soil microbial community analysis; Edible Oil adulteration studies; Microbiology (sterility testing, Microbial ID of plant & human pathogens, faster turnaround than conventional culture. FAME analysis involves multiple steps including tissue extraction, lipid class fractionation (optional), derivatization, data processing (feature extraction), multivariate statistical analysis and data reporting. Whilst individual steps in the metabolic phenotyping workflow have been automated previously, current protocols involve manual steps in sample preparation, data analysis and reporting. In addition to incurring high labour costs, these manual steps are both significant process bottlenecks and the principal source of experimental error (both bias and precision). We have recently automated all of the steps in routine FAME analysis workflows using the GERSTEL Multi Purpose Sampler for lipid extraction and derivatization, SmartSPE™for lipid class fractionation and Sherlock X (MIDI-Inc) or Masshunter/Mass Profiler Professional (Agilent Technologies) for routine feature extraction, data modelling and reporting. Combining these tools delivers an Enabling Technology for metabolic phenotyping where instrumental analysis rate limiting step in the process. Full automation of the sample preparation and data processing delivers both optimal precision & bias, and limits user input to initial sample processing and data review.

27. Kevin Mendez[1,2], Stacey N. Reinke[2], Hayley Abbiss[2], Ingrid A. Laing[3,4], Laura Coleman[3,4], Siew-Kim Khoo[3,4], Anthony Bosco[3], Peter LeSouef[5], John Moncur[6], Robert D. Trengove[2]

[1]School of Veterinary and Life Sciences, Murdoch University, [2]Separation Science and Metabolomics Laboratory, Murdoch University, [3]Telethon Kids' Institute, Australia, [4]School of Biological Sciences, University of Western Australia, [5]School of Paediatrics and Child Health, University of Western Australia, [6]SpectralWorks Limited, UK.

Trends of TMS-derivatised serum metabolites analysed by GCqTOF MS: observations from a metabolomics-based childhood asthma study

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28. Loren Olson[1] Emile Plise[2] and Baljit Ubhi[1]

[1]SCIEX, Redwood City, CA; [2]Genentech Inc. South San Francisco, CA

Evaluation of High Speed, High Resolution Data Independent Acquisition for Simultaneous Identification and Quantitative Metabolomic Flux Analysis

An evaluation of high resolution acquisition paradigms was performed for the purpose of qualitative characterization and quantitative measurement of metabolic flux in cell culture assays. High resolution "MRM-like" targeted analysis, data dependent acquisition (IDA) as well as a new data independent acquisition (DIA) mode referred to as SWATH® Acquisition was performed on time course cell assay samples. Results indicate that all three modes have utility and unique strengths for fluxomic workflows. Targeted quantitative modes which tend to be more selective and sensitive are easily transferred to nominal mass methodologies. These modes can also be useful for the targeted confirmation of putative metabolites. IDA methodologies have the advantage of being non-targeted but the MS/MS data generation is stochastic in nature. Thus, IDA modes are well suited for identifying a large number of analytes per sample but occasionally re-injection is usually required because these workflows are bias to the ion triggered for fragmentation, thus MSMS for low abundant compounds if often missed. The SWATH Acquisition approach allows MSMS of every single precursor and is not bias to an abundant compound. SWATH Acquisition also now utilizes variable window logic and shows much promise to merge and balance the advantages of the afore mentioned targeted and non targeted approaches.

29. Zuzana Demianova[1]; Cyrus Papan[1]; Joerg Dojahn[1]; Baljit K. Ubhi[2]
[1]SCIEX, Darmstadt, Germany; [2]SCIEX, Redwood City, CA

Benefits of SWATH® Acquisition, a DIA Technique over Traditional Data Dependent Analysis for High Resolution Untargeted Metabolomics Applications

A data independent acquisition (DIA) technique known as SWATH® acquisition enables the identification and quantification of a higher number of metabolites in untargeted metabolomics workflows compared to standard data dependent acquisition (DDA further as IDA) approaches thus enabling a deeper profile of the metabolome. In addition, SWATH® acquisition allows the collection of MS and MSMS data in a single injection and builds a digitized map of every detectable metabolite in your sample. This allows retrospective data mining meaning as your hypothesis changes there is no need to go back and re-run your sample but just to re-mine the data.

30. Timothy J. Garrett[1], Ranjan Perera[2], Matthew Skaley[3] and Baljit K. Ubhi[3]
[1]Department of Pathology, University Florida; [2]Sanford Burnham Prebys Medical Discovery Institute, Orlando, Florida, USA, [3]SCIEX, Redwood City, California, USA

A Simplified, Integrated Solution for Untargeted Metabolomics

Metabolomics focuses on the chemical processes central to cellular metabolism. Mass spectrometry is the tool of choice for the measurement of these metabolites. However, they can be increasingly challenging workflows to setup. Therefore, a robust solution for screening metabolites is of increased interest allowing for a more integrated and routine mass spectrometer system. A new QTOF System was developed for routine, robust workflows which require minimal MS expertise. The system integrates all data acquisition; processing and review as well as reporting into a single piece software. A prostate cancer study was used to determine whether the untargeted metabolomics workflow using the X500R System could find key differences between the samples.

31. Brian Montgomery,[1] Margaryta Makhanov,[2] Seiichiro Watanabe,[2] Arlette Lopez, [2]Maria T. Matyska-Pesek,[2] Joseph J. Pesek[2]
[1]HiChrom, Reading, United Kingdom; [2]Department of Chemistry, San Jose State University, USA

Phenyl-Hydride LC-MS Column Provides Unique Separation and Identification of Closely Related Isobaric Compounds

Potential ingredients used to manufacture illegal drugs were analyzed using high performance liquid chromatography (HPLC) and mass spectrometric detection (MS) to aid law enforcement in analyzing samples collected on the street. In terms of LC-MS, obtaining adequate selectivity and separation was crucial since some of the analytes were isobaric and could not be identified by MS alone. Investigation with various types of HPLC columns revealed that a silica hydride column with a phenyl ligand provided the best overall retention and separation in reversed phase mode. Behavior compared to other phases tested suggested that π–π interactions combined with the non-phenyl part of the ligand contributed an important role in obtaining optimal selectivity for these closely related aromatic compounds. The Cogent Phenyl Hydride column used allowed for greater stability of the phenyl ligand than would be possible with one based on ordinary silica due to the presence of direct silicon–carbon bonds and a hydrophobic support matrix.

32. Ioanna Ntai, Ralf Tautenhahn, Tim Stratton, Anastasia Kalli, Amanda Souza and Andreas Hühmer
Thermo Fisher Scientific

Advantage of High Resolution Accurate Mass Spectrometry for Metabolite Identification in Untargeted Metabolomics Studies

Purpose: Determine the mass spectral resolution required to detect and identify the highest number of compounds from a complex sample, such as human plasma, during a 15min LC/MS run. **Methods:** Human plasma (NIST SRM 1950) was analyzed at different mass spectral resolutions ranging from 15,000 to 240,000 with a Thermo Scientific™ Q Exactive™ HF Hybrid Quadrupole-Orbitrap™ mass spectrometer operated separately in both positive and negative mode. The data were analyzed using Thermo Scientific™ Compound Discoverer™ 2.1 software for metabolite identification. **Results:** Increased resolving power was extremely useful in defining isotopic distribution and determining elemental composition. Compound detection improved with increasing resolution. Our findings suggest that mass spectral resolution higher than 60,000 is essential for obtaining greater metabolome coverage.

33. Amanda Souza, Reiko Kiyonami, David Peake and Ralf Tautenhahn
Thermo Fisher Scientific

Solutions for routinely measuring metabolic knowns and identifying unknowns

Metabolomics measures all small molecules in a biological sample. These endogenous metabolites represent the biochemical phenotype for a given condition or state. The phenotype or profile of an organism is useful in understanding functional biology at the molecular level. Metabolomics experiments are often designed as comparative studies where two or multiple groups are used to determine differences between sample populations, such as control versus disease. These phenotypical differences can shed direct insight into the molecular underpinnings of the biological system. Lipidomics, or the complete profile of lipid species, is a subset of metabolomics. In turn, this information can be combined with other "omics" disciplines like genomics, proteomics and transcriptomics for a complementary readout. Metabolomics is found in many areas of research. The need for understanding human health and disease has led to metabolomics studies involving disorders such as diabetes and cardiovascular disease, and its potential for determining diagnosis by signatures or biomarkers of disease. Metabolomics provides understanding of cancer biology and the potential to determine disease progression. Further, pharmacometabolomics studies can be useful in determining an individual's response to drug therapies and the potential application of precision medicine. Other areas where metabolomics has been applied include studies of the microbiome, the exposome, diet and nutrition, and plant metabolomics. The use of metabolomics is diverse because it provides a snapshot of the biochemical network of small molecules.

34. Nicolas Di Giovanni, Cristian I. Cojocariu, Paul Silcock, Amanda Souza, Marie-Alice Meuwis, Edouard Louis, Jean-François Focant
Thermo Fisher Scientific

Untargeted serum metabolite profiling of colorectal cancer using GC-Orbitrap technology

Globally affecting more than one million new persons each year, and killing more than 700,000, colorectal cancer (CRC) is the second leading cause of cancer-related deaths in women and the third in men. Nevertheless, diagnosis is still largely based on invasive tissue sampling, while gaps remain in the understanding of its pathogenesis, with complex combinations between lifestyle, genetics, epigenetics, chronic inflammation (IBD) and microbiota. Untargeted metabolomics is one way to address these issues. Through metabolite profiling, it provides a picture of the outcome of the disease. To do so, significant variations between pathological and healthy phenotypes have to be found, and the responsible metabolites must be confidently identified. In this study, the ability of the Thermo Scientific™ Q Exactive™ GC-MS Orbitrap™ system to detect and identify metabolites related to colorectal cancer in an untargeted manner was assessed. The workflow uses the advantages of high peak capacity and chromatographic resolution of gas chromatography with the high resolution and sub-ppm mass accuracy of the Thermo Scientific™ Orbitrap™ mass analyzer. The samples analyzed belonged to two populations linked to colorectal adenocarcinoma (active and remission, 12 samples each) along with two controls cohorts of the same size matched for possible biases (gender, age, BMI, smoking status etc.), and pooled QC samples. Analytical raw data files were automatically processed through two software platforms specifically designed for the Orbitrap technology (Thermo Scientific™ TraceFinder™ software and Thermo Scientific™ Compound Discoverer™ software). Compound identification was made using existing commercial libraries as well as an in- house developed high resolution accurate mass (HRAM) Orbitrap metabolomics library.

Venue – City of Glasgow College: Riverside Campus

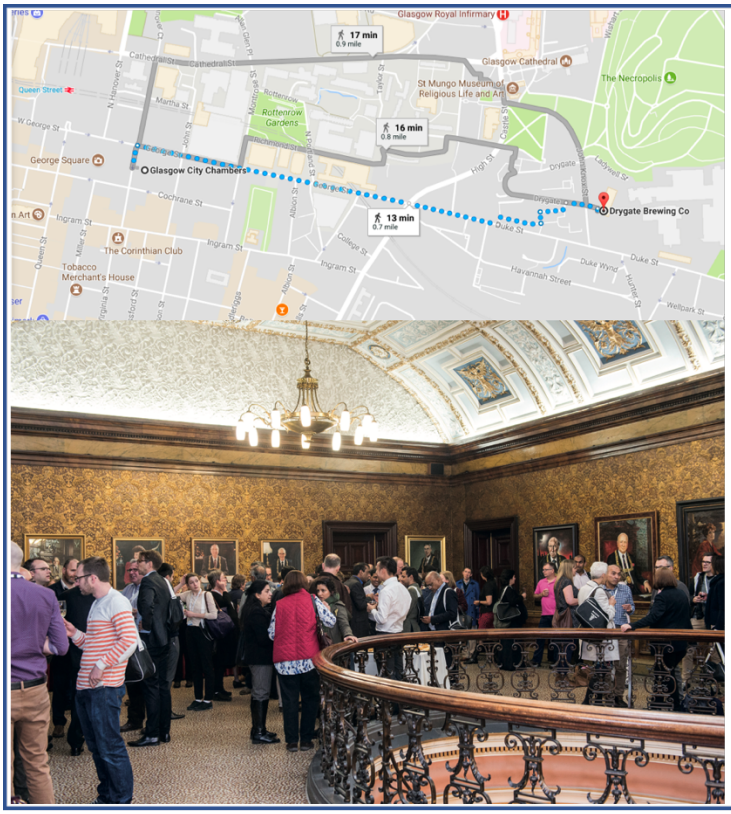
https://www.cityofglasgowcollege.ac.uk/
21 Thistle Street, Glasgow, G5 9XB
The conference will be in the Exam Hall on the seventh floor.

Situated on the banks of the River Clyde, this campus opened its doors in August 2015. The campus has won the 2017 RIAS and RIBA Award for best current Scottish architecture, as well as the Architects' Journal's top prizes; the AJ100 Building of the Year and AJ100 Client of the Year awards.

Lunch and tea and coffee will be provided (included in registration fee) on both days. Free wi-fi is available: details to be provided on the day. Please go to reception to receive a visitor badge in addition to your delegate badge. **Please wear your badge at all times so you can be identified as an invited delegate.**

Parking in available in the mosque on Thursday (£3 per day) but not Friday. Local parking is available within the city centre (Euro Car Parks on Jocelyn Square or Bridge Street Subway car park are a ten-minute walk away) but at a cost. Unless you are staying at a nearby hotel with parking we highly recommend public transport. Please see www.travelinescotland.com to help plan your journey.

Civic Reception



Thank you to the Lord Provost and Glasgow City Centre for offering to host a civic reception for the Scottish Metabolomics Network on Thursday the 2nd of November at 7 pm

Start 7 pm – 7:45 pm
City Chambers, George Square, Glasgow, G2 1DU

https://www.visitscotland.com/info/see-do/glasgow-city-chambers-p246401

The Scottish Metabolomics Network would like to thank the Lord Provost and Glasgow City Council for providing a civic reception for delegates of the Scottish Metabolomics Network Symposium 2017.

Glasgow City Chambers are a 20-minute walk (approximately) from the Premier Inn Glasgow City Centre South. George Square is in Glasgow City Centre and a short walk from Glasgow's many shops, galleries, pubs and restaurants. Please see our Glasgow Information Leaflet at http://scottishmetabolomics.net/events/ for some great information on Glasgow provided by the Glasgow Convention Bureau.

The Glasgow City Chambers overlook George Square. This prestigious building was completed in 1888 and had been the headquarters of Glasgow City Council for over 100 years. Free guided public tours are available on weekdays at 10:30 and 14:30 lasting 45 minutes.

Please wear your delegate badges to this event so you can be identified as an invited delegate

Evening Event Venue: Drygate Brewery

Start 8 pm
https://www.drygate.com/
85 Drygate, Glasgow, G4 0UT

Conference dinner is included in your registration fee

Drygate brewery is on the site of the Drygate craft brewery. The brewery has a good reputation for its food and drink as well as its relaxed and welcoming atmosphere. It is on the edge of the Glasgow Necropolis, a Victorian cemetery, popular with tourists for its views of the city and architecture. It is a 15-minute walk from the Glasgow City Chambers where we will be attending a Civic Reception. It 20-minute walk from the City of Glasgow College Riverside Campus. Please enter via the front entrance up the external staircase onto the terrace and into the beer hall (if required a lift can be found on the ground floor through the restaurant).

The Scottish Metabolomics Network would like to thank Agilent for sponsoring the desserts and Peek Scientific for providing miniature Whisky bottles. Please do not drink these on the premises – take one with you as you leave.

Please wear your delegate badges to this event so you can be identified as an invited delegate

Acknowledgments

There are a number of people that we would like to thank in assisting us with the organisation of the Scottish Metabolomics Network Meeting 2017:

- all our sponsors whose support has been crucial in allowing us to organise the meeting
- our colleagues at the Edinburgh Clinical Research Facility at the University of Edinburgh, especially Jo Merrifield, in assisting us with the organisation
- Eveline Langrell and colleagues at the City of Glasgow College for their help in organising the meeting
- The Lord Provost and Glasgow City Council for providing a civic reception for us
- Staff at Drygate Brewery for helping organise the conference dinner
- Justin Van Der Hooft and Emily Armitage for helping at the early stages before they left the University of Glasgow
- Phil Whitfield and Mary Docherty, at the University of the Highlands and Islands, Ruth Andrew, Natalie Homer and Andy Finch, from the University of Edinburgh, for their helpful advice
- Allison Jackson and Rachael Munro at Glasgow Polyomics for all their help and support
- All our speakers, poster presenters and delegates for joining us

Karl Burgess, Gavin Blackburn, Gillian Mackay and Naomi Rankin
University of Glasgow

Delegate List

A delegate list will be provided in the hard copy provided to delegates on the day.