ANALYTICAL TOOLS FOR CUTTING-EDGE METABOLOMICS

A joint meeting of the Analytical Division of the Royal Society of Chemistry and the International Metabolomics Society held at the Chemistry Centre on 30th April 2014

BOOK OF ABSTRACTS

ORAL PRESENTATIONS FROM INVITED SPEAKERS

Spectroscopy and Systems Medicine: Meeting the Challenges of 21st Century Healthcare

Professor Jeremy Nicholson, Department of Surgery and Cancer, Imperial College London, UK

There is increased demand to translate omics and related analytical technologies into the real world of the clinic as part of the move towards systems medicine and the implementation of personalised healthcare paradigms. Ultimately the success of such approaches depends on being able to measure and understand deep patient complexity using a range of analytical and spectroscopic tools that generate megavariate data that have to be interrogated, modelled and presented to physicians, surgeons and other healthcare professionals in an engaging and informative way to allow clinical decision making. So there are layered analytical challenges relating to technology choice, practicality of implementation and information recovery that have to be placed within a high quality diagnostic framework to ensure patient safety. Metabolic phenotyping has proved to be a powerful approach to understand deep patient biology as these phenotypes reflect gene-enironment interactions that underpin disease and give information on site, severity and mechanisms of disease as well as patient diversity (stratification) and metrics of therapeutic response. The presentation will explore these challenges from an analytical viewpoint and discuss the areas of medicine are practically tractable in the foreseeable future.

Good Vibrations: Shining Light on Metabolism

Professor Roy Goodacre, School of Chemistry, University of Manchester, UK

There is increasing interest in being able to measure metabolic changes in biological samples as a function of space. In order to achieve this we and others have been developing a range of powerful mass spectrometry techniques for the analysis of small molecules that effect spatial analysis from cells and tissues. However, MS is inherently destructive to the sample and this limits serial analyses on cells and tissues. By contrast methods based on vibrational spectroscopy are non-invasive and can be collected readily through transparent media. This talk will highlight our recent research in both Fourier transform infrared (FT-IR) and Raman spectroscopies. These will include: using FT-IR spectroscopy as a high throughput phenotypic screening method for generating metabolic fingerprints prior to in depth GC-MS and LC-MS analyses; enhancing Raman spectroscopy for trace detection of small molecules; and how vibrational spectroscopy can be developed for the analysis of tissues as well as single algal, mammalian and bacterial cells.

Characterization of Biomarkers in Natural Products Metabolomics

Professor Jean-Luc Wolfender, School of Pharmaceutical Sciences, University of Geneva, Switzerland

Among the different techniques enlisted for metabolome analysis, both mass spectrometry (MS) and nuclear magnetic resonance (NMR) are widely used, each having inherent advantages and disadvantages [1]. In MS and/or NMR based metabolomics, the correct identification of key biomarkers remains a challenging task because of the high chemical diversity of secondary metabolites encountered in complex natural extracts either from microbial or plant origin. In this respect we have elaborated generic metabolomic strategies based on the following steps: i) rapid UHPLC-TOF-MS fingerprinting for a sensitive detection of biomarkers with appropriate data mining methods; ii) high resolution profiling of representative pooled samples based on chromatographic gradient transfer for a precise biomarker localisation and deconvolution iii) further identification based on dereplication by MS and MS/MS with retention time prediction and cross search with chemotaxonomic information iv) de novo identification by targeted microisolation and subsequent micro NMR analyses; v) assessment of the bioactivity of the isolated biomarkers based on different HPLC at-line biological profiling methods. Some examples of this strategy will be illustrated for applications in relation with plant and fungal stress responses. In particular the challenges faced in the detection and further MS and NMR characterisation of microbial biomarkers and their microisolation will be highlighted in the context of microorganism interactions.

[1] Wolfender JL, Rudaz S, Choi Y, Kim HK 2013. Plant metabolomics: from holistic data to relevant biomarkers. Curr. Med. Chem. 20:1056-1090.

Metabolic Profiling via LC/MS & Tools for Metabolic Annotation and Identification

Dr Steffen Neumann, Leibniz Institute of Plant Biochemistry, Germany

Metabolite profiling via LC/MS can reveal "interesting" features, and subsequent tandem MS experiments provide powerful structural hints for the elucidation of these unknown mass spectral features. We encourage the community to submit spectra for metabolites to the MassBank database, but because reference spectra are often expensive to obtain (both in consumables and chemicals, but also in manpower), reference libraries will never be covering as many compounds as can be found in e.g. ChemSpider. In-silico methods such as MetFrag (http://msbi.ipb-halle.de/MetFrag/) help to identify compounds with tandem MS among candidate structures obtained from general purpose compound libraries, while the new MetFusion approach (http://msbi.ipb-halle.de/MetFusion/) can integrate several metabolite identification strategies to provide the best of both worlds.
In 2012 we invited the experimental and computational mass spectrometry community to the first CASMI, the "Critical Assessment of Small Molecule Identification", an open contest on the identification of small molecules from mass spectrometry data.
I will present details about the contest, participants, evaluation procedure, the different strategies applied and of course talk about the real winner - the field of small molecule identification.

Combining Non-Invasive Measurements: Marker Discovery & Refinement

Professor Paul Thomas, Centre for Analytical Science, Chemistry Department, Loughborough University, UK

The phrase “cutting-edge” invokes images of scalpels, wickedly sharp, slicing to the “heart-of-the-matter” so to speak. Exploratory surgery, a phrase to chill the marrow, is the acme of invasive dissection. Fortunately internal exploration now takes place most often through endoscopic images/biopsies or x-rays. If one is lucky, with access and connections, then MRI might be available. For most humans though it still remains all about penetration by radiation, blade or instrument. Even blood, the biofluid of choice, one that is rich in all possible components, still requires a penetrative wound and extensive sample work up. With the right equipment this is straightforward, provided continuous monitoring, or many samples are not needed. (In the coming era of difficult-to-treat infections taking blood may not always be the low risk option as it is currently viewed to be.) Non-invasive samples that may be repeatedly taken with no impact on the patient or participant have much to recommend them. They may be taken away from the clinic, and used with small children and vulnerable patients who provide samples as easily as anyone else. This presentation will review and demonstrate how the rich and complicated volatile organic compound profiles that are obtained from skin, saliva and breath may be obtained and integrated into metabolomic workflows.

Breath the most intimate of biofluids is invoked as a non-invasive approach par excellence and yet the transport of VOC from the distil lung to the sampler is not necessarily straightforward and the mixture that is exhaled is by no means truly representative of the original source for reactions and wall loses reduce concentrations to a significant extent. Skin in contrast is a permeation membrane that provides higher transport efficiencies although it is difficult to sample without incorporating a large exobiome component. Saliva is fresh-from-the-tap so to speak providing you capture VOC in situ. All three sample matrices can be used to provide a combined VOC profile that is rich in data and appears to offer opportunities to develop high-throughput non-invasive metabolomic methods.

To combine non-invasive measurements the data structure from each sample type needs to be the same and the registration of the data needs to be consistent across all measurement types. This can be achieved from different sampling systems provided the analytical finish is the same. Adsorbent/absorbent approaches that use polydimethylsiloxane as the sample media are compatible with the established methods developed for VOC analysis that use macroreticular adsorbents. Recovery of the sampled VOC with thermal desorption approaches enables saliva, skin, and breath, as well as urine and faeces, to be folded into metabolomic studies. With enrichments in the 10^4 to 10^5 range the resultant fidelity provides opportunities for discovery almost with every measurement. For the research community has yet to approach providing a complete description of the volatalome.

These concepts may be demonstrated by considering the use of GC-MS to prospect for non-invasive stress markers in breath and skin. A paced auditory serial addition task was used to induce psychological stress in health young adults while VOC breath and skin samples were taken. Multi-variate analysis (DFA-PLS and PCA) of the resultant data indicated complementary stress marker panels from both sample types.

An alternative approach is to interface thermal desorption to an electrospray source to produce a secondary electrospray ionisation-mass spectrometric technique. Analysis of skin VOC, with volatile fatty acids as the targets, enables participants with a single nucleotide polymorphism (SNP), 538G→A, that caused a G180R substitution in the ABCC11 gene to be identified; due to the reduced concentrations of apocrine derived axillary odour precursors.

In all such approaches it is helpful to reflect on how to evaluate the quality and validity of the resultant data, and the presentation will conclude by commenting on how cumulative distribution functions enable chemical space and VOC profiles to be objectively compared in intra- and inter-centre evaluations with the suggestion that such information should be usefully shared more widely.

From big data to knowledge – applying metabolomics to understanding type 2 diabetes"

Dr Julian Griffin[1,2], Albert Koulman[1], Luke Marney[1], Steven Murfitt[2], James West[1,2], Lee Roberts[1], Keith Summerhill[1], Laura Wang[1], Jagpreet Singh[1], Nita Forouhi[3] & Nick Wareham[3]

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[2] The Department of Biochemistry and the Cambridge Systems Biology Centre, University of Cambridge, Cambridge.
[3] MRC Epidemiology, University of Cambridge, Cambridge.

There is a global epidemic in terms of the rise in the number of sufferers with type 2 diabetes (T2DM) with the World Health Organisation estimating currently 347 million people worldwide with diabetes, and the disease representing the seventh largest cause of death globally. While a number of genes have been identified that play a role in the development of T2DM, most are associated with rare monozygotic forms of the disease, and do not model the complex interactions between genotype and the environment, and in particular diet and exercise, that is responsible for the development of T2DM in many human cases. We will present methodologies that we are applying using gas chromatography mass spectrometry (GC-MS), liquid chromatography (LC-) MS and direct infusion (DI-) MS to profile samples on an epidemiological scale to explore the link between diet, environment and genotype. These studies include the largest analysis of fatty acids by GC-MS to examine the interaction between diet and disease for type 2 diabetes risk in the EPIC cohort, DI-MS to monitor intact lipid profiles in the Fenland cohort and aqueous metabolites using a commercial kit. We will also discuss the progress in reporting large scale datasets in the MetaboLights repository to allow others access to these data in order to achieve the conversion of big data into knowledge.

ORAL PRESENTATIONS FROM EARLY CAREER SCIENTISTS

A new approach for high throughput (xeno)metabolomics based on solid phase extraction and nanoflow liquid chromatography-nanoelectrospray ionisation mass spectrometry

Dr Arthur David, School of Life Sciences, University of Sussex, UK

Current metabolite profiling methods based on liquid chromatography-mass spectrometry (LC-MS) do not detect many low abundant components in biological matrices due to poor ionisation efficiency or co-elution with highly abundant compounds. Nanoflow LC-nanospray MS platforms could overcome these limitations and significantly increase analytical sensitivity and coverage of the (xeno)metabolome (i.e., metabolites and xenobiotics) but require small (<0.5 µL) injection volumes. We developed sample preparation methods based on phospholipid removal and solid phase extraction (SPE) to remove ion suppressive components and concentrate extracts for sub-microliter injections onto nanoflow ultra-performance LC-nanoelectrospray ionisation-time-of-flight MS (nUPLC-nESI-TOFMS). This approach was used to investigate the chemical mixtures accumulating in fish exposed to effluent from a wastewater treatment works, and the associated changes in the plasma/tissue metabolomes. A nontargeted metabolomics approach was performed to

compare extracts of plasma and tissues (gonads, kidney, liver and gill) from effluent-exposed and control fish. Endocrine disruptors and mixtures of many pharmaceuticals (nonsteroidal anti-inflammatory drugs, selective serotonin re-uptake inhibitors, benzodiazepines, antipsychotics, anticonvulsants, beta blockers, fibrates, and anticoagulants) were identified in plasma/tissues of effluent-exposed fish. Effluent exposure resulted in a widespread reduction of prostaglandins in many tissues as well as reduction in androgen and increases in serotonin metabolites in the testes and plasma, indicating potential effects on immunological, reproductive and neurological endpoints. This study is the first report identifying the effects of exposure to chemical mixtures on low abundant signaling molecules using a nontargeted MS profiling method. This work demonstrated the significantly enhanced sensitivity of the SPE/nUPLC-nESI-TOFMS methodologies for untargeted MS profiling.

Habitual dietary intake impacts on the lipidomic profile

Dr Aoife O'Gorman, Institute of Food & Health, University College Dublin (UCD), Ireland

Reliable dietary assessments are essential when attempting to understand the complex links between diet and health. Traditional methods for collecting dietary exposure are highly subjective, therefore there is an increasing interest in identifying biomarkers to provide a more accurate measurement. Metabolomics is a technology that offers great potential in this area. This study aims to use a multivariate statistical strategy to link lipidomic patterns with dietary data to identify biomarkers. The relationship between lipidomic profiles and dietary data in volunteers (n=34) from the Metabolic Challenge Study (MECHE) were assessed. Principal component analysis (PCA), linear regression and receiver operating characteristic (ROC) analysis were used to (1) reduce the lipidomic data into lipid patterns (LPs), (2) investigate relationships between these patterns and dietary data and (3) identify biomarkers of dietary intake. Our study identified 6 novel LPs. LP1 was highly predictive of dietary fat intake (AUC=0.82). A random forest (RF) classification model used to discriminate between low and high consumers resulted in an error rate of >10%. LP4 was highly predictive of alcohol intake (AUC=0.81) with LPCeC18:0 identified as a potential biomarker of alcohol consumption. LP6 had a reasonably good ability to predict dietary fish intake (AUC=0.76), with LPEaC18:2 and PEaaC38:4 identified as potential biomarkers. The identification of these LPs and specific biomarkers will help to classify a person's dietary intake and in turn will improve the assessment of the relationship between diet and disease. Linking these LPs and specific biomarkers with health parameters will be an important future step.

Metabolomics of exhaled air in non-respiratory diseases

Dr Agnieszka Smolinska, School for Nutrition, Toxicology and Metabolism, Maastricht University, The Netherlands

Over 32 million European people have chronic liver disease or Crohn's disease (CD). Early diagnosis of liver cirrhosis or disease activity may prevent development of severe complications. However, in current practice only invasive methods, such as biopsy and ileocolonoscopy, are used. Moreover, they are associated with significant complications including morbidity. Therefore, a non-invasive alternative is needed. The analysis of Volatile Organic Compounds (VOCs) in breath could yield such non-invasive diagnostic. Metabolites detected in breath originate from normal and deviant (e.g. inflammatory) metabolic processes occurring in the body.

We demonstrate the use of VOCs as diagnostic and monitoring tool in two real life cohorts. In the CD cohort, we investigated whether VOCs can accurately differentiate between active and remission phase. Using Random Forest analysis, a set of 10 discriminatory VOCs correctly predicted active CD in 81.5% and remission in 86.4% (sensitivity 81%, specificity 80%) in the independent test set. In the second study we aimed to identify a specific exhaled VOCs profile to discriminate chronic liver disease (CLD) patients with cirrhosis (CIR) from those without. For that purpose we used Partial Least Square Discriminant Analysis to identify discriminatory VOCs and to predict the presence of cirrhosis. A set of 22 VOCs was found to discriminate CIR and CLD patients with an overall correct prediction of 83.3% in independent test set (sensitivity 88%, specificity 80%). Although further validation is required, these feasibility studies showed that exhaled VOCs profile is capable to monitor CD and predict the presence of cirrhosis among CLD patients.

Robust annotation of metabolite identity – maximizing the structural information from metabolites using mass spectrometry fragmentation approaches

Justin van der Hooft, Glasgow Polyomics Facilities, University of Glasgow, UK

Reliable metabolite annotation and identification is currently the biggest challenge in metabolomics analysis. Without this step, the outcome of a typical untargeted mass spectrometry (MS) experiment remains a list of significantly changed mass values and corresponding retention times with potential elemental formulas. To obtain a more robust annotation of or gain additional structural information on biomarkers, MS fragmentation approaches can be used during or following a liquid chromatography (LC)-MS run.

Fragmenting ionized metabolites eluting from the LC column can provide structural information by detection of specific fragment masses or neutral losses. The use of different collision cells and energies result in different fragmentation patterns. For example, collision induced dissociation (CID) has been used for many years to fragment metabolites and their fragments (i.e., MS2 and MSn). Alternatively, higher-energy type of collisions can be performed in many TOF instruments as well as modern Orbitraps. The aim of this study is to compare higher-energy collision dissociation (HCD) fragmentation at different energies and ion trees for its use in small metabolite annotation and identification.

We compared the ion trees and HCD fragmentation spectra of relevant metabolites present in many samples routinely measured in the Glasgow Polyomics facility, both using direct infusion (nanospray) and LC coupled to MS. HCD fragmentation resulted in additional fragment ions for many metabolites, resulting in a unique fingerprint for many isomers. This enabled robust annotation in direct infusion MS experiments of biological samples. Incorporation of more HCD spectra in mass spectrum repositories like Massbank and MzCloud is therefore encouraged.

POSTER PRESENTATIONS

POSTER ONE

Metabolomics of exhaled air reveals profiles of volatile organic compounds for asthma and sarcoidosis

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In addition to the traditional bodily fluids such as urine and blood, the analysis of exhaled air is an emerging field in metabolomics research. Exhaled air contains Volatile Organic Compounds (VOCs) that are produced during normal metabolism, but also by damaging processes such as inflammation and lipid peroxidation. VOCs are then excreted in the breath and differences in their occurrence and concentration may serve as a window of healthy and diseased organs.

The discriminative capacity of exhaled air has been demonstrated in various inflammatory diseases. Our aim is to demonstrate the value of exhaled air as diagnostic tool for two lung diseases: sarcoidosis and preschool asthma. Both disease have diverse etiology, but are both characterized by chronic inflammation. Sarcoidosis is characterized by inflammatory lesions predominantly in the lung, whereas asthma is a common chronic inflammatory disease characterized by reversible obstruction of the airways. Current diagnostics for both diseases are generally invasive and often inconclusive.

We demonstrate that 20 VOCs discriminate between sarcoidosis and healthy controls with correct classification rate for independent test set of 100%. In the study of asthma we selected 17 VOCs to discriminate between preschool asthmatic children and transient wheezing children with correct prediction rate for independent test set of 80%.

Our results suggest that the analysis of VOCs in exhaled air has potential as non-invasive diagnostics tool. This method will serve as a basis to improve early diagnosis of diseases such as sarcoidosis and preschool asthma, and subsequently improving quality of life for patients as well.

POSTER TWO

HPLC-MS method development for Fabry disease biomarkers analysis

Fahad Alharbi, University of Birmingham, UK

Introduction: Fabry disease is an X-linked lysosomal storage disease caused by the deficiency of α-galactosidase A, resulting in the accumulation of glycosphingolipids in body fluids and different organs. Globotriaosylceramide (Gb3), globotriaosylsphingosine (Lyso-Gb3) and their analogues have been identified and quantified as biomarkers for the disease severity and treatment efficacy. The current study aimed to develop HPLC-MS methods in order to identify and quantify FD biomarkers.

Methods: Human Fabry patients' plasma and urine samples were processed using solid phase extraction. The samples were then analysed for levels of Lyso-Gb3 and its analogues using HPLC-ESI-micrOTOF mass spectrometry.

Results: Urine extraction showed a recovery of 90%, while plasma samples gave a recovery of 70%. Reverse phase-HPLC methods were optimised with an isocratic elution of (0.1% formic acid/40% acetonitrile) and flow rate of 3µL/min. A multiple reaction monitoring mode MS method was optimised for identification and quantification of metabolites showing limit of detection of 40fmoles Lyso-Gb3. Lyso-Gb3 and 4 analogues were detected in plasma from an untreated male Fabry patient showing comparable

fragments. These analogues were detected at mass to charge ratio (m/z) of 758, 784, 802 and 820 and varied from Lyso-Gb3 (786) due to a modification in the sphingosine moiety giving a shift m/z of (-28, -2, +16 and +34) respectively. The predicted formula of these modifications are (-C2H4, -H2, +O and +H2O2) respectively.

Conclusion: We have established an HPLC-ESI-MS approach for analysis of Lyso-Gb3 and its analogues. Pilot data shows low levels of these biomarkers are quantified in urine and plasma.

POSTER THREE

Novel GC-MS approach for environmental metabolomics in marine polychaetes

Raquel Fernández Varela, Department of Plant and Environmental Sciences, Faculty of Science, University of Copenhagen, Denmark

The development of an appropriate extraction method for non-targeted metabolomic approach in polychaetes promotes the use of this organism for environmental monitoring purpose. Initially, we optimize the extraction strategy for untargeted GC-MS based metabolomics of marine polychaetes. We have performed a comparison of different tissue disruption methods, one solvent system and four different extraction methods with organism exposed and non-exposed to crude oil.

XCMS (peak selection software) is used for selection of important features from metabolome profile. The results are evaluated by overall multivariate clustering approaches and distributions over individual metabolite features of coefficient of variation and yield. Different normalization and scaling are discussed to demonstrate their need in GC-MS based environmental metabolomics approach in polychaetes.

Overall, we conclude that two-step extracion with 80% methanol solution on freeze dried ragworm is a good trade-off, showing a good extraction efficiency based of the yield and reproducibility of metabolites and multivariate analysis. Besides, this selected extraction strategy allow to get differences between crude oil and control worms. It is relatively high-throughput, simpler and faster and minimizes between person variations. The ultimate goal is to identify the potentially complex set of biomarkers that define the biological context and help explain the mechanisms related to tissue response.

This study suggests that the novel GC-MS based metabolomics approach is precise, sensitive and an excellent tool to measure the metabolic response of marine polychaetes to hydrocarbon exposure and can be enabled the use of polychaetes in environmental metabolomics studies for the monitoring of polluted ecosystems.

POSTER FOUR

Comprehensive metabolomics of the gut microbiome applying different separation techniques coupled to mass spectrometry

Tanja Verena Maier, Research Unit Analytical BioGeoChemistry, Helmholtz Zentrum München, Germany

Recent evidences showed that many factors (e.g. pre/probiotics) can modify the gut microbiome. These influences were investigated towards their effects on host and bacterial metabolism and the disease susceptibility. Therefore, a study was carried out to assess the impact of differently digestible carbohydrates (prebiotics) on the microbiome and metabolome of insulin-resistant (IR) individuals. Non-targeted metabolite analysis was performed and results were used to guide a subsequent series of targeted metabolite analyses.

Fecal samples of IR individuals were collected and prepared for non-targeted metabolite analysis using Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR-MS). This technique benefits from ultrahigh mass accuracy, ultrahigh resolution and allows determining fecal chemical composition.

Data elaboration performed with different pattern recognition techniques (PCA, OPLS-DA) observed that specific fatty acids and lipids were strongly affected by digestion of different carbohydrates. Hence, we extended our analysis with a Lipidomics approach using Ultra High Performance Liquid Chromatography –

Quadrupole Time-of-flight Mass Spectrometry (UPLC-QTOF-MS). Processing this dataset was performed using Genedata Expressionist® for Mass Spectrometry.

To obtain a broader range of metabolites characterizing the gut microbiome and the effects of prebiotics on IR patients, further analyses with LC and capillary electrophoresis (CE) – IonTrap Mass Spectrometry are still ongoing. Targeted metabolomics will be used to quantify metabolites of energy metabolism.

Our FT-ICR-MS based metabolomics approach is highly suitable for investigating the function of the gut microbiome and host metabolism and helped to evaluate the effect of different diets, in which analysis with LC and CE will give us even more insights.

POSTER FIVE

Metabolic phenotyping in Cerebrospinal Fluid for Alzheimer's Disease

Ana Maria Casas Ferreira, Kings College London, UK

Alzheimer's disease (AD) is a progressive and devastating neurodegenerative form of dementia, which mostly affects the elderly. Right now AD is a rapidly growing socioeconomic and public health problem contributing to 60–70% cases of dementia.

Here we proposed a non-targeted UPLC-MS/MS methodology to analyse a wide range of metabolites present in cerebrospinal fluid. CSF can reflect biological processes of the brain due to its proximity and is considered a viable source for biomarker development. Different groups of individuals (n=20) were evaluated: young healthy people, old healthy people, Alzheimer diagnosed patients and Frontotemporal dementia diagnosed patients. Any possible correlation between the metabolites analysed in CSF samples and three known biomarkers for Alzheimer's disease (Aβ1-42, Total Tau or P-Tau181) have been evaluated. Besides, any possible association between AD, normal aging and gender in terms of the metabolites of the CSF samples have been investigated through multivariate modelling.

Three unidentified metabolites have been found to significantly discriminate between groups, two of them between Alzheimer patients and the other four (p values < 0.001, Mann Whitney test), and one between Frontotemporal dementia patients and the other four groups (p value < 0.001, Mann Whitney test). Good correlations to Aβ1-42 and total Tau protein concentrations have been observed, with sensitivity and specificity values of 0.95 and 0.86 respectively (AUC 0.90) when both dementias were compared.

POSTER SIX

Metabolic profiling of Acronychia species by means of NMR and UPLC-ESI-LTQ-Orbitrap platform

Eirini Kouloura and Maria Halabalaki, University of Athens, Greece

Acronychia species have been traditionally used in folk medicine for their anti-inflammatory and antipyretic effects and to treat asthma, ulcers and rheumatism[1]. Previous phytochemical studies on *A. pedunculata* have resulted in the identification of a number of compounds belonging to a specific chemical category called *Acronychia*-type acetophenones (AtA) with interesting anti-inflammatory and cytotoxic properties against tumor cell lines[2]. Nevertheless, limited information is available for the majority of *Acronychia* species.

In this context, a metabolomic profiling study was performed for the development of a methodology for the discrimination and classification of diverse species, origins and organs of *Achronychia* genus. Therefore, 20 different species and organs of *Achronychia* species collected in Malaysia and Vietnam were extracted and analyzed by means of pJES-NMR and UPLC-ESI-LTQ-Orbitrap platform. The stability and the repeatability of the developed UPLC-LTQ-Orbitrap methodology were determined by pooled QC samples during the analysis. Supervised and unsupervised multivariate analysis methods were applied for data mining purposes. Discriminating signals were correlated to the literature data resulting in the identification of several biomarkers, among them AtA compounds, responsible for the classification of the different extracts. In addition, species closely related according to the literature could be distinguished using pJES-NMR and UPLC-ESI-LTQ-Orbitrap fingerprinting.

[1]. Hartley, T.G. (1974) J. Arnold Arbor. Harv. Univ. 55: 2

[2]. Kouloura et al, (2012) J. Nat. Prod, 75 (7):1270–6

POSTER SEVEN

Automatic metabolite annotation in complex LC-MS$^{(n \geq 2)}$ data using MAGMa

Lars Ridder, Wageningen University, The Netherlands

High-resolution multistage LC-MS^n data contains detailed chemical information of (unknown) compounds observed in metabolite profiling studies. To support full exploitation of this data we have developed the MAGMa algorithm for automatic substructure-based annotation of multistage spectral trees. The algorithm yields a hierarchical tree of substructures of a candidate molecule to explain the fragment peaks observed at consecutive MS levels. The resulting candidate score indicates how well the observed hierarchical fragmentation pattern is explained and can be used to rank extensive lists of candidate molecules, e.g. retrieved from the PubChem database.

The method is evaluated on the basis of a published benchmark dataset and we present recent results on the spectral data from the CASMI contest for small molecule identification. Furthermore, we present applications to LC-MS^n metabolite profiling data from green tea as well as from human urine samples obtained after green tea consumption. More than 100 compounds found in the green tea sample data were systematically converted by *in silico* biotransformation rules defining possible modifications in the human gut and liver before their excretion in urine. This systematic virtual library of potential tea metabolites was used as candidate set for automatic annotation of the urine LC-MS^n datasets. In addition to 74 compounds previously identified in the urine samples, 26 additional urinary metabolites originating from green tea consumption were putatively identified. 77% of the annotated metabolites were not present in the Pubchem database, indicating the importance of combining automatic structure annotation methods with *in silico* biotransformation to discover novel metabolites.

POSTER EIGHT

Selecting chromatographic system for LC-MS fingerprinting of plant metabolites of high and intermediate polarity

Nikoline J. Nielsen*, Giorgio Tomasi, Jan H. Christensen

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The strategy comprises: screening of generic reversed phase systems varying in column chemistry, mobile phase pH, gradient steepness and eluotropic strenght, column temperature and electrospray mode of ionisation. The chromatographic systems were evaluated with respect to the number of features detected as well as the consensus of feature detection across replicate analyses. Since the validity of the experiment depends not only on the quality of the chemical analyses, but also on the appropriateness of the data processing approach, we also devised a feature detection procedure based on the combined matched second derivative Gaussian filter[1] and median measures[2] for threshold determination (COMFIRMED), and bench-marked it against the commonly used XCMS[3] package. XCMS was parameterised to use the same filter, but a mean measure based threshold. The metabolome coverage could be improved by a factor of 3 for negative ion mode and by a factor of 1.5 for positive ion mode, simply by selecting the better chromatographic system. The XCMS and COMFIRMED procedures performed differently depending on the nature of the LC-MS data. The reversed phase systems with MS detection is to be employed for fingerprinting of *Brassica napus* seedlings exposed to the herbicide glyphosate and environmental stressors, alone or in combination, with the overall purpose of identifying specific biomarkers of glyphosate exposure.

[1] Danielsson, Bylund, Markides (2002): *Anal. Chim. Acta* 454: 167-184.

[2] Ullsten, Danielsson, Bäckström, Sjöberg, Bergquist (2006): *J. Chrom. A* 1117: 87-93.

POSTER NINE

NanoflowUPLC-nanoESI-TOFMS combined with SPE sample preparation methodologies for improved global urine metabolomics.

Andrew Chetwynd, University of Sussex, UK

Current LC-MS methods for urine metabolomics focus on rapid sample preparation and high-throughput analyses in order to screen many samples in a short timescale. These methods however, are limited by poor sensitivity to low abundance metabolites such as estrogens and eicosanoids. This is in part due to sample dilution and ion-suppression caused by co-elution of highly abundant compounds in the samples. In this work, we developed SPE pre-concentration combined with direct nanoflowUPLC-nanoESI-TOFMS (nUPLC-nESI-TOFMS) methodologies to increase the coverage of the urinary metabolome. The SPE methodology was compared to traditional sample preparation techniques which use neat/diluted urine, analysed by conventional UPLC-ESI-TOFMS. Compared with neat/diluted urine samples, SPE pre-concentration using mixed mode polymeric cation/anion exchange phases resulted in detection of many other metabolites. These included bile acids, lipids, pharmaceuticals and lifestyle factors. Compared with conventional UPLC-ESI-TOFMS, the nanoflow/nanospray MS platform resulted in the detection of additional signalling compounds including eicosanoids, glucocorticoids, estrogens and melatonin metabolites. The increased sensitivity of nUPLC-nESI-TOFMS was due to its superior ionisation efficiency at lower flow rates (700 nL/min), and enhanced peak resolution on the nanoflow column. The combination of SPE pre-concentration and nUPLC-nESI-TOFMS methodologies allowed the detection of many more low abundance signalling metabolites, enabling more metabolic pathways to be analysed in a single analytical run, thereby improving the potential for biomarker discovery.

POSTER TEN

Coffee – what else? Combining non-targeted and metabolic pathway driven targeted Metabolomics in Food Metabolomics

Alec Kettle, Bruker UK

Metabolomics is an integral part of the whole OMICS picture. To interpret results derived from Proteomics or Metabolomics experiments biologists often think in terms of biological pathways. In contrast scientists often do not want to risk missing a significant change in metabolite abundance when concentrating the analysis and data evaluation on known compounds only. Both researchers' requests can be answered using the same dataset acquired using modern full scan high resolution QTOF instruments.

Here a coffee metabolomics experiment was conducted and the acquired LC-MS data initially evaluated using an untargeted workflow. This pointed to N-methylnicotinic acid as being characteristic for weak coffee types. A targeted screening list was automatically generated using a novel software tool which enables to query compounds in a metabolic pathway database. The query for N-methylnicotinic acid returned the nicotinic acid metabolism as characteristic pathways. To build a target screening list compounds derived from this pathway were subsequently extended by metabolites described to be present in coffee. Subsequently the full scan data files used for the untargeted workflow were screen for the presence of the compounds in the target list. Compounds with significant changes between coffee cultivars were tentatively identified taking into account accurate mass and isotopic pattern information.

In summary a proof of concept food metabolomics example will be presented to demonstrate a novel workflow enabling pathway driven targeted metabolomics based on the same high resolution MS data files used for a parallel non-targeted data evaluation.

POSTER ELEVEN

Development of an analytical workflow for the characterisation of the liver metabolome.

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The Liver Metabolome Project is funded by the NIH to fully characterise and quantify the lipid and small molecule components of liver tissue using a series of open-profiling and targeted chromatographic, mass spectrometric and NMR spectroscopic methods. We have designed an analytical workflow to investigate a wide range of lipid classes including (but not exclusively) glycerolipids, phospholipids, sphingolipids, ceramides, cholesterol esters, non-esterified fatty acids, eicosanoids, carnitines and bile acids. Open-profiling NMR methods (including both ^{1}H and ^{13}C) alongside targeted LC-MS assays have been used to investigate the small molecule metabolite profiles and cover classes including amino acids, organic acids, sugars, nucleotides and nucleosides. The first stage of the analytical workflow has involved optimisation of metabolite extraction which has been achieved by comparing liquid-liquid extraction and protein precipitation methods. Certain assays, such as those used for profiling eicosanoids, require a further Solid Phase Extraction step to clean up and concentrate the class of metabolites prior to the application of a targeted LC-MS assay. In addition, open-profiling Direct Infusion Mass Spectrometry (DIMS) has been employed to investigate lipid profiles of the high concentration intact lipids, and to date has identified >500 potential lipid molecules in liver tissue. Further targeted LC-MS and MS/MS experiments have been employed for structural elucidation and quantitation. Overall, this project will provide a comprehensive database of the components of the liver metabolome which could be of great interest for researchers involved in biochemical, toxicological and medical fields as well as providing a valuable resource for the metabolomic community.

POSTER TWELVE

Identification of novel biomarkers of dietary intake

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Self-reported dietary intake assessment methods can be influenced by random and systematic errors. Advances in these methods are therefore necessary to improve the classification of a person's dietary intake and enhance our understanding of the link between diet and disease. The identification of novel biomarkers of dietary intake, through the application of metabolomics, offers the potential of a more objective measure of dietary intake.

This study aims to identify and quantify specific metabolites that reflect food group intake. Dietary intake data and ^{1}H nuclear magnetic resonance (^{1}H NMR) urine spectra from 565 participants of the National Adult Nutrition Survey (NANS) were used for this analysis. Dietary intake data was obtained from 4-day food diaries and reduced into 34 food groups. Heat map analysis was performed to identify correlations between ^{1}H NMR spectral regions and food group intakes. Positively correlated peaks within the spectral regions were compared between low and high consumers of the specific food groups. Receiver operating characteristic (ROC) analysis was performed.

Heat map analysis identified sugar sweetened beverages (SSB) as having strong correlations with a number of spectral regions. Spectral peaks which were positively correlated with SSBs and significantly increased in the high consumers were identified as formate, citrulline, taurine and isocitrate. This panel of biomarkers had an area under the curve of 0.8 for ROC analysis and a sensitivity and specificity of 0.7 and

0.8 respectively. Validation of these markers is currently underway. Future work will ascertain how to translate these markers for use in nutrition epidemiology.

POSTER THIRTEEN

Targeted HILIC LC-MS/MS for environmental metabolomics: successes and current limitations

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Non-targeted metabolomic studies are typically conducted using high-resolution mass spectrometry. They can generate hypotheses that subsequently require targeted and quantitative measurements of metabolites using LC-MS/MS. Many of the metabolic pathways to be targeted can be dominated by polar compounds which cannot be separated well by reversed-phase resins. Here we report the successful application of targeted and quantitative HILIC, and discuss the limitations of this approach.

An FTICR-MS based study of liver carcinogenesis in flatfish discovered significant changes in the 1-carbon methylation pathway (1). A targeted LC-MS/MS method for 10 metabolites was initially developed on a 2.1 mm Thermo WAX-1 column, but was scaled down to a 0.3 mm WAX-1 column to improve sensitivity. In a second study, analyses of archaeological wine residues revealed the importance of phenolic and small polar acids as biomarkers, which were then separated using a ZIC-pHILIC column. This column, which is resilient to elevated pH, is also capable of separating compounds such as sugar phosphates or nucleotides.

Scaling down HILIC chromatography leads to peak broadening, which decreases separation efficiency and complicates quantitation. This problem is inherent to the pure HILIC mechanism. Also only a low number of commercially-available columns are resistant to a wide pH range; therefore the choice of chemistries to optimise separations is low. While the studies presented demonstrate the suitability of HILIC LC-MS/MS approaches for low abundance or highly anionic compounds, the limitations show that improvements in column chemistries are desirable.

(1) Mirhabai *et al.* 2013, *J. Proteome Res.* **12(6)**, 2895–2904.

POSTER FOURTEEN

Linking metabolism and cell identity: a voyage from the Arabidopsis root to embryonic stem cells

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Cell differentiation involves a shift in the metabolic machinery. Deeper knowledge of the metabolic switches and molecular circuitry underlying cell differentiation may provide a means for monitoring and controlling such processes. This requires the study of metabolism in specific cell types. The metabolic profiling of cell populations within the *Arabidopsis* root was used as a model for cell-type metabolic analysis. The interactions between metabolism, epigenetics, and cell identity were then examined during human embryonic stem cell differentiation. New insights into metabolic regulation of cell differentiation will be discussed.

POSTER FIFTEEN

New Standards and Tools for the Analysis of Central Metabolites

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Central metabolites and their analysis are of fundamental importance in the understanding of metabolic pathways and their regulation in healthy and disease states. As many central metabolites of well-established pathways and their analyses have not been available, an initiative to fill these gaps has been started. The challenges in the development of inclusive analytical methodologies with high resolution and sensitivity, e.g. for finding deviations along a metabolic pathway or for the direct separation of chiral metabolites instead of summarizing optical rotation measurements, are due to the tremendous diversity in molecular structure and information content. The three-stage process for establishing new chiral separation methods starts with the preparation of the racemates, continues with the development of the chiral separation methods and finishes with the preparation of the pure enantiomers for assigning peaks to structure. New chromatographic, electrophoretic and NMR tools for the analysis of the enantiomers of highly polar carboxylated and phosphorylated metabolites will be presented and have proven useful for the development of new metabolite standards. These newly developed analytical methodologies and metabolite standards can however also be of use in such widely different applications like analysing inborn errors of metabolism, cancer- and other disease-related metabolites.

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