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University of Wollongong NSW
9-13 July, 2023

ABSTRACT BOOK

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Oral Presentations

Posters

Australian and New Zealand Society for Mass Spectrometry Conference

University of Wollongong NSW
9-13 July, 2023

Plenary Speakers

Plenary Lecture 1

Metallo-supramolecular aggregates in the gas phase: avoid and provoke fragmentation

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Self-assembly has led to numerous fascinating supramolecular structures and functional architectures based on an impressive variety of subcomponents. One way to design self-assembly processes uses the metallo-supramolecular approach based on well-chosen organic ligands interconnecting two or more metal centers. There are impressive examples in literature of highly selective self-assembly processes of multicomponent mixtures even leading to complex heterometallic assemblies.
Mass spectrometry has become an indispensable tool to determine the stoichiometry of self-assembled aggregates. This task however is often very challenging as very soft ionization conditions are needed to avoid in-source fragmentation, although analysis of the detected fragments can also confirm the structure of the aggregate in fortunate cases. Hence, it is tempting to use induced fragmentation in the gas phase to deduce the structure of metallo-supramolecular aggregates in analogy to the well-established routes of structure elucidation of covalent compounds by mass spectrometry. However, the combination of rather weak non-covalent bonds that form the aggregate and strong Coulomb interactions between the charged metal centers and between metals and counter anions can result in unforeseen fragmentation pathways and rearrangements that easily can be misleading in structure determination. Thus, a much broader experimental basis is currently needed to better understand the gas-phase fragmentation patterns of metallo-supramolecular aggregates.
The talk will present results from gas-phase fragmentations of metallo-supramolecular squares of the classic Stang-type as well as of heterobimetallic ones that exhibit much more complex fragmentation patterns which strongly depend on the ligand length and thus aggregate size. Metallo-supramolecular rhombs formed out of six metal centers showed a very general and simple fragmentation scheme, whereas rhombs and helicates formed from chiral ligands based on a pseudo-meta-difunctionalized [2.2]paracyclophane skeleton exhibited a more peculiar fragmentation behaviour. In addition, one-electron reduction in the gas-phase enables us to study the fascinating behavior of highly reactive open-shell metallo-supramolecular aggregates.

Keywords: Metallo-supramolecular aggregates, Electrospray, Mass spectrometry, Collision-induced dissociation, redox-active ligands

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Plenary Lecture 2

Advancing Structural Characterization Methods in Mass Spectrometry for Protein Aggregation Analysis

Iuliia Stroganova, Agathe Depraz Depland, Raya Sadighi, Dave Orlemans, Sjors Bakels, Melissa Bärenfänger, and Anouk M. Rijs

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Neurodegenerative diseases, such as Alzheimer's, Amyotrophic Lateral Sclerosis and Parkinson's disease, are directly correlated with the aging process. On a molecular level, this is translated by the development of protein and peptide aggregates. However, the mechanism of aggregation, and especially the transition from soluble oligomers to insoluble fibrils remains unclear. This is particularly important as neurodegenerative toxicity originates from the conformational intermediates that are formed along the aggregation pathway. The challenge here is to obtain structural information of these elusive intermediate oligomers from their complex and heterogeneous environment.

Therefore, at the MS-LaserLab of the BioAnalytical Chemistry Division (VU Amsterdam), we have developed the Photo-Synapt, which interfaces droplet-based microfluidics electrospray ionization and ion mobility mass spectrometry with IR ion spectroscopy in a single experiment. This hyphenated approach allows us to reveal the identity, the structure, and the temporal evolution of key intermediates during the aggregation process. In this presentation, I will focus on our advances on studying the early steps of protein aggregation of alpha-synuclein, amyloid-beta, and the peptide segments of tau and TDP43. Our ion mobility mass spectrometry measurements provide more insight on the global picture of oligomer formation, while the IR signature reveal information on the local structure.

The gain in molecular understanding of the aggregation process will lead to insights in the toxic nature of the observed intermediates and provide the basis for validating inhibitors during the aggregation process. Therefore, in addition to commissioning our Photo-Synapt, we aim to use our knowledge and technology to screen future therapeutics.

Keywords: peptide aggregation, Photo-Synapt, ion mobility mass spectrometry, IR action spectroscopy, neurodegenerative diseases

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Plenary Lecture 3

MALDI-MS imaging at high spatial resolution – challenges and recent developments

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Over the last 10-15 years, MALDI-MS imaging has developed into a valuable tool that is now used in many medical and biological applications. By scanning a focused laser beam across a sample surface, it adds spatial information to each MALDI mass spectrum recorded. The combined data allows for the reconstruction of “molecular maps” across samples, most often tissue sections. Pixel size and thereby spatial resolving power of the method are largely dictated by the size of the employed laser spot on the one hand and suitable sample preparation techniques on the other. Consequently, the demand for improving spatial resolving power down to the cellular and sub-cellular level opens up new challenges in both fields. Design and construction of conventional MALDI-MS ion sources impose physical limits to the numerical aperture (NA) of the employed focusing optics; thereby limiting the achievable spot size to 5-10 µm. Transmission mode (t-)MALDI circumvents these limitation by illuminating the sample from behind using a high NA microscope objective and can produce ablation craters down to 1 µm. The analysis of such minute sample amounts that correspond to 6 to10 pg of tissue, however, requires sensitive ion sources and greatly benefits from post ionization strategies such as MALDI-2. Here, a second laser pulse intersects the evolving MALDI plume and resonant two photon ionization of matrix molecules initializes the effective post ionization for a number of analyte classes. With cellular resolving power now in sight, co-registration of MS imaging results with light microscopy at a sufficient accuracy becomes increasingly important for single-cell analysis. Putting the microscope objective, employed in the t-MALDI setup, to dual use allows to establish a slide scanning microscope inside the ion source and intrinsically couples the spatial coordinates of optical and MS modalities for automatic co-registration with sub-µm accuracy.

Keywords: MALDI-MS imaging, transmission mode MALDI, post-ionization, MALDI-2

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Plenary Lecture 4

Delivering the CanTEST Health and Drug Checking Service

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In July 2022 Australia's first fixed site drug checking service CanTEST opened as a six month pilot program in central Canberra, operated by Directions Health Services and funded by the ACT Government. In January 2023 the pilot was extended for a further six months to August 2023. This builds on the two successful trials of drug checking at the Groovin the Moo festival in Canberra in 2018 and 2019, developed and delivered by Pill Testing Australia. So, what is drug checking?

Drug checking, or pill testing is a harm reduction intervention that seeks to reduce the risks associated with drug use. Drug samples presented by members of the public are subject to chemical analysis with the results returned directly to the client. The information provided by the client and chemical analysis of the sample is used to inform conversations between the client analysts and health or harm reduction workers about sample contents and potential risks associated with consumption. Drug checking can also provide people who use drugs withy avenues to engage with health professionals and associated services more broadly, and also serves as a powerful approach to monitor the unregulated drug market.

This seminar will discuss how the service was developed and delivered through the close collaboration between a range of government and non-government stakeholders. It will also describe the analytical technologies employed, some of the lessons learned through service delivery, a selection of the more interesting findings arising from the pilot program to date, and a reflection on the future of drug checking in Australia.

Keywords: Drug checking, pill testing, harm reduction, drug analysis

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Plenary Lecture 5

Unravelling Virus Glycoprotein Structure and Function across Molecular Scales

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Enveloped virus spikes are among the most glycosylated proteins in nature and glycans function to hide antigenic sites as they are derived from the host glycosylation machinery and therefore perceived as immunologically “self”. Virus glycosylation is fundamental for efficacy of structure-based and mRNA/DNA-derived vaccines as many broadly neutralising antibodies (bnAbs) incorporate glycans as part of their binding epitopes. Consequently, antigenic similarity between viruses and vaccines is crucial, however the molecular design of spike trimer complexes used for structural studies and immunogens are engineered to preserve their pre-fusion states. These mutations, which are absent on circulating virions, restrict conformational dynamics, affect spike glycosylation and influence macromolecular interactions within the host. Here, we explore these principles to 1) shed light on how HIV and SARS-CoV-2 spike assemblies are glycosylated and 2) understand the mechanisms by which obligate receptors and bNAbs recognise spikes. Our methodological approach involves glycomics, glycoproteomics and native mass spectrometry to characterise HIV-1 envelope (Env) and SARS-CoV-2 spike (S) glycosylation as well as single molecule imaging and tracking via mass photometry to quantify diffusion and binding by bnAb to Env/S spikes immobilised on supported lipid bilayers. Overall, these data help shed light on immune evasion and vaccine efficacy across molecular scales, from site-specific glycosylation to antibody-mediated crosslinking of viral spikes and the formation of immune complexes.

Keywords: HIV-1, SARS-CoV-2, glycomics, glycoproteomics, mass photometry

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Keynote Speakers

Keynote Lecture 1

Resolving Macromolecular Complexity Using Ion Mobility and Mass Spectrometry

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Over the last century, synthetic co-polymers have become a ubiquitous aspect of daily human life – in roles ranging from food storage to medical implants to synthetic fabrics. Understanding the intrinsic chemical transformations and intermediates involved in polymer synthesis and degradation represents a key challenge for designing next-generation materials. Hampering this understanding is the inherent molar mass distribution, which means that even simple polymers are comprised of a complex mixture of distinct macromolecular structures. This mixture complexity is compounded by the presence of topological or structural isomers, *i.e.*, polymers of the same composition but different arrangements of monomer units. Such subtle changes can significantly influence molecular packing, optoelectronic, or other physical properties, so analytical tools capable of discriminating polymer isomers and assigning their molecular structure(s) are essential. This talk will describe our efforts to address this problem using both ion mobility and mass spectrometry to separate and structurally interrogate isomeric polymers.

Experiments were performed using a cyclic ion-mobility enabled quadrupole time-of-flight mass spectrometer (Waters Select Series CyclicIMS). Diluted polymer samples were infused via electrospray ionization and precursor ions mass selected using the quadrupole mass filter and passed to the ion storage array. The mass selected ions were subsequently injected into the cyclic ion mobility array and separated over multiple passes around the device.

The utility for ion mobility to resolve macromolecular architectures was demonstrated for several novel photoactive monomers containing photoactive end groups, which have been shown previously to reversibly dimerise under UV wavelengths of light, creating the possibility for intramolecular cyclisation. Cyclic ion mobility revealed that the morphological transformation from linear to cyclic was accompanied by a detectable change in collision cross section, with two isomeric forms (*i.e.* cyclic and linear) of the monomer resolved. Moreover, the cyclic form of the monomer was revealed to have two different isomeric forms itself, revealing the underlying structural complexity even within a reasonably simple structure.

Keywords: Ion-mobility, polymers, isomers

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Keynote Lecture 2

Zero-pressure photolysis quantum yields determined by photoionisation mass spectrometry

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Quantum yields have long eluded the practitioners of experimental photodissociation reaction dynamics in molecular beams. Routinely we can characterise, in exquisite detail, the dynamics of a chemical reaction pathway, yet conclude nothing about its significance -- our state-selective and universal photoionisation techniques are so sensitive…

However, several techniques have been presented over the years that require either measurement of detection efficiencies, external calibration measurements, internal standards, or comparison to another compound with known quantum yields. We present a new technique, using photoionisation mass spectrometry, with none of these limitations, that can explicitly determine absolute photodissociation quantum yields in molecular beams: Proportion of Photoproducts by Tetralaser-measurement and Analysis of REMPI and Translational Spectra (POPTARTS).

This talk will give some background and history on photodissociation yields in molecular beams, discuss some particular atmospheric chemistry problems that necessitate such a technique, then walk through the development, benchmarking and application of, possibly, the most complicated photoionisation experiment you've seen.

Keywords: photoionization, photodissociation, quantum yields, molecular beams

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Keynote Lecture 3

Characterising the interactions of biomolecules associated with health and disease using native mass spectrometry

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Characterising interactions between biomolecules associated with health and disease (e.g., proteins, RNA, DNA, viruses) is crucial to multiple stages of therapeutic discovery, development, and translation. Native mass spectrometry (nMS) is one of the most powerful technologies available to gain these insights as it allows experimental conditions that are sufficiently gentle to allow the analysis of intact and natively folded biomolecules and their non-covalent interactions with binding partners such as ligands or other biomolecules. Our research group has a strong focus on the application of nMS in drug discovery and in this presentation I will overview three current projects: (i) development of a fragment screening platform using nMS to enable fragment based drug discovery; (ii) assessing the suitability of nMS for the analysis of RNA as an emerging class of therapeutic drug targets; and (iii) using nMS for the analysis of peptide-protein binding and assessment of structure-activity relationships to guide drug discovery. These projects span infectious disease (antibiotic resistance and emerging viral threats) and cancer to demonstrate an expanding role of native mass spectrometry in facilitating a route to therapeutic drug discovery and research impact.

Keywords: Native mass spectrometry, drug discovery, fragments, biomolecules, RNA

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Keynote Lecture 4

Proteome-wide systems genetics in mouse populations

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Proteomic technologies now enable the rapid quantification of thousands of proteins across genetically diverse samples. Integration of these data with systems-genetics analyses is a powerful approach to identify new regulators of economically important or disease-relevant phenotypes in various populations. We have performed a proteomic analysis of various tissues from 73-107 genetically distinct inbred mouse strains, and integrated the data with genomics and >300 molecular/phenotypic traits via quantitative trait loci mapping and correlation network analysis. These data identified thousands of associations between genetic variants, protein abundance and phenotypic traits. Here, we will present our data focused on skeletal muscle which we used to prioritize targets for a functional genomic screen in human bioengineered skeletal muscle. This identified several negative regulators of muscle function including UFC1, an E2 ligase for protein UFMylation post-translational modification. We show UFMylation is up-regulated in mouse and human models of muscle atrophy, and in vivo knockdown of UFMylation improved muscle function. Finally, we present a new proteomic assay to site-specifically quantify *in vivo* UFMylation sites.

Keywords: proteomics, systems genetics, multi-omic integration, skeletal muscle, protein post-translational modifications, UFMylation

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Keynote Lecture 5

Immuno-mass spectrometry imaging – potential and pitfalls

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Immuno-mass spectrometry imaging is an emerging technique for the in situ quantification and localisation of biomolecules that is based on the combination of laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) with metal-conjugated antibodies. High levels of quantitative multiplexed imaging of up to 40 targets in formalin-fixed, paraffin-embedded or frozen tissue sections is possible using standard immunolabeling protocols. The resulting multidimensional images provide single-cell resolution and quantification simultaneously across all targets and may include cell segmentation. Despite its rapid uptake, it is a field that is still in its infancy, and many factors require optimisation and validation before it reaches its full potential.

This presentation will provide an overview of immuno-mass spectrometry imaging and the technology underpinning it, with a focus on outlining where improvements are required. Promising applications in the clinical domains of neurodegeneration, neuromuscular diseases, oncology, cardiovascular disease and infectious disease will be highlighted, and future perspectives discussed.

Keywords: mass spectrometry imaging, immunolabelling, multiplexing, quantification

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Keynote Lecture 6

Exploring DNA triplex assemblies for antigene technologies using structural mass spectrometry

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Variations from the canonical Watson–Crick double helical DNA structure are thought to play key roles in a range of cellular processes. For example, nucleic acid triplexes, which results when a third oligonucleotide strand binds within the major groove of a double helix, have been proposed as under-explored regulators of gene expression and potential candidates for development in antigene therapeutic strategies.

In contrast to other non-covalent biomolecular assemblies, nucleic acid triplexes are relatively poorly characterised, and reliable techniques to investigate them in detail are lacking. We have performed foundational mass spectrometry-based studies on model oligonucleotide systems to probe the formation of biologically relevant DNA triplex assemblies. Utilising a suite of structural mass spectrometry-based experiments, including native mass spectrometry, ion mobility and collision induced dissociation, in conjunction with molecular modelling and solution phase measurements, we have probed fundamental features controlling formation, structure and stability of oligonucleotide triplex assemblies. Our experiments emphasise the utility of mass spectrometry in the structural study of nucleic acid triplexes, however, importantly, this work also highlights key considerations for analysis of these structures in the gas phase. For example, experimental factors including buffer composition and ionisation mode can significantly impact detection of higher order DNA structures and reveal differences between solution and gas-phase behaviour for some systems.

New structural understanding afforded by these mass spectrometry studies has underpinned our ongoing efforts towards rational design of chemically modified oligonucleotides for applications in antigene technologies, particularly aimed at development of novel antibacterial agents.

Keywords: Structural mass spectrometry, oligonucleotides, DNA triplexes, antigene technology, antibacterial agents

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Keynote Lecture 7

Towards clinical applications: proteomics of patient derived cells and tissues

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Cancer treatments have the potential to save lives. Medical professionals utilize a comprehensive approach to make informed decisions about the most suitable clinical interventions for cancer patients. However, there are areas where clinical needs remain unmet, such as accurately determining the most effective therapy for each individual patient. We are currently investigating how analysing the proteome of patient-derived samples can contribute to the decision-making process. Through retrospective MALDI-MSI studies using formalin-fixed, paraffin-embedded (FFPE) samples of gynaecological cancers, we have successfully predicted chemotherapy response in Ovarian Cancer and identified metastasis in Endometrial Cancer based on the proteome of the primary tumour. To accomplish this, we have refined sample preparation, workflows, and data analysis techniques for MALDI-MSI. Furthermore, we have utilized MALDI-MSI to monitor drug penetration in primary Ovarian Cancer spheroids and evaluate the response to standard chemotherapy. We are currently exploring how proteomics can be used to assist patient-derived cell testing, correlating chemoresponse to multiple agents and combinations thereof with the proteome of the primary tumour. To facilitate the potential application of these findings in a clinical setting, we are actively developing novel enabling technologies to support this transition.

Keywords: proteomics; MALDI-MSI, patient-derived samples;

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Award Presentations

Morrison Medal Award 2023

The role of mass spectrometry in understanding beneficial plant root microbe interactions

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The evolution of the application of mass spectrometry for metabolomics and lipidomics and the ability for the analysis of biomolecules such as metabolites and lipids in much greater depth allows us now to tackle biological questions difficult to address before. Our research group focuses on unravelling the signalling and communication processes between plant roots and soil microbes. Roots anchor plants, absorb nutrients and water, produce and store compounds essential to plant growth and productivity. They also host a diverse microbiome that influences plant physiology, morphology, and biochemistry. It is known that abiotic stresses, including salinity and temperature, impact root performance via changes in cell membrane stability and metabolism. We propose that harnessing beneficial microorganisms from the soil can mitigate the negative effects of stress on the lipidome of roots and improve overall plant performance.

We know now that the plant lipidome is significantly more complex than in mammals, is defined by tissue type and developmental stage, and is highly responsive to abiotic stress, including salinity and temperature. I will present our experiments to understand how beneficial root-microbe interactions are influenced by salinity, temperature, and nutrient stress, and the molecular mechanisms by which beneficial microbes help relieve stress on plant growth and metabolism. Our findings have implications for developing sustainable agricultural practices and enhancing crop resilience in challenging environments.

Keywords: lipids, metabolomics, plant roots, microbes, interaction

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Guilhaus Award 2021

Imaging metabolic flux using mass spectrometry imaging

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Kinetic mass spectrometry imaging (kMSI) has emerged as an innovative technique integrating the imaging capability of mass spectrometry imaging (MSI) with stable isotope labelling to determine metabolic flux in a spatiotemporal manner within tissues. Efforts to study flux are hampered by the complexity and high volumes of data generated by kMSI. To address these challenges, we developed KineticMSI, an open-source bioinformatics workflow written using base R programming language providing an semi-automated pipeline for processing and analysing kMSI data. Several examples of metabolic flux using a deuterated water (^2H_2O) labelling strategy in mouse models of disease are presented.

Mice (C57BL/6 (WT) or R6/1 model of Huntington's disease (HD)) were injected with deuterated water (99% ^2H_2O and 0.9% NaCl) *via* intraperitoneal injection. Additional, 8% ^2H_2O was maintained in drinking water for between 4-50 days post-injection. Tissues were harvested, immediately flash frozen, then cryo-sectioned at 8-15 µm, vacuum dried and MALDI matrix (norharmane or BPYN) applied using a TM Sprayer. A Bruker SolariX XR FT-ICR-MS was used to acquire spectra in negative ionisation mode, over *m/z* 100-2500, using a 30x30 µm raster. Data pre-processing was performed using SCiLS Lab prior to analysis with KineticMSI.

We present a comprehensive method, incorporating a range of statistical tools that can be applied to systems to conduct relative quantification of isotopic tracer incorporation across different treatment groups displaying intra-tissue spatial heterogeneity. KineticMSI allows users to take data-driven decisions by providing a tool for the elucidation of affected pathways that are associated with detected metabolic turnover changes, thus providing mechanistic insights into biological systems.

By segregating pixels into clusters based on tracer incorporation and comparing the cluster means between two conditions, we uncovered distinct metabolic states in diseased mice, where conventional approaches failed. Highlighting early changes to lipid metabolism prior to phenotypic changes.

Keywords:stable isotope labelling, mass spectrometry imaging, metabolic flux, MALDI, FT-ICR-MS

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Guilhaus Award 2023

Investigating Photochemical Reactions with Photodissociation Action Spectroscopy

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Photochemical reactions can provide synthetic pathways to novel functionalised polymers. Optimising photoligation reactions requires detailed knowledge of the photoactivation profiles of the reagents, which can only partially be determined from UV-Vis spectroscopy. Importantly, absorption spectroscopies do not probe the *reactivity* of the reagent(s) and are thus inadequate for fully optimising reaction outcomes. Photodissociation action spectroscopy (PDAS) monitors absorption events as a function of photoproduct ion yield, thereby uniting the advantages of spectroscopy and mass spectrometry. Herein, we apply PDAS to investigate the wavelength-dependent formation of nitrile-imine intermediates from substituted 2,5-diaryl-*2H*-tetrazoles.

PDAS was implemented on a linear ion trap mass spectrometer, modified to enable optical interrogation of trapped ions. Thermalised, mass-selected poly(ethylene glycol) (PEG) ions from electrospray ionisation were irradiated with the tuneable UV output from a Nd:YAG-pumped optical parametric oscillator, and photodissociation mass spectra were recorded over the desired wavelength range. Photoproduct yields calculated from each mass spectrum were plotted as a function of wavelength to produce action spectra.

The predominant photoproduct observed upon UV irradiation of PEG-tagged diaryl tetrazoles is consistent with nitrile-imine formation *via* loss of N_2. The photodissociation onset is approximately 318 nm for a tetrazole with a phenyl substituent, in agreement with the UV-Vis absorption spectrum and solution-phase reactivity studies. Moreover, the action spectrum is largely independent of charge state and polymer size. PDAS demonstrates that introduction of a *p*-methoxy substituent induces a redshift in the dissociation onset to 335 nm, and an *N*-ethylcarbazole derivative further shifts the onset to 365 nm.

These discoveries demonstrate the utility of PDAS in providing critical mechanistic insight into the efficiency and selectivity of photoligation reactions. Moreover, using PEG-tagged substrates enables screening of the neutral-like photochemistry in the quest toward optimising reagent structure for efficient visible light ligation in biological applications.

Keywords: Photodissociation, lasers, action spectroscopy, ion activation

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Oral Presentations

Speaker 01

Diastereomer Resolution of M_4L_6 Coordination Cages by Ultra-High-Resolution Ion Mobility-Mass Spectrometry

M. C. Pfrunder[1,2], D. L. Marshall[1,3], B. L. J. Poad[1,2,3], T. M. Fulloon[1,2], J. K. Clegg[4], S. J. Blanksby[1,2,3], J. C. McMurtrie[1,2], K. M. Mullen[1,2]

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Metallosupramolecular cages are 3-dimensional assemblies that often feature internal cavities and have myriad applications including enantio- and regioselective catalysis, molecular sensing, stabilisation of reactive species and drug delivery. These species are notoriously difficult to unambiguously characterise on account of their dynamic behaviour in solution, such as isomer interconversion, reversible guest binding and other structural rearrangements. While traditional techniques including nuclear magnetic resonance, single crystal X-ray crystallography and electrospray ionisation mass spectrometry (MS) are typically employed to achieve this end, another technique, namely ion-mobility MS, has begun to grow in popularity as it can provide information about the shape and size of many co-existing species simultaneously without requiring the growth of single crystals. By utilising the separating power of cyclic ion-mobility, we have extracted and resolved diastereomers of a series of M_4L_6 cages from a mixture of kinetically labile interconverting molecules. We have also extended this methodology to investigate cages containing paramagnetic ions such as octahedral Ni^{2+} revealing information regarding the equilibrium ratio of isomers that is not possible to discern using traditional techniques. The potential applications for this technique as both a specialist tool and a standard characterisation technique for the analysis of metallosupramolecular cages will be explored and our progress to date on this will be discussed.

Keywords: supramolecular, metallosupramolecular, paramagnetic, guest binding, gas phase

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Speaker 02

Investigating small molecules as templating agents for Coordination Driven Self-Assembly with Ion Mobility Mass Spectrometry

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Coordination driven self-assembly is a powerful supramolecular synthetic approach for creating complex structures. Simple ligands and metals come together to create ordered structures that open the door for functional materials, enhanced catalysis, and sensors. The primary challenge of self-assembly is controlling the outcome of the supramolecular systems. To moderate the structures formed, small molecules are often added, with the intention that they will act as templating agents and guide the self-assembly. Decoding the impact of templating agents can often present an analytical challenge, as the non-covalent interactions that govern formation and the intermediates involved are hard to isolate and analyse.

Ion mobility mass spectrometry enables a unique method of analysis where the structures formed can be separated and measured directly through arrival times in ion mobility. A large library of bis-β-diketonates, metals and templating agents was analysed by coupling an established high throughput analytical approach with the capabilities of a cyclic ion mobility instrument. Using automated experiment generation, it was possible to perform many ion mobility-mass spectrometry experiments rapidly. Ion mobility was used both as a separative tool and to obtain information about the size and shape of the ions analysed.

Through this approach it was possible to directly observe the impact of templating agents by tracking the “up regulation” of critical species separated by their size and shape. The upregulation of these desired species can be directly linked to the structures of the templating agents and their non-covalent interactions with the metals and ligands. Remarkably, it has been observed that some of these templating agents are acting catalytically, where the structures formed through self-assembly switch when a trace amount of templating agent is added.

Keywords: Cyclic ion mobility, high throughput, self-assembly, metal ligand

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Speaker 03

Enhancing Metabolite Coverage in MALDI-MSI Using Laser Post-Ionisation (MALDI-2)

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Matrix-assisted laser desorption/ionisation mass spectrometry imaging (MALDI-MSI) has received increasing interest for the spatial mapping of metabolites from biological tissue. The spatial mapping of metabolites is a powerful tool for obtaining comprehensive information on changes in altered metabolism throughout heterogeneous tissues, such as those originating from the Warburg effect in cancer. However, as is the case with all desorption/ionisation techniques used for MSI, analyte coverage is often limited by low ionisation efficiencies, an effect that is further magnified by the complex nature of biological tissues leading to ion suppression effects. In recent years post-ionisation techniques have received increasing interest to overcome the poor ionisation efficiencies of many analytes. Herein we present the optimisation of an intermediate pressure MALDI ion source coupled to an Orbitrap Elite mass spectrometer that has been combined with laser-induced post-ionisation (MALDI-2) enabled by a pulsed 266 nm laser introduced parallel to the sample surface. When applied to kidney tissue, MALDI-2 was capable of detecting almost double the number of tissue-specific signals compared to conventional MALDI. When compared against ~140 publically available kidney datasets on METASPACE using the same NEDC matrix, MALDI-2 was shown to detect 63 metabolite signals not that were not previously annotated, demonstrating the dramatic increase in metabolite coverage achieved using MALDI-2. MALDI-2 was also applied to metabolic imaging of liver tissue containing a tumour originating from metastasised breast cancer at a spatial resolution of 20 µm. Again MALDI-2 provide significantly improved metabolic coverage, providing up to 20-fold improvements in sensitivity for many tumour-specific signals such as *m/z* 146.0461 (glutamate) and *m/z* 174.0408 (*N*-acetyl-aspartic acid), as well as detection of 6 tumour-specific metabolites not detected at all using conventional MALDI.. This work provides the first report of MALDI-2 applied to metabolite imaging and demonstrates the dramatic improvements in sensitivity and metabolite coverage it provides.

Keywords: MALDI-MSI, metabolites, MALDI-2, post-ionisation, cancer

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Speaker 04

Metal organic frameworks for enhancing the analysis of volatile organic compounds in breath by high resolution mass spectrometry

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The human breath profile consists of thousands of volatile organic compounds (VOCs), originating primarily from metabolic processes in the body. The presence of diseases, such as cancer, can alter these metabolic pathways and impact the concentration and type of VOCs present in the breath. Therefore, the analysis of breath may be useful for earlier and less-invasive disease diagnosis. However, such volatile compounds often exist at exceedingly low concentrations (ppb to low ppt), and therefore ultrasensitive methods are required for confident diagnosis. Thus, the development of preconcentration methods to extract biomarkers from breath for accurate, high-performance chemical analysis may make breath analysis more robust, reliable, and accurate for enhancing disease diagnosis. Metal organic frameworks (MOFs) are a potential selective and sensitive preconcentration material due to their high chemical versatility, large surface areas and the presence of permanent pores. This work describes the proof-of-concept for the use of metal organic frameworks (MOFs) as efficient adsorbent materials for breath VOCs. An analytical platform that integrates MOFs for preconcentration and a hydrazide derivatisation reaction to convert carbonyl-containing biomarkers to fixed-charge quaternary ammonium ions for rapid and sensitive detection by nano-electrospray ionisation mass spectrometry was developed and optimised. UiO-66 and ZIF-8 MOFs were compared to commercial adsorbent material Tenax for their carbonyl adsorption capabilities in both breath and ambient air. UiO-66 was determined to be the optimal preconcentration material, detecting over 4.5 times the number of chemical features in breath compared to Tenax. Using this method, a signal-to-noise ratio of over 100 can be obtained for breath biomarkers which are predominantly in the low ppb_v range, indicating that ppt detection limits are possible. These results indicate that early-stage disease diagnosis via breath analysis is possible with this workflow, and that MOFs offer superior preconcentration ability due to their high surface areas and chemical tunability.

Keywords: Preconcentration, breath analysis, disease diagnosis, metal organic frameworks, nano-electrospray ionisation

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Speaker 05

Space for (Molecular Weight) Growth: A tricyclic product in the reaction of distonic benzonitrylium-2-yl radical cations with acetylene

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Benzonitrile is one of only a few larger (>10 atom) molecules that has been detected in the interstellar medium (ISM). Nonetheless, polycyclic aromatic molecules are known to be abundant in the ISM. Much like soot formation in combustion processes, molecular weight growth from monocyclic species to bi- and tricyclics is a key step in generating yet larger molecules. As such, benzonitrile and its close derivatives are good candidates for experiments investigating their molecular weight growth chemistry. Room-temperature ion-trap mass spectrometry is used to measure the reaction of distonic benzonitrylium-2-yl radical cations with acetylene. 2-iodobenzonitrile is protonated *via* electrospray ionisation, and then deiodonated with a pulsed 266 nm laser, generating the benzonitrylium-2-yl radical cations. Traces of acetylene at known concentration are introduced into the ion trap, reacting over time with the distonic cations to form a variety of C_9H_7N isomers. Of these, only distonic isoquinolinium-1-yl radical cations are both abundant and reactive enough to undergo secondary addition with acetylene. The major product from this secondary addition is the tricyclic cyclopenta[ab]isoquinolinium ion, identified using action spectroscopy as well as SCS-CC2/TZVP calculations.

Keywords: Distonic, Molecular Weight-Growth, Spectroscopy, Kinetics

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Speaker 06

High-Resolution DBS-Based Lipidomics Platform

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Dried blood spots (DBSs) are a minimally invasive and cost-effective method for collecting and storing blood samples for metabolic phenotyping research that can enhance our understanding of human metabolism and lead to development of personalised interventions that improve patient outcomes. This is because DBSs allow for more frequent and longitudinal sampling, enable better tracking of metabolic changes over time, and are easily shipped, making them suitable for remote or resource-limited settings. However, differences between traditional dried sample carriers (Whatman 903®, Perkin Elmer 226®, and Ahlström 222®) have not been investigated using a lipidomics platform. In this study, we aimed to develop an untargeted 4D lipidomic analytical method for DBS samples using ultra-high-performance liquid chromatography (UHPLC), high-resolution mass spectrometry (HRMS) with a trapped ion mobility spectrometry (TIMS) module (Bruker timsTOF Pro). We optimised the extraction method to obtain maximum lipid yield and compare each of the DBS materials. Data was acquired on an optimised TIMS-TOF method to identify lipid species from 20 subclasses including free fatty acids, monoacylglycerides, diacylglycerides, triacylglycerides, cholesteryl esters, lysophosphatidylcholines, lysophosphatidylethanolamines, lysophosphatidylglycerides, lysophosphatidylinositols, lysophosphatidylserines, phosphatidylcholines, phosphatidylethanolamines, phosphatidylglycerides, phosphatidylinositols, phosphatidylserines, ceramides, dihydroceramides, hexosylceramides, lactosylceramides, and sphingomyelins. We further evaluated the method using DBS samples obtained from 20 healthy individuals using different commercially available filter papers commonly used in microsampling literature. In conclusion, our untargeted 4D lipid analysis method using UHPLC-TIMS-TOF is a powerful tool for the comprehensive analysis of lipids in DBS, and may precipitate the potential to facilitate identification of lipid biomarkers for metabolic disorders using DBS.

Keywords: dried-blood-spots (DBS), microsampling, metabolic-phenotyping, metabolomics, lipidomics

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Speaker 07

Isomer resolution of lipid biomarkers in human plasma

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Lipids are a structurally diverse class of biomolecules that play important roles across the span of biological functions. To maintain healthy function, the identity and quantity of lipids in cells and organisms is strictly controlled and thus changes in the lipidome can be associated with human disease, making them potential candidates for biomarkers. Since the emergence of modern lipidomics, numerous promising lipid biomarker panels have been identified, with a panel for cardiovascular disease successfully translated to the clinic. Despite substantial interest in lipid biomarker discovery, most of the lipids present within such panels have only been identified at a sum composition level. Consequently, the molecular structures of many of these putative biomarkers have yet to be determined and furthermore, the associated responses with disease states may reflect variable responses across a number of lipid isomers. Here we deploy isomer-resolved mass spectrometry technologies to describe the molecular structure(s) of putative cancer biomarkers in human plasma.

Changes in the plasma-abundance of the phosphatidylcholine, PC 34:1, have been reported as a part of biomarker panels for prostate cancer. To elucidate the structure(s) of PC 34:1 in plasma, reference plasma was extracted and analysed using direct-infusion nanoelectrospray ionisation on a Thermo Scientific Orbitrap Elite mass spectrometer. This instrument had previously been modified to introduce ozone into the instrument. To resolve lipid regioisomer populations, target lipids were mass-selected and subjected to ozone-induced dissociation (OzID), collision-induced dissociation (CID), and sequential collision-induced dissociation/ozone-induced dissociation (CID/OzID). These scan functions revealed that PC 34:1 extracted from human reference plasma was comprised of two double bond isomers (*i.e.*, PC 34:1*n*-9 and PC 34:1*n*-7) and two *sn*-positional isomers differing in the relative location of fatty acid chains on the glycerol backbone (PC 16:0/18:1 and PC 18:1/16:0). Taken together, PC 34:1 was found to be present as a mixture of at least three (and most probably four) distinct isomers suggesting that the measurements of the resolved isomers would be required to describe the disease state. Given that the preponderance of lipid biomarkers have to-date only been characterised at the sum composition level, results from these preliminary, isomer-resolved investigations provide a clear motivation to map the isomer profile of lipid biomarkers in plasma.

Keywords: lipids, biomarkers, cancer, human plasma, isomers

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Speaker 08

Synergistic Approach to Lipid Structure Elucidation Using Selective Fragmentation Methods

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Alterations to the cellular lipidome are associated with the onset and progression of numerous pathologies, including cancer. Consequently, detailed structural characterisation of complex lipids in cellular extracts is increasingly desirable. Complete lipid structure elucidation requires identification of lipid class, sum composition (*i.e.*, total carbons and double bonds), relative position of acyl chains, and the double bond location(s). Contemporary MS/MS approaches based on collisional activation do not yield structurally conclusive spectra that fully resolve all these features. Here, we demonstrate the utility of implementing multiple emerging ion activation techniques on a common platform for lipid identification. Specifically, ozone-induced dissociation (OzID) and 193 nm ultraviolet photodissociation (UVPD) were deployed on a modified Q-Exactive Orbitrap mass spectrometer for complementary and comprehensive structure elucidation of lipids extracted from DLD1 colorectal adenocarcinoma cancer cell lines.

Mass-selected lipid ions were reacted with ozone or irradiated at 193 nm in the HCD cell of the instrument prior to mass analysis in the Orbitrap. Trapping gas pressure, OzID reaction time, and number of laser shots were optimised to maximise the abundance of diagnostic product ions that facilitate lipid structure elucidation without significantly compromising duty cycle and throughput.

The consistency of results across both ion activation methods increases confidence in the assignment of lipid structures, even in the absence of standards. For example, both techniques indicate that PC 16:0_18:1 from each of the DLD1 cell line extracts is a mixture of at least four isomers. Comparing the relative abundances of isomeric lipids across isogenic parental, wildtype and mutant cell lines, significant remodelling of oleic and vaccenic acid-containing phosphatidylcholines is observed due to the pro-tumorigenic KRAS mutation, but not in an anti-tumorigenic MEK knockout. The results obtained from UVPD and OzID both provide insights into oncogenic cellular lipid synthesis and metabolism that are inaccessible with traditional tandem mass spectrometry methods.

Keywords: Lipids, double-bond, OzID, photodissociation

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Speaker 09

Mass spectrometry-based assays for measuring bound Infliximab in patient sera. Can we improve patient outcomes?

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Therapeutic drug monitoring (TDM) of anti-tumour necrosis factor-alpha drug, infliximab (IFX) is used increasingly in patient management protocols for several autoimmune diseases. Traditionally both drug and anti-drug levels are measured using immunoassays. Measurement of serum drug levels of IFX and anti-infliximab antibodies (ATI) improve patient outcomes over clinical assessment alone. However, the presence of IFX prevents detection of the ATI, and vice versa owing to the formation of complexes between drug (IFX) and ATIs. This produces a gap in information for the clinician in cases of non-response to mAb therapy.

Pathology labs currently cannot readily monitor the early development of ATIs. We are interested in determining the extent that patient outcomes can be improved by quantifying ATIs early. Thus, the development of assays that can quantify ATIs in the presence of IFX are required. Recently, assays for the measurement of IFX drug levels have been developed using LC-MS/MS. Such assays are highly sensitive and specific, providing information on drug levels and the potential development of ADAs. The aims of this study are to develop and validate LC-MS/MS assays for measuring IFX drug levels (both total and free) in patient serum to quantitate bound IFX.

We have developed both total and free IFX assays using LC-MS/MS, which are currently being fully validated. We have also compared free, total IFX, and traditional immunoassays using serum spiked with increasing concentrations of both infliximab and commercial anti-infliximab antibodies. This is proof-in principle that free and total IFX LC-MS/MS assays can detect bound infliximab and can lead to the indirect measurement and possible early detection of anti-infliximab antibodies in the presence of drug. This paves the way for NSW Health Pathology to improve patient outcomes by adjusting treatment with IFX in cases of non-response due to sub-therapeutic dosing or immunogenic reactions.

Keywords: Infliximab (IFX), mAb, anti-infliximab antibodies (ATIs), LC-MS/MS, primary non-response

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Speaker 10

The first accurate measurement of somatostatin concentration in human blood

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The primary structure of the cyclic peptide which inhibits the secretion of growth hormone from the pituitary gland was first reported in 1973 by Roger Guillemin and co-workers, through extraction of the ovine hypothalamus. Later named somatostatin, discovery of this and other hypothalamic hormones resulted in Guillemin being jointly awarded the Nobel Prize for Physiology or Medicine in 1977. Since then, at least 19 studies have been published on the measurement of somatostatin concentration in mammalian blood plasma. Unfortunately, every single reported measurement is wrong, many grossly so, the values ranging from 2.5-360 pg/mL and averaging 60 pg/mL. This current study will detail the measures taken to arrest endogenous somatostatin degradation, selection of a suitable internal standard, relevant technical innovations, and development of LC-MS/MS methods on state-of-the-art instrumentation. Finally, the newly developed analytical method was applied to a pilot study employing ten healthy human subjects. Fasting somatostatin levels were 0.87 ± 0.03 and 0.59 ± 0.11 ng/mL in the male and female cohorts respectively. These concentrations increased to 1.44 ± 0.07 (M) and 1.33 ± 0.18 ng/mL (F) following breakfast.

Keywords: somatostatin, neuropeptide, blood plasma, LC-MS/MS

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Speaker 11

Comprehensive Lipidomics Workflow for Population Phenotyping and Application to Non-Severe Burns

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Dysregulated lipid metabolism is involved in many diseases and injuries, including cardiometabolic diseases and burn trauma. Mass spectrometry-based lipidomics is an important tool for understanding the mechanisms underlying lipid dysfunction and is widely used in epidemiology and clinical studies. With increasing cohort size, single batch acquisition is less feasible, requiring strategies to make analytical methods robust against long-term experimental variations (batch-to-batch). Here, this is achieved by combining stable isotope internal standard dilution, automated sample preparation, and reversed phase ultra-high performance liquid chromatography-mass spectrometry (UHPLC-MS) to quantify 1163 lipid species spanning 20 subclasses in plasma/serum.

The resultant method has been tested for batch-to-batch and inter-instrument variations across 16 independent batches (6142 total injections) and two identical LC-MS systems. This approach was found robust to common sources of analytical variation during sample handling and preparation, including blood tube collection type, multiple freeze-thaw cycles, storage stability at -80°C and analyte extraction technique, except for sample haemolysis where lipid concentrations were impacted by lysis ≥0.4%. A total of 820 lipids were reported with a relative standard deviation of <30% in 1048 plasma quality control samples. This protocol is suitable for large-scale, multi-batch applications in precision medicine and for intra- and inter-lab parallelisation.

To demonstrate clinical applicability, lipid profiles were investigated between non-severe burns (total body surface area burnt <15%) and non-burn controls. Multivariate modelling and univariate analysis found decreases in anti-inflammatory phosphatidylcholines, phosphatidylglycerides, phosphatidylinositols and phosphatidylserines, and increases in monoacylglycerides (MAGs), particularly pro-atherogenic MAG(20:4), that persisted up to 6 weeks post-injury. This lipid signature could potentially be indicative of an increased risk of cardiovascular disease and long-term inflammatory complications after non-severe burns. The validation of these findings by analysing and appending new cohorts to our existing data will provide insights into the mechanisms underlying systemic diseases and actionable clinical knowledge to improve patient recoveries.

Keywords: Lipidomics, Lipid profiling, Liquid chromatography-mass spectrometry, Non-severe burns, Cardiovascular disease risk

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Speaker 12

Interpretable machine learning on metabolomics data reveals biomarkers for Parkinson's disease

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The use of machine learning (ML) with metabolomics provides opportunities for the early diagnosis of disease. However, the accuracy and extent of information obtained from ML and metabolomics can be limited owing to challenges associated with interpreting disease prediction models and analysing many chemical features with abundances that are correlated and 'noisy'. Here, we report an interpretable and computationally efficient neural network-based framework for analysing datasets generated by untargeted mass spectrometry-based methods (Figure 1) entitled, 'CRANK-MS' (Classification and Ranking Analysis using Neural network generates Knowledge from Mass Spectrometry). CRANK-MS has several built-in features including: (i) integrated model parameters that allow the high dimensionality of metabolomics datasets to be analysed without the need for pre-selecting chemical features; (ii) SHAP to retrospectively 'mine' key chemical features that contribute the most to an accurate model prediction; and (iii) benchmark testing with five well known ML methods to compare diagnostic performance and further verify significant chemical features. Using CRANK-MS, we report the highest diagnostic performance to date for binary classification of PD *vs* healthy control with a mean area under the curve of > 0.995. Additionally, NN-driven predictions trained on a prognostic PD study were used to reveal new PD-specific chemical features which were not previously identified and can be considered indicative of pre-PD diagnosis. The program for implementing this approach is freely available online at https://github.com/CRANK-MS. The use of CRANK-MS should enhance the accuracy of disease prediction models based on metabolomics and many other types of '-omics' experiments, and facilitate biomarker discovery.

Keywords: metabolomics, machine learning, neural networks, Parkinson's disease, Shapley Additive exPlanations (SHAP), PFAS

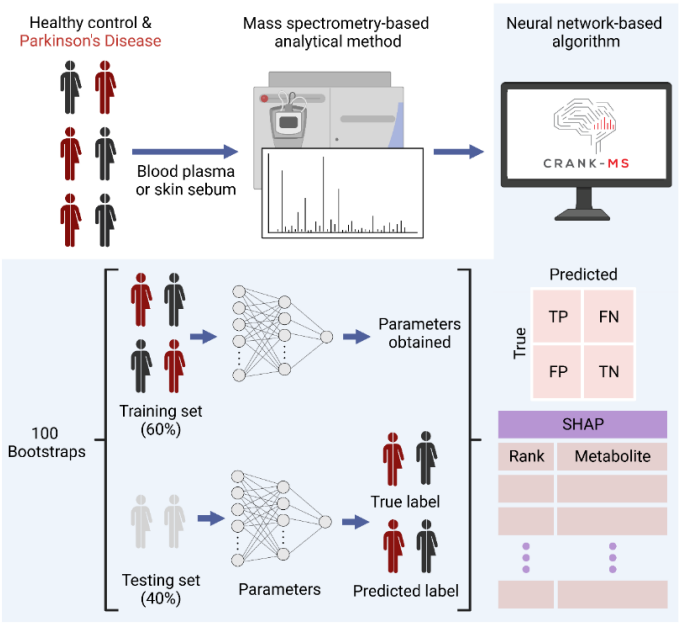

Figure 1. Neural network (NN) framework for predicting Parkinson's disease using large mass spectrometry-based metabolomics data. Whole metabolomics datasets without feature selection can be analysed directly by NN for the binary classification of Parkinson's disease. Using a 100-iteration bootstrap model, 60% of the data was randomly distributed for training and 40% for testing. Diagnostic performance for each bootstrap was calculated based on the absolute values obtained for true positive (TP), false negative (FN), false positive (FP), and true negative (TN). SHapely Additive exPlanations (SHAP) analysis of NN was used to rank chemical features based on the extent of their contribution to a positive Parkinson's disease prediction.

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Speaker 13

Spectral deconvolution of All Ions Fragmentation data for improved structural elucidation of low-abundance compounds in the plasma metabolome.

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Liquid chromatography with mass spectrometry provides the best coverage, sensitivity and dynamic range for acquisition of metabolomics data. Recent developments in Data Independent Acquisition [DIA] techniques, such as Sequential Window Acquisition of All Theoretical Mass Spectra [SWATH], have advanced coverage of complex parts of the metabolome, such as phospholipids. However, even new, fast-scanning instruments may struggle to capture sufficient windows to avoid chimaeric spectra resulting from multiple adducts or isomers falling within the SWATH window. Particularly when combined with the widest m/z ranges. Analysis of human metabolites of dietary-derived compounds is an example of a particularly challenging application of metabolomics. The complexity of the plasma metabolome, the diversity of metabolites synthesised, and their low typical concentration in peripheral plasma necessitates maximum sensitivity and wide coverage. In order to identify such metabolites in plasma and urine samples from a dietary intervention with tea made from kawakawa leaves (Piper excelsum) we applied an old concept from GC-MS data processing- spectral deconvolution- to extract fragmentation spectra from DIA data acquired using All Ions Fragmentation. While this technique provides the same coverage of the metabolome as other DIA techniques the spectra compare well to those acquired using Data Dependent Analysis. These spectra produced more library hits and better match factor scores than spectra extracted using correlation-based spectral deconvolution in MS-DIAL. When used for in-silico structural elucidation they yielded more putative structures. This technique has higher data processing overheads than SWATH but demands shorter cycle times from the instrument, which should equate to more sensitive and wider coverage of the metabolome as well as better quantification of the narrow peaks modern LC instruments can produce.

Keywords: Metabolomics, structural elucidation, chemoinformatics,

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Speaker 14

Investigation of small-molecule drug uptake in pancreatic ductal adenocarcinoma tumours via MALDI-MSI

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There is a strong clinical need for novel therapeutic options for pancreatic ductal adenocarcinoma (PDAC), which has among the worst prognosis of all solid malignancies and is characterised by high metastatic burden. Increased expression of urokinase plasminogen activator (uPA), a cell-surface expressed mediator of extracellular matrix degradation and tumour cell invasion, is significantly associated with poorer survival and worse clinicopathological features in patients with locally advanced PDAC.

We have developed a novel, non-cytotoxic 5,6-disubstituted amiloride analogue, BB2-30F, that potently inhibits human uPA activity and completely inhibits metastasis in an orthotopic xenograft model of PDAC. Here, we aimed to confirm extent of tumour uptake of small-molecule uPA inhibitor, BB2-30F, and visualise its spatial distribution in tumour tissue for the first time, using matrix-assisted laser/desorption ionisation mass spectrometry imaging (MALDI-MSI).

Tumours were embedded in 2% carboxymethylcellulose, prior to obtaining 15-µm tissue sections for analysis. Sections were coated with 2,5-Dihydroxybenzoic acid matrix using a TM-Sprayer (HTX Technologies, USA) and analysed on an Orbitrap Elite mass spectrometer coupled to an intermediate pressure MALDI-ion source, in positive-ion mode. Preliminary MALDI-MSI results show a signal consistent with the protonated drug analyte $[(BB2\text{-}30F)+H]^+$ *m/z* 386.2042 in treated tissue, which is absent in control tissue. Furthermore, the drug signal intensity is heterogenous in histological compartments of tissue sections, which potentially correlates with uPA rich areas of tumour tissue, where parallel detection of lipids would provide further insight into heterogeneity in the tumour microenvironment. The MALDI image will be spatially compared to adjacent uPA stained tumour sections to correlate uPA localisation with tissue drug disposition.

In summary, our novel mass spectrometry-based experimental approach demonstrates that BB2-30F successfully reaches and penetrates PDAC primary tumour, validating the efficacy of uPA targeting approaches as a therapeutic opportunity to limit metastatic potential in PDAC.

Keywords: Pancreatic cancer, drug delivery, MALDI-MSI, urokinase plasminogen activator, metastasis

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Speaker 15

Plasma Post-Ionisation combined with Atmospheric Pressure Matrix-Assisted Laser Desorption/Ionisation on a timsTOF Pro for the analysis of Lipid Spatial Distributions and Turnover in APOE-knockout Mouse Brains

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While MALDI has been widely investigated for its use in mass spectrometry imaging (MSI) for examining the distribution of hundreds of metabolites and lipids simultaneously across the spatial dimension, alternative source arrangements and ionisation technologies offer unique advantages such as enhancing detection of different lipid classes or increasing ion yields. Dielectric barrier discharge (DBD) devices can be coupled to the sampling capillary of a mass spectrometer, enabling efficient interaction of ablated neutrals with ions formed in the cold plasma to allow for post-ionisation. Here we present the development of a timsTOF Pro mass spectrometer equipped with an atmospheric pressure (AP)-MALDI and plasma-post-ionisation (PPI) source. We find that AP-MALDI-PPI is found to yield similar benefits to alternative post-ionisation technologies, with the signals of many phospholipids, sterols and sphingolipids increasing by several orders of magnitude over conventional MALDI, which we exploit for lipid imaging of tissues at spatial resolution at low as 10 µm. This presents the first coupling of PPI with ion mobility, providing additional chemical information to assist in identification and allowing in many cases at least 8 isobaric and isomeric lipid-related features to be resolved within a 0.2 Da mass window. This unique system was deployed to study region-specific lipid turnover in both wild-type and APOE-knockout mouse brains, with a focus on myelin-localised hexosylceramides, shedding light on the link between APOE function and dementia susceptibility. The use of ion mobility is shown to assist in deconvoluting complex spectra containing numerous deuterated lipid peaks produced by D2O feeding, assisting in determination of turnover rates by change in deuteration level for individual species over multiple timepoints. This work demonstrates the power of plasma post-ionisation for spatial lipidomics, including for lipid classes that are poorly ionised by conventional MALDI.

Keywords: Lipidomics, MALDI, MSI, Post-ionisation

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Speaker 16

Discovery of n-10 monounsaturated fatty acids in pancreatic ductal adenocarcinoma by ozone-induced dissociation: a new target for improving chemotherapy efficacy?

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Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive disease with a survival rate of less than 10% at 5 years, a figure that has not improved in more than 25 years. This poor prognosis is due to late diagnosis, its asymptomatic and heterogeneous nature, and resistance to common chemotherapies like gemcitabine. New treatments are desperately needed to improve PDAC patient survival time.

In this work we have generated gemcitabine-resistant (GEMR) PDAC cell lines and discovered upregulation of *de novo* lipogenesis in GEMR cells through immunoblotting for total and activated lipid synthesis enzymes and by tracing radiolabelled glucose incorporation into lipid. Untargeted lipidomics revealed increased amounts of lipids containing saturated and monounsaturated fatty acids in GEMR cells.

Alternative desaturation pathways have recently been discovered in other cancer types but untargeted lipidomics using tandem mass spectrometry cannot determine which specific fatty acid isomers are present in PDAC. An example of such a pathway is that catalysed by fatty acid desaturate 2 (FADS2), which normally desaturates polyunsaturated fatty acids in the Δ6 position but has also been shown to produce 16:1n-10 and 18:1n-10 in several other cancer types. Here we report FADS2Δ6 activity in PDAC for the first time in PDAC following the detection of 16:1n-10 & 18:1 n-10 isomers by ozone-induced dissociation. FADS2 expression is also upregulated in GEMR PDAC making it a novel target for improving gemcitabine treatment. Knockdown of key enzymes in lipid synthesis such as acetyl-CoA carboxylase, fatty acid synthase (FAS), stearoyl-CoA desaturase 1 (SCD1) and FADS2 reduce the growth of PDAC cells when combined with gemcitabine treatment above that of gemcitabine monotherapy, with FAS or combination SCD1/FADS2 knockdown producing the largest decreases in cell growth. These data demonstrate that targeting lipid metabolism can sensitive PDAC cells to gemcitabine treatment paving the way for new molecular therapies for PDAC.

Keywords: pancreatic cancer, lipidomics, ozone-induced dissociation, lipid synthesis, gemcitabine

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Speaker 17

Uncovering non-canonical unsaturated fatty acids in the brain

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By weight, the human brain is made up of 50% lipid. Despite its major contribution to the mass of the brain, at the molecular level, the brain lipidome remains incompletely described and the functions of many of the species identified to-date are unknown. Recently for example, non-canonical unsaturated fatty acids –with carbon-carbon double bonds in positions not predicted by canonical lipid metabolism– were identified in mouse brain. The extent to which these unusual lipids contribute to neurochemical processes presents a significant gap in knowledge that is challenging to close due to technological impediments to the detection and tracing of such non-canonical fatty acids. Here we deploy state-of-the-art mass spectrometric methods that enable the detection and relative quantification of non-canonical fatty acids in both *in vitro* and *in vivo* brain models.

Free fatty acids (FFA) were extracted from PC-12 cell lines –which model neurosecretory cells –that had been stimulated with high potassium. FFA stable-isotope tagging (FFAST) was employed to differentially label FFAs from stimulated and unstimulated PC-12 cells. Thus labelled, fatty acids were separated using reversed-phase liquid chromatography (RPLC) with explicit sites of unsaturation assigned using ozone-induced dissociation (OzID) mass spectrometry and changes in relative abundance of canonical and non-canonical unsaturated FFAs were assessed. In parallel, both desorption electrospray ionisation and RPLC were deployed, in combination with OzID, to search for complex lipids carrying non-canonical fatty acids within rat brain sections and extracts, respectively.

Application of the FFAST-OzID method revealed the presence of abundant populations of the non-canonical FA 16:1*n*-10 (sapienic acid) and FA 18:1*n*-10 (dihomosapienic acid) in extracts from PC-12 cell lines. Neither of these fatty acids have previously been reported in neurons or neurosecretory-like cells, with both demonstrated to increase between 6-10-fold in relative abundance upon active stimulation. Surveying the complex lipids in rat brain revealed the presence of double-bond positional isomers. For example, phosphatidylcholine lipids of composition PC 34:1 were identified to be present with both *n*-7 and *n*-9 isomers with each expressed in differing abundance across different sections of the brain.

Keywords: Lipidomics, LC-MS, MS-Imaging, fatty acid isomers, stable isotope tagging

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Speaker 18

Establishment of Reliable and Convenient GC-MS Analysis of Human Faecal Short Chain Fatty Acid (SCFA)

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Short chain fatty acids (SCFA) are generated via fermentation of indigestible carbohydrates by endogenous microbiota within the gastrointestinal tract. The large intestine harbours the largest microbial community in the human body and is its main source of SCFA. As well as important metabolic precursors, research has highlighted the influence of SCFA on multiple physiological pathways. The concentration of SCFA in the colon is influenced by various factors, in particular microbiota composition. Consequently, SCFA have become important targets in research that examine the association of gut microbiota to health and disease. Analysis of SCFA in faecal samples represent a convenient measure of microbiome metabolism and colon SCFA generation. To satisfy requests from multiple researchers, a robust and accurate method was developed, that enabled faecal samples to be conveniently prepared in each researcher's laboratory before transport to BMSF for GC-MS analysis.
Full analytical validation demonstrated strong accuracy, precision and reproducibility. BMSF trained each researcher and supplied heavy isotopic internal standards and calibrations to maintain quality control. Initially, a small aliquot of supernatant from a 10% faecal homogenate was derivatised (aqueous) with pentafluorobenzylbromide (PFB-Br) to generate more stable SCFA – PFB esters. After hexane extraction, SCFA and other organic acid metabolite PFB derivatives were monitored by single quadrupole GC-MS using selective ion monitoring and negative chemical ionization.
Over 400 human faecal samples collected at MRC, as a part of the Australia IBD Microbiome Study have been analysed to date. Preliminary data analysis of 90 healthy individuals (18-80 years) confirms that acetate, propionate and butyrate are the most abundant SCFA in the colon and that there is considerable inter-individual variation in all SCFA. Faecal samples collected from each volunteer at baseline, six and twelve months demonstrated that SCFA composition was stable over time amongst healthy individuals, with non-significant time shifts.

Keywords: GC-MS, SCFA, Microbiome, Faecal, Organic acid

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Speaker 19

Lipid mass spectrometry in a nutshell: deep profiling of fatty acids in edible nuts

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The unsaturated fatty acids contained in edible nuts, including *w*-3 and *w*-6 fatty acids, have been studied extensively over past decades and a plethora of studies report associations to their health-promoting characteristics. Beyond nutritional investigations, there is a growing mechanistic understanding of the differing role(s) of unsaturated fatty acids in cellular biology and the distinct biochemical and biophysical effects of fatty acids differing only in the position(s) of carbon-carbon bonds. In order to better link nutritional outcomes with biochemical composition we have deployed recently-introduced isomer-resolved mass spectrometry methods to enable a deeper profile of fatty acids in a wide distribution of edible nuts.

Nine commercially available edible nut varieties were cryo-milled and subjected to lipid extraction. Two orthogonal analysis strategies were deployed for fatty acid identification. In the first, (i) extracts were hydrolysed and derivatized to methyl esters before being subjected to gas chromatography covalent-adduct chemical ionisation tandem mass spectrometry (GC-CACI-MS/MS); while in the second (ii) hydrolysed extracts were derivatized with the fixed charge aminomethylphenyl pyridinium moiety before being subjects to liquid chromatography ozone-induced dissociation mass spectrometry (LC-OzID-MS/MS). Results revealed both assays to generate detailed qualitative descriptions of fatty acids present at trace and ultra-trace concentrations, while notably LC-OzID-MS/MS demonstrated a wider dynamic range. Taken together, sixty-six distinct fatty acids were identified across all nut varieties. The most diverse fatty acid population was found in pine nuts with 38 distinct structures whilst the least diverse were Brazil and cashew nuts with 30 each. Importantly, the ability to resolve isomeric fatty acid isomers empowered the discovery of five novel fatty acids including, FA 18:3*n*-3,5,8 (in walnuts), FA 18:3*n*-3,5,9 (in cashews, pecans, pistachios, and walnuts), FA 18:3*n*-6,8,13, FA 18:3*n*-7,9,13, and FA 20:3*n*-6,8,15 (in pine nuts). These discoveries of hitherto undescribed unsaturated fatty acids in these, previously well-studied nut varieties, implies that the lipid metabolism of the parent plants that has not yet been fully described and, moreover, these distinctive species may serve as markers for the consumption and metabolism of nuts from dietary sources.

Keywords: nuts, fatty acids, isomers, chromatography, mass spectrometry

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Speaker 20

Large Scale Monitoring of Illicit Drug Use via Mass Spectrometry Based Trace Residue Analysis of Discarded Drug Packaging and Paraphernalia

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Due to their unknown origin, composition and lack of regulation, the use of illicit drug substances present significant risks of harm. These risks may be exacerbated when a drug sample has an unknown quantity, is adulterated or substituted with other pharmacologically active compounds, or when present in poly-drug mixtures. Here, I will describe a strategy involving the use of Direct Analysis in Real Time (DART) coupled with mass spectrometry (MS), tandem mass spectrometry (MS/MS) (including UV-photodissociation MS/MS), and automated database analysis software, for rapid and large-scale trace residue identification, characterization and semi-quantitative analysis of a diverse range of illicit drugs and adulterant substances found in Discarded Drug Packaging Samples (DPS) (e.g., plastic reusable ziplock bags) and Discarded Drug Paraphernalia (DDP) (e.g., used syringes, plastic spoons and metal trays). Results from two large-scale pilot studies will be presented, involving the analysis of (i) >1300 DPS collected at large public events e.g., music / dance festivals, including analysis performed on-site and in close to real time using a 'transportable' triple quadrupole MS instrument housed within a custom modified mobile analytical laboratory [West, et al. (2021) *Am. Soc. Mass Spectrom.* 32, 2604-2614.] and (ii) >10,700 DDP sourced from service providers throughput Melbourne during and after Covid-19 lockdowns, from which 28 different substances in 231 different poly-drug combinations and containing up to 9 different substances were observed, including a novel synthetic opioid drug, β-U10 that was identified for the first time anywhere in the world while Covid-19 lockdowns were in place [West et al. (2022) *Drug Testing and Analysis. 14*, 1576-1586.]. Finally, I will show that the number and weekly 'Average Signal Intensity' (ASI) values of identified substances can be used as proxy indicator for semi-quantitative monitoring of dynamic changes in the availability, relative purity and compositions of local illicit drug markets.

Keywords: drug monitoring, trace-residue analysis, ambient ionization, DART-MS, high-throughput

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Speaker 21

Search and recovery of human remains in mass disasters

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Globally over the past two decades, there has been a significant increase in incidences of natural disasters (e.g. earthquakes, tsunamis) and heightened threats of man-made disasters (e.g. explosions, air crashes, suicide bombings) resulting from terrorism. Locating and processing victims from mass disasters presents a major challenge to search and recovery responders as it is still unknown how remains will degrade and decompose in these unique environments. The aim of this project was to simulate disaster events, to investigate the process of human decomposition and develop methods for the successful location and recovery of victim remains.

Simulated mass disasters using donated human remains were conducted at the Australian Facility for Taphonomic Experimental Research (AFTER). The donors were left to decompose for a predetermined time, before a recovery simulation by trained personnel was run. Tissue samples were collected on the day of placement and again on the day of recovery and analysed via gas chromatography tandem mass spectrometry (GC-MS/MS). The odour profile emitted from the mass disaster area was collected over the duration of the disaster and analysed using comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC-TOFMS).

The information obtained from this study will provide the baseline for the development of protocols for best practice which will be crucial for disaster recovery situations.

Keywords: Mass disasters, GC-MS/MS, GC×GC-TOFMS, taphonomy

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Speaker 22

Data-Dependent Analysis of PFAS using Mass Defect and Real-Time Library Searching

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PFAS comprise a category of synthetic chemicals characterised by a carbon backbone with near or complete saturation with fluorine. Valued for their hydrophobic and oleophobic traits, PFAS have been used for decades in non-stick cookware, waterproof clothing, and fire-fighting foams. These compounds resist degradation and readily bioaccumulate in plants and animals and have emerged as a prolific environmental contaminant. Characterising these chemicals can be difficult due to the thousands of variations of the synthesized chemicals, isomeric structures, and the limited availability of reference standards. In our work we guided data dependent analysis of PFAS to optimize data acquisition. A filter was used to limit data dependent acquisitions to compounds with a negative mass defect such as PFAS compounds. In addition, the Real-Time Library Search feature on the Thermo Scientific™ Orbitrap™ IQ-X™ Tribrid™ mass spectrometer was used to target PFAS compounds for additional data analysis. Results were processed using a new workflow in Compound Discoverer that was designed to simplify characterisation of PFAS compounds.

Keywords: HRAM, PFAS, Orbitrap, Environmental

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Speaker 23

Metal organic frameworks for improving the analysis of perfluoroalkyl substances by mass spectrometry

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Per and polyfluorinated alkyl substances (PFAS) are a group of molecules that resist heat, oil, stains and water. PFAS are exceptionally effective at fighting liquid fuel fires, leading to its use in aqueous fire-fighting foams and subsequent groundwater contamination near airports and military bases where fire-extinguishing training often occurs. This is of concern as PFAS are persistent organic pollutants, characterized by the many strong C-F bonds throughout their structures that do not readily breakdown in the environment. Owing to the continual development of PFASs for many different industrial applications since at least the 1950s, thousands of distinct PFAS compounds have been detected in the environment, presenting a major analytical challenge for characterising and monitoring PFAS pollution. In current commercial analytical labs, the estimated lowest detection limit is ~1 ppt, which is much higher than the 2022 lifetime drinking water health advisory levels set by the US EPA at 0.004 ppt for PFOA and 0.02 ppt for PFOS. As a result, there is a strong need to lower detection limits of PFAS to accurately assess environmental PFAS pollution.

To improve PFAS analysis at lower concentrations, metal organic frameworks (MOFs) are highly promising for the pre-concentration of PFAS prior to detection by mass spectrometry. MOFs are permanently porous materials that have been used for gas separation, sensing, and catalysis. Although MOFs such as MIL-101, UiO-66, and ZIF-8 have been explored for adsorbing PFAS, such studies only investigated a limited number of PFAS often at high concentrations that are not environmentally relevant. In addition, such studies involved a wide range of different testing protocols which makes it challenging to identify the most promising MOFs for PFAS preconcentration.

In this project, we have developed a standardised LC-MS/MS protocol for 33 different PFAS to allow the direct comparison of the most promising MOFs to be used for the adsorption of PFAS from water samples. We have focused our studies on six MOFs: ZIF-8, ZIF-8A-61%, ZIF-8A-SO_3H, UiO-66, UiO-66-NH_2 and UiO-67 to determine the effects of functional groups (methyl, amine and sulfonic acids) and/or pore sizes on the adsorption of different types and sizes of PFAS. By testing across different PFAS functional groups, anionic and neutral species, a better understanding of how MOFs can be used for PFAS adsorption will be achieved. This will guide the design of materials that are strongly suited to absorbing PFAS from water to further lower the detection limit of PFAS in aqueous samples.

Keywords: Perfluroalkyl substances, Metal organic frameworks, Solid phase extraction, Liquid-chromatography tandem mass spectrometry (LC-MS/MS)

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Speaker 24

Wild-Caught Freshwater Turtles Exposed to Elevated PFAS: Omics-Based Ecosurveillance Reveals Atypical Scute Formation and Biological Impact.

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PFAS (Per- and Polyfluoroalkyl substances) are synthetic contaminants that are persistent and pervasive. Exposure to PFAS can cause toxic effects in wildlife, but little is known about how it affects egg-laying species. To address this gap and provide better ecosystem health metrics, we collected 26 freshwater turtles from three different waterways, each with different levels of PFAS contamination. The turtles were tested for PFAS and analysed using omics-based approaches, which assess chemical contamination impacts through proteomics, lipidomics, and metabolomics. We revealed that the turtles taken from the PFAS-impacted site had serum PFAS concentrations ten times greater than those taken from the control site, while those from the reference site had no measurable PFAS. Additionally, the distribution of PFAS within tissues showed a high bioaccumulative effect of perfluorooctanesulfonic acid (PFOS) in the liver and ovary tissues compared to serum values. Specific biochemical profiles of serum, tissues, eggs, and hatchlings showed that the impacted turtles exposed to elevated PFAS had an enhanced purine metabolism, glycerophosphocholines, and an elevated innate immune response, suggesting inflammation, metabolic preservation, and rerouting of central carbon metabolites. Lipid transport and binding activities were negatively correlated. Purine metabolism metabolites were significantly elevated in the PFAS-impacted eggs, while yolks were depleted in lipids tied to growth and development. This resulted in emerged hatchlings at both the impact (17 out of 44 hatchlings) and control sites (23 out of 71 hatchlings) showing atypical intergular scales, while only 2 out of 34 hatchlings from the reference site demonstrated this feature. This study highlights the impacts of PFAS exposure on wildlife and provides data-driven ecosystem management for informed decision-making for regulators.

Keywords: Metabolomics, lipidomics, proteomics, wildlife, ecosurveillance

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Speaker 25

What's in a Vape? Chemical Analysis of Electronic Cigarettes by GC-MS

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Disposable electronic cigarettes (e-cigarettes or vapes) have rapidly increased in popularity in Australia, particularly amongst the younger population. Despite the regulations restricting the sale of nicotine-containing e-cigarettes, these devices are still available illegally from retailers. The liquid contained in these devices (e-liquid) generally contains the carrier fluids propylene glycol and vegetable glycerine, flavouring molecules, synthetic cooling molecules and nicotine. Reactions within the e-liquid have been shown to occur between certain flavouring molecules and the carrier fluids to form adducts in situ. This study, which has analysed over 1000 devices including: e-cigarettes donated by Australian users; devices illegally sold and seized from retailers; devices confiscated from students in schools; and those purchased online or from retailers within Australia; aims to investigate the chemical content of these products. Identification and quantification of their chemical contents was completed by gas chromatography-mass spectrometry for the targeted quantification of 34 common flavouring chemicals, nicotine, synthetic coolants, adducts formed in situ (compared against laboratory synthesised standards) and chemicals prohibited by the current Therapeutic Goods Administration Regulation for Nicotine containing products (TGO110). Other non-targeted compounds were tentatively identified by comparison to compound spectral libraries against the National Institute of Standards and Technology (NIST17). The data presented will provide insights into trends and prevalence of nicotine-containing e-cigarette products in Australia and chemical content of these products in comparison to the enforced regulation (TGO110, October 2021). These findings will have significant implications for the future of Australian e-cigarette legislation and the potential risk evaluation of e-cigarettes.

Keywords: Gas Chromatography-Mass Spectrometry, Electronic Cigarettes, Nicotine, Synthetic Coolants, Flavours.

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Speaker 26

Untargeted metabolomic analysis with direct electrospray ionisation mass spectrometry for discrimination of *Botrytis cinerea* infection in grapes

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Infection of grapes (*Vitis vinifera*) by *Botrytis cinerea* (grey mould) occurs frequently in vineyards exposed to wet and humid conditions and leads to detrimental impacts on yield and quality. Growth of *B. cinerea* in grapes causes the oxidisation of phenolic compounds resulting in loss of colour and formation of a suite of off-flavours and odours in wine made from infected fruit.

In this study, metabolites were extracted from sample homogenates using acetonitrile. Our data set comprised a total of 140 healthy and infected bunches of grapes representing different vintages, cultivars, regions, and maturity stages. Sample extracts were randomly analysed by direct injection into a Q trap mass spectrometer, including regular quality assurance samples, with data collected from 50-2000 *m/z* for 1 min. Molecular feature abundances were summed from 0.1 to 0.4 min and normalised prior to PCA for quality assurance. Samples were randomly assigned to a calibration and independent test data set, with feature reduction, a two-class model PLS-DA, cross-validation and permutation testing performed with the calibration data set. Prediction of sample class in the independent test samples demonstrated an overall predictive error of less than 5%. Feature importance was assessed using a combined VIP and selectivity ratio plot which demonstrated a high level of correlation with standard volcano plots. Annotation of important molecular features was undertaken using a high-resolution Orbitrap MS detector, and LC/MS -QToF of selected samples from healthy and infected extracts. The rapid sample preparation, analysis and data workflow presented could be applied for analytical demands requiring discrimination of complex samples including environmental or biological specimens.

Keywords: Rapid analysis, non-targeted metabolomics, high-resolution mass spectrometry, fruit quality

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Speaker 27

Optimisation and investigation of different options for HILIC-MS/MS based metabolomics

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Comprehensive untargeted LC-MS/MS analysis of metabolites is complicated, with many metabolomic studies choosing to apply only one LC-MS/MS method on a non-fractionated sample to be time efficient; however, this potentially results in fewer identifiable metabolites. There are many variables involved in the analysis of metabolites including, different types of liquid chromatography, the pH conditions and ionisation polarity. Many choose to run the same chromatographic conditions for positive and negative mode, with most employing polarity switching, despite chromatographic conditions typically favouring only one of the ionisation modes. This wide array of choices is also extended to mass spectrometry conditions, with many metabolomic methods opting to leave the source parameters at their default settings. Regardless of the choice, some sacrifice has to be made for a realistic analysis. By filtering out combinations which yield limited results, feasible comprehensive LC-MS/MS methods can be established.

The aim of this research was to optimise a range of mass spectrometry source and chromatography conditions to enhance polar metabolite signal. Following optimisation, a comparison of multiple metabolomic methods determined whether a single method or combination of methods would yield the greatest coverage of identifiable metabolites. In this study, polar metabolites from neuroblastoma cells (SH-SY5Y) were extracted and subjected to analysis from several different methods. Methods for individual ion polarities, polarity switching, and the unique application of solvent switching were examined under acidic, basic and neutral pH conditions using hydrophilic interaction liquid chromatography (HILIC). Results showed that while different metabolites mostly prefer different source parameters, some parameters see a universal increase in signal over multiple chemical classes. The comparison of multiple methods yielded different pros and cons. Additional factors, such as cost, time and data curation also impact the determination of what could be deemed the preferred method for the analyst.

Keywords: Metabolomics, Method Development, Method Comparison, HILIC-MS/MS,

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Speaker 28

Investigation into the metabolomic effects of the non-protein amino acid azetidine-2-carboxylic acid (AZE)

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Azetidine-2-carboxylic acid (AZE) is a non-protein amino acid (NPAA) homologue of the protein amino acid proline. AZE is produced by several plant families including some cultivars of the *Beta vulgaris* species which includes the sugar beet and garden beet. Toxic and teratogenic effects of AZE have been demonstrated in a broad range of organisms and it has been shown to readily misincorporate into proteins in place of proline. AZE could therefore negatively impact human health due to its ability to generate non-native and potentially immunogenic proteins.

Human exposure to AZE could occur through the food chain since sugar beet is fed to livestock that provide meat and dairy products. It has been hypothesised that the incorporation of AZE into proline-rich proteins such as myelin basic protein (MBP) may play a role in the pathogenesis of multiple sclerosis (MS). There has been limited research however on the broader toxicity of AZE to humans, however recent studies have shown that other NPAAs impact important metabolic pathways in the cell, suggesting that toxicity may not be solely attributed to their misincorporation into proteins.

This aim of this research was to examine the effects of AZE treatment on the polar metabolome of a human neuroblastoma cell line (SH-SY5Y). Polar metabolites were extracted from AZE-treated cells and analysed by hydrophilic interaction liquid chromatography tandem mass spectrometry (HILIC-MS/MS). Mass spectrometry data was analysed with MS-Dial for peak identification and pathway analysis was performed using MetaboAnalyst 5.0. AZE was determined to impact several metabolic pathways important in correct neurological function including glutathione, arginine, and proline metabolism, that may suggest possible alternative mechanisms of toxicity. These results suggest that metabolomic response to AZE may also contribute to its toxicity which may have further implications in how AZE contributes to disease pathogenesis such as in MS.

Keywords: Metabolomics, Azetidine-2-carboxylic acid, AZE, Neurotoxin, Non-protein amino acid

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Speaker 29

Direct injection ion mobility-mass spectrometry for structural analysis and identity confirmation of anthocyanins in *Brassica oleracea* extracts

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Anthocyanins are ubiquitous flavonoids that impart red, blue, and purple hues to plant tissues. Possessing diverse bioactive effects including anticancer, antioxidant and neuroprotective properties, anthocyanins are typically utilised commercially as natural food colorants and in nutraceutical formulations. Red cabbage (*Brassica oleracea*) contains anthocyanins with various glycosylation and acylation patterns which are challenging to structurally interrogate in high throughput approaches. Here we show that direct injection ion mobility-mass spectrometry can provide wholistic structural information on anthocyanins in red cabbage extract with a data acquisition time of less than 2 minutes. Post-IM drift time-aligned fragmentation generates MS/MS data for observable anthocyanins, with conserved fragments providing rapid identifiers of these compounds. Separation of anthocyanins and their isobars into discrete drift time regions based on their number of structural modifications enables structural prediction of unknown anthocyanins. Finally, CCS and *m/z* values for red cabbage anthocyanins were used to perform rapid identity confirmation of anthocyanins in other *Brassica oleracea* extracts, with nine individual compounds identified. Developing high throughput approaches to characterise the polyphenolic component of plant extracts will prove increasingly important in agriculture, metabolomics, and drug discovery.

Keywords: ion mobility, *Brassica oleracea*, anthocyanin, identity confirmation, structural analysis

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Speaker 30

In-depth characterisation of *Naja nivea* venom using a multifaceted mass spectrometry approach

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Snakes possess extremely complex venoms consisting of a mixture of bioactive proteins and peptides. This complex mixture has evolved to allow snakes to efficiently immobilise, kill, and digest prey, and has been of interest to scientists for decades due to both the potential pharmacological significance and the envenomation of humans. In 2017, the World Health Organisation added snakebite envenomation to their list of neglected tropical diseases, highlighting the importance of research in this area. While antivenom treatment is readily available in developed countries, individuals in developing countries do not experience this luxury. Snakebite envenomation is responsible for more deaths per annum than any other neglected tropical disease due to the expense and instability of current antivenoms.

Our work utilised bottom-up proteomics and complementary transcriptomics to characterise the proteome of Naja nivea, a snake from South Africa classed as medically important due to the lethality of its venom and its tendency to enter human settlement. We have found that the venom proteome of Naja nivea contains proteins belonging to eight toxin families, the most abundant being three-finger toxins. In addition to this fundamental proteomic characterisation, we have investigated the presence of quaternary structures in Naja nivea venom using complementary native and denatured mass spectrometry experiments. We have reported the first evidence of both covalent and non-covalent quaternary structures in Naja nivea venom. This work will provide a starting point for new antivenom development, specifically targeting quaternary structures within venom that have been shown to exhibit greater toxicity than their tertiary counterparts.

Keywords: proteomics, venom, proteins

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Speaker 31

Identification of bioactive natural products using multiplexed, multistage native mass spectrometry

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Natural products are a rich source of bioactive molecules for drug discovery. However, their identification from complex crude extracts is challenging due to limitations in isolation and screening. The Donald group at UNSW has recently developed a method for directly identifying small-molecule binders of druggable proteins from crude natural product extracts using native mass spectrometry (MS).

In this approach, a target protein is incubated with a crude natural product extract containing thousands of small molecules. Unbound small molecules are removed from the mixture by gel filtration and salt adduction to protein ion complexes is minimized using nanoscale ion emitters. Ligands can then be identified by comparing the ligand mass to separate metabolomics data of the natural product extract or by multistage ion activation experiments. This workflow represents a major increase in the number of compounds simultaneously screened using native MS from less than 300 to ~9,000.

To address some key limitations in this workflow in terms of cost of consumables, sample consumption, and throughput, an alternative gel filtration approach is used that reduces the cost of filtration and amount of protein consumed per extract by 55-fold and 10-fold, respectively. The sample preparation was also adapted to a 384-well plate format that is compatible with automated liquid handling systems suitable for large-scale drug discovery campaigns.
Furthermore, the use of an alternative hybrid mass spectrometer enables low mass ligands that are more representative of small-molecule drugs to be directly identified from a single native MS measurement. By integrating a computational approach for *de novo* molecular formula and compound class identification, active compounds from complex natural product mixtures can be rapidly characterized, potentially eliminating the need for separate metabolomics data.

Overall, by incorporating these advances to the established workflow, active compounds from crude natural products can be identified in a highly efficient manner, facilitating natural product-based drug discovery.

Keywords: Native mass spectrometry, Natural products, Protein-small molecule interactions, Tandem mass spectrometry

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Speaker 32

Characterising virus-like particles with native charge detection mass spectrometry

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Native mass spectrometry (nMS) allows the characterisation of intact, natively folded biomolecules and their interactions, as sample conditions and instrument parameters are optimised to maintain non-covalent interactions. The interpretation of nMS spectra from highly complex, heterogeneous samples is challenging due to the *m/z* overlap of peaks from the different species responsible for the heterogeneity. Previously to overcome this, sample clean up and simplification (i.e. via chromatography) and/or advanced data analysis deconvolution methods were required to interpret these complex spectra. More recently, instrumentation and software improvements have allowed the introduction of native charge detection mass spectrometry (nCDMS), whereby the charge state on an ion is measured, which together with the measured *m/z* allows accurate empirical determination of the absolute mass of the ion. This leads to simple, easily interpretable, empirically determined, absolute mass spectra for complex, heterogeneous samples.

Here, nCDMS was utilised to characterise virus-like particles (VLPs); self-assembled protein capsids that are utilised to encapsulate cargos for a variety of synthetic biology and drug discovery applications. Using the Thermo Fisher Q Exactive Ultra High Mass Range Hybrid Quadrupole Orbitrap Mass Spectrometer with Direct Mass charge detection, empty and cargo loaded VLPs self-assembled *in vivo* from human JC polyomavirus capsid protein subunits were characterised. nCDMS revealed the absolute mass range of the VLPs providing evidence on the subunit stoichiometry of the particles, the sub-species of particles within the population, and the efficiency of cargo loading when GFP was employed as the cargo. Analysis over an approximately 1-week time frame revealed mass changes in the VLPs, trending towards a lower molecular weight population, which had previously not been observed with other characterisation techniques. Further studies are ongoing to understand the mechanism of this VLP mass rearrangement. This work demonstrates the enormous potential for nCDMS to characterise and reveal new insights into complex biomolecules.

Keywords: Native mass spectrometry, charge detection, virus-like particles, drug discovery, biomolecule characterisation

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Speaker 33

Dietary Restriction Induces a Sexually Dimorphic Type I Interferon Response in Mice with Gene-Environment Interactions

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Intermittent fasting (IF) is an established intervention to treat the growing obesity epidemic. However, the interaction between dietary interventions and sex remains a significant gap in knowledge. In this study, we used unbiased proteome analysis to identify novel diet-sex interactions. We discovered unique sexual dimorphism in response to intermittent fasting, within both type I interferon signaling that was strongly induced in females, alongside differences in many aspects of lipid and cholesterol metabolism. We verified that secretion of type I interferon was induced during the IF response in females. Gonadectomy differentially altered the EODF response and demonstrated that sex hormone signaling can either suppress, or enhance, the interferon response to IF. IF failed to potentiate a stronger innate immune response when IF-treated animals were challenged with a viral mimetic. Lastly, the IF response was altered by genotype and environmental changes. These data reveal a novel interaction between diet, sex, and the innate immune system in a model animal.

Keywords: intermittent fasting, interferon alpha, sexual dimorphism, cholesterol, proteomics

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Poster Presentations

P-01

Delving deeper into the metabolome with cIMS, sub ppm mass accuracy and High-Resolution Mass Spectrometry

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Metabolomic studies involve the analysis of complex, mulit-class matrices. Deconvolving these complex mixes requires combining multiple separation techniques, including liquid chromatography and ion mobility separations, with high mass resolution mass spectrometers (MS), contribute to increasing the number of compounds that can be accurately detected. Metabolomic extracts resulting from Human plasma samples, which originated from COVID-19 cohort were analysed in both positive and negative ion ESI modes with the data acquired on a multi-reflectron ToF (MRT) using a data independent acquisition (DIA) mode. Chromatographic separation was achieved rapidly over 10 mins using a ACQUITY TM Premier BEH TM amide (2.1 x 100 mm) and HILIC gradient elution profile. The biological samples were further characterised by analysis on the Select Series™ Cyclic™ IMS where isomeric compounds of interest were investigated using IMS^n multipass, enhancing mobility resolution alongside analysis using a CCS calibrated, ion mobility DIA acquisition mode. All data underwent pre-processing using Progenesis™ QI software, where data were aligned, peak picked and normalised prior to statistical analysis. The mass accuracy of example metabolites features were extracted from pooled quality control (QC) samples across the whole sample set and demonstrated an accuracy of <500 ppb. Furthermore, the mass resolution enabled the detection of the fine isotopic pattern of the selected compounds, provided more detailed spectra with improved feature determination and peak picking alongside compound annotation following database searching. Bile acid levels between mild and sever cases of COVID-19 were one example class of significance. Possible annotations of sulphated bile acid isomers examined, using the IMS^n multipass functionality to determine the presence of both isomer annotations. Collisional cross section (CCS) values for each compound improving the confidence when searched against in-house and in-silico databases. Statistical analysis highlighted clear separation of the various samples/patients based upon severity of disease observed by PCA analysis.

Keywords: Mass accuracy, Ion mobility, Advanced HRMS, metabolomics

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P-02

Cannabinoid and Terpene Content of Homemade DIY Cannabis Extracts for Vaporisation

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There has been an increase in the use of electronic devices for vaporising cannabis products. Forms of cannabis that are vaporised include dry herb, cannabinoid containing e-liquids and extracts. Cannabis extracts (or concentrates) are often produced by users at home using different DIY methodology. The key methods for making extracts are solventless rosin press extraction; solventless hash; gas-solvent extraction; and various liquid-solvent extractions. This project involved investigation and compilation of online sources (YouTube, Reddit, online forums) showcasing individuals' methods for creating cannabis extracts for vaporisation to identify different variables for extraction procedures (temperatures, times, solvents etc.). Common DIY methods were then replicated in the laboratory using commercially available medicinal cannabis products and materials which can be obtained by the general public from sources such as supermarkets, hardware stores and online vendors. Differences of cannabinoid content in cannabis extracts prepared using different methodologies was investigated using LC-MS/MS (MRM). Variances in terpene levels within cannabis extracts prepared using different methodologies was also investigated using GC-MS. This project is the first of its kind examining and comparing the range of methods currently employed by the general public to generate cannabis homemade extracts for vaporisation. Differences in cannabis extract preparation such as: solvent selection; temperature; and extraction time have an effect on the cannabinoid and terpene levels and profiles in extracted products that are subsequently vaporised by users.

Keywords: Liquid Chromatography-Mass Spectroscopy, Gas Chromatography-Mass Spectrometry, Cannabinoids, Terpenes, Vaporisation

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Understanding fate and behaviour of PFAS in the Victorian environment

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The group of synthetic chemicals known as poly and per-fluoroalkyl substances (PFAS) are currently of high concern to environmental regulators and the public due to their widespread occurrence, resistance to degradation, and reported toxicity. However, little data exists on the major sources and sinks of PFAS in Victoria as well as seasonal variation in input (if any). This lack of information hampers the effective management of these compounds. Here we use liquid chromatography high-resolution mass spectrometry to conduct a detailed analysis of the contamination of over 52 PFAS compounds across Victoria. Samples were taken from surface water (rivers, lakes, Port Philip Bay) and sediment cores around Port Philip and the Gippsland Lakes. While Multiple PFAS compounds were found a small subset of four to five compounds Perfluorooctanesulfonic acid (PFOS), Perfluorooctanoic Acid (PFOA), Perfluorohexanesulphonic acid (PHFHxS), and Perfluorohexanoic acid (PFHxA) and, in some cases, 6:2 Fluorotelomer Sulfonic Acid (6:2 FTSA) made up the majority of PFAS detected. There were differing distributions of compounds across the state, indicating varying sources of inputs. The PFAS type and amount also varied by season. The western side of Port Philip showed the highest PFAS concentrations while Gippsland had low levels of PFAS contamination. Analysis of sediment cores from Port Philip Bay showed an interesting trend in that the top 5-10cm often had little to no PFAS while deeper levels held significant amounts. The finding means that surface grab samples may well underestimate the amount of PFAS present with implications for effective monitoring. Future work will involve more detailed sampling campaigns to give a better understanding of PFAS movement in the bay as well as detailed measurements of other potential sources of contamination, such as ground and surface waters.

Keywords: contamination, environment, PFAS, sediment

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P-04

Structural Characterisation of Glyphosate and AMPA Metal Complexes Using Ion Mobility-Mass Spectrometry

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N-(phosphonomethyl)glycine or Glyphosate is the most used herbicide globally. First developed in the 1970s, it quickly gained popularity due to its extreme efficacy and its non-selective trait. Recent studies have also linked Glyphosate and its primary metabolite, aminomethylphosphonic acid (AMPA), to multiple adverse health effects from metalloenzyme inhibiting activities to non-Hodgkin's lymphoma. Both Glyphosate and AMPA are zwitterionic and have multiple reactive functional; groups that can readily participate in coordination bonding, and polymerisation, allowing them to easily form various complexes with divalent metal cations, including multiply charged species. The metal complexes formed possess extraordinarily rich structural variability. Structural characterisation of these complexes is highly desirable as that would provide better mechanistic understanding of their behaviour, characteristics, interactions, and biological and environmental fate. Ion mobility spectrometry-mass spectrometry (IMS-MS) is fit for this purpose as it can separate isomers allowing users to access an additional analytical dimension over mass spectrometry. alone that is the measurement of ions' collisional cross section. Here, target $[Glyphosate+M-H]^+$ and $[AMPA+M-H]^+$ complexes where M = Mg^{2+}, Ca^{2+}, Sr^{2+}, $Ba^{2+}Mn^{2+}$, Co^{2+}, Cu^{2+}, and Zn^{2+}were structurally characterised. Multiple IMS-MS techniques including drift tube (DTIMS), trapped (TIMS) and travelling wave (TWIMS) were used to robustly characterise the Glyphosate and AMPA metal complexes under different instrumental conditions. Density functional theory (DFT) structures were generated to computationally predict potential collisional cross sections These theoretical values were compared to the experimentally obtained values to gain further structural insight. Experimental results have confirmed that there is a common structure preferred by the complexes across the different metals investigated. However, there are also additional isomers with different CCS values indicating the capriciousness of Glyphosate and AMPA which is supported by the computational results and hints at the origins of adverse events and fate in the environment.

Keywords: Glyphosate, Metal complexes, Structural characterisation, Ion Mobility, Mass Spectrometry

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P-05

Imaging tissue lipids using histological dyes for laser desorption and plasma post-ionisation

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Eukaryotic tissues comprise a matrix of cells. In order for organoid tissues to fulfil complex functions, multiple cell types, fit with unique physical and chemical properties, are discretely organised. One class of biomolecule that contributes to cellular property differences, including to membrane structures and signalling, is lipids. The multifunctional nature of these molecules is owed to diversity in their molecular structures, and subsequently cellular lipid profiles are known to differ between cell types.

To observe the organisation of cell types in tissues and their molecular profiles, different imaging techniques need to be employed. Most commonly, cell identification is achieved by using histological stains and optical imaging, while molecular information is obtained using mass spectrometry imaging (MSI), including matrix assisted laser desorption/ionisation (MALDI)-MSI. Typically, MALDI is performed first, however, MALDI sample preparation and matrix deposition can be detrimental to tissue-stain quality.

Due to bond conjugation, histological dyes are photoactive and absorb light at discrete wavelengths. Herein, we have exploited a timsTOF Pro mass spectrometer fit with a modified 355 nm MALDI source capable of atmospheric pressure desorption and plasma post-ionisation to image tissues using histological dyes as the matrix. Decoupling the desorption and ionisation processes allows for histological stains, such as H&E, to desorb tissue lipids separately to their ionisation downstream using the low temperature plasma source. Tissue staining was achieved without the “defatting” process and remains of comparable quality to conventional staining workflows. This approach resulted in high-intensity signals for many lipid species, including phospholipids and glycosphingolipids, and displayed minimal lipid delocalisation as seen by the close correlation between ion images and tissue morphology. Additionally, lipid signal-to-noise was shown to be improved with dye-assisted desorption compared to matrix deposited samples, which allowed for the observation of lipid species not commonly seen in positive polarity mass spectrometry, such as protonated phosphatidylinositol.

Keywords: Imaging, histology, MALDI-MSI, lipidomics

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P-06

Structural Elucidation of Branched-Chain Fatty Acids in Plasma through Charge-Remote Fragmentation

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Branched-chain fatty acids are critical as signalling molecules for various health issues and diseases. Branched-chain fatty acids are difficult to distinguish from their isomeric straight-chain counterparts by tandem-mass spectrometry strategies, with the only structural difference being a methyl group(s) at different sites along the acyl chain. The introduction of a positive fixed charge site to promote charge-remote fragmentation in the fatty acid is therefore beneficial for inducing fragmentation patterns characteristic for the carbon-carbon bonding arrangements of the acyl chain.

Herein, we adopt the charge-inversion strategy of complexing straight and branched-chain fatty acid standards with the doubly charged tris-5-nitro-1,10-phenanthroline magnesium (II) complex by combining the compounds in a T-infusion set up post chromatographic column separation and analysed using a Thermo Fisher Linear Ion Trap LTQ-XL. The non-esterified fatty acids in citrated human plasma were extracted and analysed using the same method.

We demonstrate that collision-induced dissociation of these ionic complexes yields unique spectra for each fatty acid studied, including the ability to differentiate between isomeric branched-chain fatty acids. The dissociation of the combined $[Mg(NO_2Phen)_2(FA - H)]^+$ yields a loss of one NO_2Phen ligand, and subsequent dissociation of the $[Mg(NO_2Phen)(FA - H)]^+$ produces unique spectra for each FA and allows for unambiguous identification of the branch-chain location.

Enhancing charge-remote fragmentation processes maximises the structural information obtained in the analysis and enables chain branching assignment for even low abundance fatty acids, including branched chain fatty acids in human plasma. Combining this technique with chromatographic separation of a wider range of branched-chain fatty acids will allow for further investigation into human plasma.

Keywords: Lipidomics, BCFA, plasma, charge-remote fragmentation, T-infusion

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Elucidating novel fatty acid structures in v*ernix caseosa* by combining photodissociation and ozone-induced dissociation mass spectrometry

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Vernix caseosa is a protective biofilm that covers newborns at birth that is comprised of a diverse array of lipid classes such as triacylglycerols, wax esters, sterol esters, ceramides, among others. In addition, the *vernix* lipidome is known to be rich in branched-chain fatty acyls including those exhibiting unusual sites of unsaturation. These fatty acyls are also characterised by ultra-long carbon chains (>24 carbons) that are uncommon in other mammalian lipidomes. The complexity of the lipidome and the diversity of structural motifs conspire to present a significant challenge to conventional chromatography-mass spectrometry methods in providing for the unambiguous identification of known lipids and the elucidation of potentially novel structures. Here we describe the application of reversed-phase liquid chromatography (LC) in conjunction with ultraviolet photodissociation (UVPD) and ozone-induced dissociation (OzID) mass spectrometry to surmount this challenge and enable robust *de novo* identification of the fatty acid (FA) building blocks of the *vernix* lipidome.

Preliminary results have revealed fatty acids (4-I-AMPP derivatized) with carbon chains ranging from 9 to 35 with chromatographic separation using C18 RPLC with a 230min gradient, identifying straight-chain and methyl-branched isomers in all cases. UVPD (266nm) mass spectrometry identified site(s) of chain branching that followed a broad trend of even carbon-number exhibiting methyl-branching preferentially at the penultimate position (*iso*) while odd carbon number variants were predominantly branched at the antepenultimate position (*anteiso*). To our knowledge evidence for very-long chain FAs with carbon numbers of 32-to-35 with chain, branching is presented here for the first time. Interestingly, methyl-branching was also observed at site(s) other than the classical *iso* and *anteiso*-positions. Notably, evidence is presented for methyl-branching at the 4-position (with respect to the carboxylic acid) spanning carbon chains from 9-to-18 carbons. Alignment of UVPD and OzID spectra also identified trends in unsaturation with differing sites of unsaturation along with evidence for polyunsaturated, branched-chain lipids that will be presented for the first time.

Keywords: *vernix caseosa*, lipidomics, photodissociation, ozone-induced dissociation, fatty acid derivatization

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Exploring The Philippine Endemic *Hornstedtia conoidea* Frut and *Etlingera elatior* Inflorescence: Its Mass Spectrometry- Based Metabolomics Profile

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Zingiberaceae plants, commonly known as gingers, are one of the most interesting families of plants to study due to their widespread distribution in tropical countries and multiple plant part usage. Hornstedtia conoidea Ridl, (HC) and Etlingera elatior (Jack) R.M. Smith (EE) are some of the species belonging to this ginger family. The reported ethnomedicinal uses of H. conoidea remain a claim since no study was done to profile the chemical constituents of the fruit despite being eaten by local people. E. elatior, commonly known as torch ginger plant, is among the most common species of Zingiberaceae in South and Southeast Asia.

Most of the plants belonging to this Zingiberaceae family have an aromatic odor when crushed. The inflorescence of E. elatior has been used for a variety of applications as a culinary ingredient and condiment, essential oil source and as an ornament in other countries. Hence, exploring the mass spectrometry-based metabolite profile of these understudied species is of paramount importance.

Untargeted LC-MS profiling analysis of samples was carried out using SCIEX ZenoTOF 7600 system with EAD fragmentation capability. Chromatographic separation was performed using a LC gradient with mobile phases comprised of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile. Gradient: A 5 min linear gradient from 5% to 30% mobile phase B, followed by a 5-minute gradient to 100% mobile phase B and then a 5 min hold, then re-equilibration at 5% mobile phase B.

The metabolite profiling of two ginger species revealed significand differences of the polar metabolite profile in between two species. The SCIEX ZenoTOF 7600 provide increased number of compound identification with higher confidence. This novel finding will guide to discover new compounds with medicinal properties and open new area for further discovery of metabolite of these exotic plant species in future.

Keywords: Metabolomics, Untargeted, ZenoTOF 7600, EAD, Ginger metabolites

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P-09

Direct observation of phospholipase activity by mass spectrometry

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Phospholipase-A1 (PLA1) and -A2 (PLA2) enzymes catalyse ester hydrolysis at the *sn*-1 or *sn*-2 position (respectively) of a glycerophospholipid, releasing a free fatty acid and a lysophospholipid. With their varying roles in pro- and anti-inflammatory responses, the importance of rapid differentiation between the action of PLA1 and PLA2 in cells and tissues cannot be overstated. Monitoring enzyme function requires a method for the rapid separation and unambiguous identification of lysophospholipids that includes the resolution of regioisomers differing only in the (*sn*-1 or -2) position of substitution on the glycerol backbone. Though isomer resolution remains challenging for conventional mass spectrometry, here we deploy high-resolution cyclic IM-MS for separation and identification of lysophospholipid regioisomers and demonstrate its applications in direct monitoring of enzymatic reactions.

Experiments were conducted using a cyclic ion-mobility enabled quadrupole time-of-flight mass spectrometer (Waters Select Series Cyclic IM-MS). Methanolic solutions of lysophosphatidylcholine (LPC) standards were infused via electrospray ionisation in positive ion mode. $[M+Na]^+$ precursor ions were mass selected using the quadrupole mass filter and separated over multiple passes around the cyclic IM device. Following separation, ions were subjected to collision-induced dissociation (CID) in the transfer region, prior to time-of-flight mass analysis. Complete separation of LPC isomeric pairs (of ranging acyl chains) was achieved after 40 passes (total drift time ~1 s), presenting two clearly resolved IM features. Integration of the CID product ions from each arrival time feature revealed unique fragmentation patterns that could further differentiate between isomers. Enzymatic reactions of PLA1 or PLA2 with a range of diacylphosphatidylcholines were undertaken in methanol (1:30) with 2 hours incubation at 37 °C, combined, and directly sampled by direct-infusion electrospray ionisation IM-MS. Successful separation and identification of regioisomers was achieved for lysophospholipids substituted with a diverse array of fatty acyl groups thus, enabled direct assessment of enzyme fidelity and efficacy across a broad substrate scope.

Keywords**:** phospholipase, lipids, isomer-resolution, ion-mobility

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Single-cell lipidomics of neurons

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Metabolic dysfunction is linked to a variety of neurodegenerative diseases (Bourgognon and Steinert 2019, *Neural. Regen. Res*). Bulk metabolomics has revealed distinct metabolic signatures during neurodegeneration, with dysregulation of lipid metabolism, redox homeostasis, bioenergetics, and central carbon metabolism key homeostatic processes that become impaired (Dong *et al.* 2018, *Front. Mol. Neurosci.*; Sonntag *et al.* 2017, *Sci. Rep.*). However, the diversity of different cell types in the brain, each with highly integrated and complex morphology, results in an extraordinary degree of cellular heterogeneity. This makes it challenging to understand which specific cell types may be implicated with disease (Fitzner *et al.* 2020, *Cell Rep.*), as is previously exemplified using lipids (Neumann et al. 2019, *Angewandte Chem Int Ed*). To address this need, we have developed a workflow for lipidomic profiling of single neurons by MALDI-mass spectrometry imaging (MSI). Live neuronal cultures were stained with CellBrite Green, a fluorescent cytoplasmic membrane dye, to improve cell segmentation needed for single-cell profiling. Data was generated using a high resolution MALDI-enabled Orbitrap Elite mass spectrometer from samples coated in 2,5-DHA matrix. MALDI-MSI data was metabolically annotated using the METASPACE engine against LipidMaps, SwissLipids and HMDB, allowing for robust detection of a broad panel of metabolites and lipids. The overlay of MSI with microscopy data and construction of single-cell profiles was done using the SpaceM software (Rappez et al 2021, *Nat. Methods*). Our protocol allows for single-cell interrogation of neuronal cultures under physiologically relevant conditions to maintain *in vivo*-like neuronal metabolism, such as inter-cellular interactions. The development of single-cell metabolomic and lipidomic profiling of neurons has broad reaching applications, including identifying the contribution of specific cell types to metabolic dysfunction in multiple neurodegenerative diseases, and the detection of novel low-level disease-specific biomarkers, whose signal would otherwise be lost during bulk analysis.

Keywords: Metabolomics, Lipidomics, Mass Spectrometry Imaging, Single-cell Analysis

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APOE Regulated Myelin Lipid Turnover in Healthy Brains

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Apolipoprotein E (ApoE) is the major lipid transporter in the brain, and inheritance of the ε4 allele of the APOE gene (APOE4) is the most significant genetic risk factor for Alzheimer's disease and dementia overall. Turnover of myelin sheaths, which are comprised of 80% lipids, is crucial for the maintenance of myelin integrity. This study employed proteomic and lipidomic analyses of mouse and ageing human brain tissue samples with no significant dementia pathology to investigate how APOE genotype affects the physiological brain to modulate the risk of developing dementia. A novel stable isotope mass spectrometry approach involving administration of deuterium oxide to mice was used to probe the role of APOE in myelin lipid turnover in vivo.

Significant accumulation of the myelin-enriched sphingolipids hexosylceramides and sulfatides was observed in the hippocampus of human APOE4 carriers relative to carriers of the protective APOE2 allele and risk-neutral APOE3/3 individuals. These findings were corroborated in the hippocampus of mice bearing targeted replacement of Apoe with human APOE variants (hAPOE2/2, hAPOE3/3, hAPOE4/4). More pronounced myelin lipid accumulation was observed in the hippocampus of Apoe knockout mice, suggesting that APOE4 is a loss of function allele with regard to lipid turnover. Untargeted proteomics revealed myelin proteins MAG and MOG, and proteins in the phagocytic microglial pathway (C1qA) are increased in Apoe knockout mice, indicating defective clearance of myelin debris and microglial activation. Global metabolic lipid labelling with deuterium oxide drinking water indicated that Apoe knockout mice have slower rates of myelin lipid turnover compared to wild-type mice.

This study provides evidence for defective clearance of myelin debris in the hippocampus of non-pathological, cognitively normal humans and mice carrying APOE4, and points to a physiological role of APOE in myelin lipid clearance. Impaired myelin debris clearance may underlie the increased susceptibility of APOE4 carriers to dementia.

Keywords: Fluxomics, Lipidomics, ApoE, Brain, Myelin

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The effectiveness of molecular glue and E3 ligase oligomeric remodelling revealed using native mass spectrometry and mass photometry

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Molecular glues (MGs), discovered fortuitously in recent studies, bring together E3 ligases and a target protein, resulting in subsequent ubiquitination and proteosome-mediated degradation of the target. These proteins are interesting targets in cancer treatment but previously thought “undruggable” by conventional occupancy-driven pharmacology. However, a rational design for novel molecular glue is challenging due to the unresolved mechanism of molecular glue induced E3 ligase reconfiguration for stable interaction with neosubstrates. Therefore, analytical approaches for characterizing the effect of molecular glue on E3 ligase and ternary complex formation (E3:MG:neosubstrate) are required for accelerating molecular glues development. Here, we demonstrate that native mass spectrometry and mass photometry can provide unique insights into the physical mechanism of molecular glues, revealing the oligomeric rearrangement of E3 ligase. For example, DCAF15 self-assembles into dimers (K_d of 7 ± 3 nM) and trimers (K_d of 9 ± 4 nM) at physiologically relevant low nanomolar concentrations. And the stoichiometric addition of a molecular glue significantly lowered the binding affinity (K_d of 16 ± 1 and 26 ± 4 nM for dimers and trimers respectively), suggesting a secondary mechanism for how molecular glue regulates E3 ligase activity. Native mass spectrometry accurately assesses the order of molecular glue potency and the binding specificity towards different neosubstrates, correlating well with solution-phase techniques, such as isothermal titration calorimetry, fluorescent transfer energy resonance and amplified luminescence proximity homogeneous assay-based screening. Furthermore, the weak interactions between E3 ligase and target proteins, that is enhanced by molecular glue, are also observed by native mass spectrometry, affording a more rational selection for E3 ligase and neosubstrate pairs for molecular glue development. Such detailed mechanistic insights should be beneficial in developing molecular glue as powerful therapeutic agents.

Keywords: Native mass spectrometry, E3 ligase, molecular glue, mass photometry, targeted protein degradation

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Biophysical characterisation of DNA triplexes for antigene technology

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Deoxyribonucleic acid (DNA) is an essential biomolecule that contains the genetic information required to build and maintain a living organism. While canonical duplex DNA is well-characterised, the structure and function of higher order DNA structures such as triplex DNA are comparatively poorly understood. It is broadly proposed that these triplex structures, in which a third strand known as the triplex forming oligonucleotide (TFO) binds in the major groove of the double helix, have genetic repressive traits at a transcriptional level, making them a suitable candidate for design of antigene therapies. A decrease in expression of an essential gene in a target such as an antibiotic resistant bacteria would help combat issues of antibiotic resistance and ameliorate hospital-acquired infections. In order to better exploit this, it's necessary to have a better understanding of triplex formation.

Our investigation of Y-type (pyrimidine-rich TFOs) have provided new insight into the factors that affect triplex stability and quality of mass spectrum such as ion mode, oligonucleotide sequence and solution conditions including salt concentration and pH. The mentioned factors need to be optimised for mass spectrometry analysis by assessing the amount of triplex formed and the signal to noise ratio. Furthermore, utilising a bioinformatics approach we have identified a candidate antigene site based on a specific triplex sequence corresponding to the *rlmN* gene in methicillin-resistant *Staphylococcus aureus* (MRSA). Triplex formation using this specific sequence has been demonstrated *in vitro* under native conditions. To facilitate the antigene strategy via cell-penetration, we have added a cell-penetrating peptide to the TFO using bioconjugation chemistry. Formation of triplex using the synthetic TFO was successful and now *in vivo* assays have been undertaken to determine whether these TFOs have antibacterial capacity.

Keywords: DNA, Triplex, Antigene strategy, Mass spectrometry, MRSA

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Looking at the Forest Cobra (*Naja melanoleuca*) Venom Through the Lens of Mass Spectrometry

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Snake venoms are comprised of highly bioactive proteins and peptides that are responsible for the lethal physiological effects of snakebite envenomation. While antivenom administration is the primary treatment, the proteomic complexity as well as the inter- and intra-species variability of venoms pose a tremendous challenge in developing effective and consistent antivenoms. To guide the development of targeted antivenom strategies, robust and high-throughput analytical pipelines are necessary for comprehensive understanding of venom compositions and the various proteoforms that are often not captured by traditional bottom-up proteomic workflows. Here, we are developing an integrated 'omics' and top-down mass spectrometry (MS)-based approach to characterise snake venoms. We report the use of venom gland transcriptomic-guided proteomics to catalogue the venom composition of the medically significant forest cobra (*Naja melanoleuca*) and we explore the subtle diversity of venom proteoforms using intact MS and native MS strategies for the first time. Importantly, our integrated workflow reveals a significant increase in the number of venom proteoforms within various toxin families that were not captured in previous studies.

Keywords: Snake venom, proteins, proteomics, top-down MS

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Characterising proteoforms by top-down proteomics and (cyclic) ion mobility

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A proteoform describes the precise form of a protein that exist in biological systems including any changes due to *in vivo* processing events and post-translation modifications (PTMs). Proteoforms define protein structure and function and therefore effective proteoform characterisation methods are required to understand cellular function and processes. Characterising intact proteoforms by top-down proteomics in their native or denatured state is becoming more widely available, but has not yet attained the same rigour as bottom-up proteomics. As intact analysis retains information about PTM site occupancy relative to the rest of the protein structure, it enables insights that are not possible with traditional bottom-up approaches.
Additionally, separation of closely related proteoforms, such as structural isomers, by applying ion-mobility separation provides an important tool for probing proteoform complexity. Ion mobility has proven to be a valuable tool for top-down proteomics methods as ion mobility can enhance the information extracted from the mass spectra to return critical information about the primary amino acid sequence and PTMs, as well as insight into protein structure. Cyclic ion mobility provides further opportunity for in-depth probing of proteoforms, due to its mobility cell allowing unrestricted reiteration.

We demonstrate the characterisation of proteoforms of three proteins (carbonic anhydrase, β-casein and ribonuclease B) using nanocapillaries acquired on a ThermoFisher Fusion Lumos and Waters Cyclic IMS, using ion mobility separation and multiple fragmentation methods. We demonstrate and assess various data analysis platforms in the characterisation of proteoforms by native and top-down proteomics, including capacity of data analysis programs to resolve and identify proteoforms, and assess capacity for performing sequence coverage and PTM site localisation. These results highlight the capability of native and intact analyses to characterise proteoforms, and provide guidance for optimising experiments for future studies.

Keywords: Top-down proteomics, native MS, cyclic ion-mobility, proteoform, data processing

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Your stable isotopes will be taken down and may be used as evidence against you.

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This presentation will use examples of determining the ratio of stable isotopes by Isotope Ratio Mass Spectrometry (IRMS) and amounts of trace elements by Inductively Coupled Plasma Mass Spectrometry (ICPMS) to provide intelligence and evidence in a range of cases.

Pairs of light stable isotopes of hydrogen ^{2}D and ^{1}H, carbon ^{13}C and ^{12}C, nitrogen ^{15}N and ^{14}N, oxygen ^{18}O and ^{16}O and sulphur ^{34}S and ^{32}S are the building blocks of life and so determination of their ratios can indicate the source of food, drugs, pollutants, and bodies. When coupled with trace element analysis by ICPMS the combination of elements and isotopes can add corroboration to the evidence. Examples to be presented include the use of variation in natural isotopes to determine the relative sources of water and nutrients to the bays around the threatened oyster growing area around Coffin Bay on the Eyre Peninsula of South Australia and distinguishing between the contamination in homes due to smoking and/or synthesising methamphetamine.

The forensic environmental investigation - ‘Catching the Cockle Killer of Coffin Bay’ - involves distinguishing sources of water and nutrients by determining the isotope ratios in water from groundwater bores, seasonal creeks and various jetties around the bays in conjunction with elemental concentrations of key elements including boron and strontium, compounds including nitrate, nitrite and ammonia and electrical conductivity, total dissolved solids and salinity.

For distinguishing between illicit activities causing contamination the different products produced from synthesising and from smoking methamphetamine were investigated using normal methamphetamine and D3, D5 and D9 labelled methamphetamine and analysis by Pyrolysis-GCMS, GCMS and LCMS.

Keywords: IRMS, ICPMS, Methamphetamine, Environmental Forensics

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Increased image clarity and specificity using dual MSI sources and Multi-Reflecting Time of Flight analysing lipid metabolites in mouse testis

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Tight regulation in lipid homeostasis is critical for male fertility and in the normal process of germ cell development, extensive lipid remodelling occurs in different cell types which is poorly understood. Mass spectrometry imaging provides spatial localization of different molecular species in the adult mouse testis but there are challenges with conventional imaging given the highly complex tissue structure and marked heterogeneity in cell types. We have investigated lipid metabolites of the different compartments within a mouse testis using high resolution mass spectrometry imaging (MSI) for increased specificity and high mass accuracy. MSI experiments were carried out on a Q-oaTOF and multi-reflecting Q-Tof (MRT) mass spectrometer equipped with a DESI source and MALDI source. DESI was imaged a stage speed was 100 µm/sec. MALDI was acquired with laser with a repetition rate of 1KHz and α-Cyano-4-hydroxycinnamic acid (CHCA) matrix was used. Mainly glycerophospholipids and triglycerides were detected for DESI positive mode with a mix of potential cation types such as H^+, Na^+ and K^+, increasing the complexity in lipid identification. Principal component analysis (PCA) was performed using eight ROIs drawn on the three main compartments of the testis using MetaboAnalyst. From the score plot (PC1 vs PC2), the groups were clearly separated. The MRT instrument yields ppb mass accuracy, allowing possible identification of PC (36:1) and PC (38:4) (both detected with a sodium and potassium cations) which was strongly localised in the interstitial regions where Leydig cells and blood vessels reside. PC (34:1) and PC (36:4) (Na^+ and K^+) ions were mainly localised in the Sertoli cells/early germ cell type and PC (38:5) and PC 38:6) were localised in the more mature germ cells. Similarly, for m/z 830.55, four peaks were detected with the MRT and localised within the testis tissue.

Keywords: DESI, MALDI, HRMS, imaging, MRT

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Mapping the distribution of sterols in the mouse brain - integrating mass spectrometry imaging with the Allen Mouse Brain Atlas

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Approximately 25% of the entire body's cholesterol is found in the brain. Different sterols are involved in brain functions, e.g., 20S-hydroxycholesterol and 24S,25-epoxycholesterol in the hedgehog signalling pathway, and the glucocorticoid corticosterone. Knowing the distribution of cholesterol and metabolites across the brain can provide insight to their biological functions. We combined cholesterol-specific mass spectrometry imaging (MSI), histology, and bioinformatics to map cholesterol and its metabolites in different brain regions. Sagittal sections (15 µm) from fresh-frozen adult C57Bl/6 mouse brain separated at 200 µm were taken across an entire hemisphere and mounted on histology slides for Nissl staining, and on ITO-coated or glass slides for MSI. A modified enzyme-assisted derivatization for enhanced sterol analysis approach was used with or without coating with 5 mg/mL CHCA in water/propan-2-ol/acetonitrile (3:4:3, v/v/v). MSI was carried out on an Orbitrap IDX mass spectrometer (ThermoFisher Scientific) coupled with an AP-MALDI UHR source (MassTech, Maryland USA) or LESA-ESI over m/z range of 400–1000 in positive-ion mode. Spatial resolution was 50 µm in MALDI-MSI and 800 µm in LESA-MSI. Landmarked-based co-registration was used to map the histological and MSI data to the Allen Mouse Brain Atlas (AMBA).

Collecting complementary MSI and histological data from serial sections enabled us to register sterol content with anatomical regions, derive the spatial coordinates of where each cholesterol MSI spectrum was acquired, and view the expression of cholesterols for different anatomical regions such as the cerebellum, hippocampus, and striatum. This approach allows generation of a molecular atlas and computation of aggregate statistics for different cholesterol metabolites across specific anatomical regions, and enable future studies e.g., the comparison the presence or differential expression of a certain molecule between regions (e.g., cortex vs. white matter).

Keywords: Sterols, Mass spectrometry imaging, Multi-modal imaging, Data integration, Lipidomics

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Quantitative Mass Spectrometry Imaging of Lipids in Mouse Brain Tissue using an (Optimised) Internal Standard Mixture

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Mass spectrometry imaging (MSI) is a powerful tool for spatially mapping compounds within biological tissues. In lipidomics, it is known that ionisation efficiencies depend on both individual molecular properties and the presence of other lipid classes, leading to varying ion intensities for given lipid species across tissues, even when present at equal concentration. The use of internal standards (IS), where an analogue of the analyte(s) of interest is sprayed across a tissue sample, has been used to overcome this issue in the quantitative MSI (Q-MSI) of drugs. This study utilised a custom formulated lipid IS reflecting the endogenous brain concentrations of 13 major lipid classes to enable Q-MSI of multiple lipid classes simultaneously.

Mouse brains from C57BL/6N animals were cryosectioned (Leica Biosystems) and mounted on indium tin oxide-coated slides (Delta Technologies). The optimised 13-lipid IS mixture (AVANTI Polar Lipids) was diluted 1:10 in LC-MS-grade methanol and deposited onto tissue sections using a TM-Sprayer (HTX Technologies) at known surface concentration. MALDI matrices were applied using the TM Sprayer: norharmane negative mode analysis or DHB for positive mode. MSI was conducted using regular MALDI (negative mode) and laser post-ionisation MALDI-2 (positive mode) on both an Orbitrap Elite (Thermo Fisher) equipped with a reduced pressure ESI.MALDI ion source (Spectroglyph), and a timsTOF flex MALDI-2 (Bruker). Detected lipids were normalised against their respective IS reference peaks, and *m/z* images plotted with a ±3 ppm theoretical mass window of the chosen lipid species.

The dual polarity approach allowed detection and imaging of hundreds of unique lipid species, including phospholipids and, glycosphingolipids. IS normalisation allowed region-specific lipid concentrations (pmol/mm^2) to be determined and revealed subtle changes in distribution compared to standard TIC normalisation. This demonstrates the ability to perform Q-MSI on multiple different lipid species, greatly expanding the utility of MSI approaches for spatial lipidomics.

Keywords: Lipidomics, Mass spectrometry imaging, Data analysis, Quantitation

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Optimized Extraction Protocol for Two Commercial Polyphenol-Enriched Extracts: Identification by LC-QTOF-MS and Quantification by LC-QQQ-MS

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Plant-derived foods are a rich source of polyphenols, which are popularly known for their antioxidant properties. This study focuses on two commercial polyphenol-enriched extracts originating from the sugarcane by-product molasses, specifically formulated to serve as dietary bioactives. The present study aims to compare the efficacy of a crude Liquid-Liquid Extraction (LLE) method with improved LLE techniques involving six different sets of aqueous-organic solvent mixtures, along with the optimization of pH and temperature. This study further improves the initial LLE by adding further clean-up steps, consisting of one or a combination of solid-phase extraction (SPE), Supported-Liquid Extraction (SLE), and Ion Exchange Chromatography (IEC). The extracts obtained under various extraction conditions are subjected to Liquid Chromatography-Quadrupole Time-of-Flight Mass Spectrometry (LC-QTOF-MS) to identify the specific polyphenols present in each extract using an in-house standard polyphenol library. Polyphenols identified in each extraction condition are first quantified using LC-QTOF-MS and the polyphenol enrichment is further confirmed with total polyphenols assay, total flavonoids assay, and radical scavenging activity assay. The optimized extract of each commercial product underwent Liquid Chromatography-Triple Quadrupole Mass Spectrometry (LC-QQQ-MS) analysis for the final quantification of all polyphenols present, with additional reporting of spike recoveries, linearity, Limit of Detection (LOD), and Limit of Quantification (LOQ) for each polyphenol in the final extract. The results of the study unequivocally indicate that the incorporation of at least one supplementary clean-up step subsequent to LLE is indispensable for enhancing the extraction yield of polyphenols. The optimized extract of both polyphenol-enriched products will be applied to biological assays based on two monocyte cell lines to evaluate their potential anti-inflammatory effects.

Keywords: Polyphenols, Bioactives, Extraction, LC-QTOF-MS, LC-QQQ-MS

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Development and Validation of an LC-MS/MS Method for the Quantification of PFAS in Drinking Water at Trace Levels: Implications for the New EPA Health Advisory Guidelines.

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Per- and polyfluoroalkyl substances (PFAS) are a group of synthetic chemicals used in many industrial and consumer products. Exposure to PFAS has been associated with numerous adverse health effects, including cancer, immune system dysfunction, and developmental issues. Of particular concern are the PFAS compounds perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS), perfluorobutane sulfonate (PFBS), and hexafluoropropylene oxide dimer acid (HFPO-DA, known as Gen-X), which have been found ubiquitously in drinking water worldwide. In June of 2022, the U.S. EPA issued new lower health advisory levels for PFAS in drinking water (PFOA = 0.004 parts per trillion (ppt), PFOS = 0.02 ppt). Currently, no published methods demonstrate confident analysis at such low levels. To address this issue, we propose to develop a highly sensitive method for measuring the previous PFAS in drinking water samples. Our method will be based on solid-phase extraction (SPE) and liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), which has been shown to be a highly effective approach for the analysis of PFAS in various matrices. Parameters of both SPE and LC-MS/MS are optimised such that the method's limit of detection and quantitation incorporate the newly defined levels. Following this, validation of the method using both spiked and real-world samples, to ensure accurate and reliable measurements of these compounds in drinking water. Once the method is developed and validated, analysis of drinking water samples from various locations in Australia will be conducted, in order to assess the prevalence of PFOA, PFOS, PFBS, and HFPO-DA in these water supplies and trace potential sources of contamination. In conclusion, our proposed method for measuring these compounds in drinking water has the potential to improve our understanding of PFAS contamination. By identifying the sources and levels of these contaminants, we can develop effective solutions to protect our water supplies and public health.

Keywords: PFAS, Drinking Water, EPA Method, Detection Limit, Health Advisory Levels

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LC-MS/MS of the lung cancer drug Osimertinib and its Metabolites from Plasma and Microsampled Dried Blood Spots

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Osimertinib is a third-generation oral small molecule tyrosine kinase receptor inhibitor used to treat non-small cell lung cancer (NSCLC) with a sensitising epidermal growth factor receptor (EGRF) mutation. Up to 40% of patients receiving Osimertinib develop Grade 3 or higher toxicity which may require hospitalisation and dose de-escalation which cannot be predicted. Therapeutic drug monitoring may address this problem. The aim of this work was to quantitate osimertinib and two active metabolites AZ5104 and AZ7550 from microsampled dried blood spots (DBS) collected using a hemaPEN™ device in NSCLC patients and compare to plasma drug levels. Here we developed a fast ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) method and validated using plasma and DBS. The assay was validated over a concentration of 1-729 ng/mL with a LLOQ of 1 ng/mL for all compounds in both plasma and DBS. The matrix effect in plasma at four QC levels were in the range of 1.5-9.1% relative standard deviation (RSD), while in DBS the range was 1.7-9.2% RSD. Overall mean recovery in plasma were in the range 92.7-101.8% and 91.9-103.8% for DBS, indicating no significant loss during extraction process. Stability experiments revealed that osimertinib and its metabolites are poorly stable in the biomatrix, and sample preparation should be performed on ice, and stored at -800C to avoid degradation. Median steady state plasma trough concentrations of osimertinib, AZ5104, and AZ7550 was 205 ng/mL (range 166.6 – 285.8ng/mL), 20 ng/mL (13.2 - 23.9 ng/mL), and 18 ng/mL (13.2 - 23.2ng/mL), respectively, and consistent with previous reports. In DBS, median concentrations of osimertinib, AZ5104, and AZ7550 were 203 ng/mL (range 183.4-256.6 ng/mL), 19 ng/mL (14.9 – 23.6ng/mL) and 18 ng/mL (14.7 – 20.6ng/mL), respectively. Different hematocrit values did not significantly affect the accuracy of the measured concentrations. We demonstrate that osimertinib at its metabolite concentrations in cancer patients can be reliably determined from DBS obtained by microsampling.

Keywords: Tyrosine Kinase inhibitors, Therapeutic drug monitoring, Liquid chromatography-tandem mass spectrometry (LC-MS/MS), osimertinib, non-small cell lung cancer.

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A liquid chromatography-mass spectrometry (LC-MS) method for the determination of methamphetamine for surface wipe sampling and sorbent tube analysis

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Methamphetamine as a powder or in crystalline form (commonly referred to as 'ice') is becoming an increasingly prevalent problem in several countries and is one of the highest used illicit drugs in some Australian states and territories. Numerous liquid chromatography-mass spectrometry (LC-MS) methods have been developed for determining methamphetamine and related amphetamines from biological matrices. In this work an LC-MS method was designed for identification and quantitation of methamphetamine for analysis of wipe samples of surfaces and airborne material from sorbent tubes. Reversed phase chromatographic separation was performed using a Thermo Scientific™ Vanquish™ Horizon Ultra-High Performance Liquid Chromatography (UHPLC) instrument with a Phenomenex® Kinetex® UHPLC C18 core shell column with appropriate guard column. Mobile phases were water with 0.1% (v/v) formic acid (solvent A) and methanol (solvent B) with gradient elution at a 0.2 mL/min flow rate. Ionisation was performed using a Thermo Scientific™ ISQ™ EC Single Quadrupole Mass Spectrometer with electrospray ionisation in positive mode and collision induced dissociation (CID) voltage at 30 V. For identification and quantitation, the extracted ion chromatogram (EIC) at 150.1 *m/z* and the EIC for methamphetamine ions at 91.1, 119.1, 135.1, and 150.1 *m/z* were used. A calibration curve was prepared using a certified reference standard of methamphetamine (hydrochloride) in initial starting mobile phase composition at a 0.2 to 5 µg/mL concentration range. A deuterated analogue, methamphetamine-D_5 (hydrochloride), was used as an internal standard with the EIC at 155.1 *m/z*. Calibration linearity, the detection limit, and the quantitation limit were reported. This method will be applied to analyse wipe samples of surfaces and sorbent tube air samples collected from methamphetamine contaminated belongings to help determine the extent of contamination.

Keywords: Methamphetamine, LC-MS, detection

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Comparison of High-Resolution Mass Spectrometry Acquisition Methods for the Simultaneous Quantification and Identification of Per- and Polyfluoroalkyl Substances (PFAS)

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The aim of this study was to compare quadrupole time-of-flight mass spectrometry (QTOF) data acquisition modes for the simultaneous quantification and identification of per- and polyfluoroalkyl substances (PFAS). The acquisition methods investigated were data independent (DIA) scan mode or (MS-Only) and all ion fragmentation (All-Ions), and the automated data-dependent (DDA) tandem mass spectrometry (Auto-MS/MS). Methods were compared by analyte response, limit of quantification (LOQ), accuracy, precision, and identification limit (IL), of the 25 PFAS listed in US EPA Method 533. All three acquisition methods met the accuracy and precision criteria set within EPA Method 533 for each of the 25 PFAS. Individual differences in LOQ were marginal, with the LOQs of all individual PFAS within an order of magnitude (irrespective of differences in analyte response) across acquisition methods. All three acquisition methods tested provided adequate LOQs, with averages around ~1 ng/mL (MS-Only: 0.99 (0.25 – 5.0) ng/mL; All-Ions: 1.3 (0.10 – 5.0) ng/mL; Auto-MS/MS: 1.1 (0.10 – 5.0) ng/mL). MS-Only is recommended for faster data processing and tentative identification as it does not capture any product ion data and may lead to more false-positives. Auto-MS/MS is recommended for concentrated or pooled samples where both quantification and high-confidence identification are desired, but cycle time considerations should be considered where large numbers of compounds are present. Lastly All-Ions is recommended for comprehensive screening workflows, sample archiving and retrospective analysis, whilst still facilitating quantification. This study validated HRMS acquisition approaches for quantification (based upon precursor data) and exploration of identification workflows for a range of PFAS compounds, in efforts to explore the strengths and weaknesses of different QTOF acquisition methods and provide further comparisons to current triple quadrupole mass spectrometry (TQ) multiple reaction monitoring (MRM) acquisition.

Keywords: Quadrupole Time-of-Flight Mass Spectrometry (QTOF), Emerging Contaminants, Suspect Screening, Analytical Chemistry, EPA Method 533.

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Evaluation of Monometallic and Bimetallic Rhodium Catalyst Activation by ESI-MS/MS

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When used as catalysts, bimetallic homogeneous complexes have frequently been shown to outperform their monometallic analogues. However, the reasons for this are not fully understood. For this, a set of mono and bimetallic carbene/phosphine ligated rhodium(I) complexes were synthesised. Importantly, the design ensured the electronic and steric environments for all metal centres were effectively identical. Further, for the bimetallic complex, flexibility was incorporated into the design to allow for metal-metal proximity (ca. 3.6 – 6 Å) usually required for the bimetallic enhancement. Interestingly, the bimetallic enhancement was only seen when the coligand (ligand removed to activate the catalyst) was 1,5-cyclooctadiene (COD), resulting in ~8 times faster catalysis reaction. When CO-s were used as the coligands, no improvements were seen.

The challenge in this work was understanding the reasons behind these coligand removal-mediated catalyst activation differences. As the metal centres for the catalysts were effectively identical, a suitable analytical approach was crucial. The solution to the challenge was found using ESI-MS methodology. The binding energy strength of the COD coligands was evaluated using collision-induced dissociation with ESI-MS/MS in an ion trap. This setup required using air-free solutions and low dry gas temperatures (60 ^{o}C) in fluorobenzene as a less coordinating solvent. From the results, a key trend was identified – the mass normalized collision energy at 50% parent loss was lower (0.269 mV) for the bimetallic complex compared to the monometallic (0.335 mV), suggesting that the COD coligand is less strongly attached to of the bimetallic complex. Additionally, the data suggested that some H-transfer could be occurring.

Overall, the use of the ESI-MS/MS setup acted as a crucial tool providing a direct comparison of the mono- and bimetallic systems during the catalyst activation step – a concept that has never been investigated prior to this work, providing new knowledge for future catalyst design.

Keywords: bimetallic catalyst, binding energy, collision induced dissociation

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Origins of Antibiotic Protomers: Tuning and Separation in Plasma AP-MALDI

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Matrix assisted laser desorption ionization (MALDI) is one of the most advantageous ionization methods to produce gas-phase ions in biological tissue imaging mass spectrometry (MS)—boasting unprecedented high spatial fidelity and preservation of analyte molecules. One key drawback in MALDI-MS is poor ionization yields and this has sparked the development of hyphenated secondary ionization methods including MALDI-2, MALDESI and in-line plasma sources. For plasma-based sources, the mechanisms of ionization are poorly understood and requires experimental and theoretical intervention. For molecules with multiple protonation sites, this poses an additional challenge to identify the key conditions that decide which site the proton binds to. Fluoroquinolones are a well-known class of antibiotic drug molecules that exhibit multiple protonation sites with unique structural properties, which exhibit well-defined collisional cross sections and bifurcated fragmentation profiles.

In this study, we determine the key ionization source parameters that control the site of protonation in ten fluoroquinolone target molecules. The first setup is a MALDI timsTOF flex mass spectrometer. The second setup consists of a custom-built atmospheric pressure MALDI stage coupled with a Plasmion plasma source attached to a Bruker timsTOF Pro mass spectrometer. Traditional MALDI (timsTOF flex) experiments revealed that all fluoroquinolones exhibit the lowest energy keto protomeric form. However, fluoroquinolone ions generated with plasma (timsTOF Pro) exhibited protomers (keto and amino protonated) and their ratios were molecule dependent. This highlights that there are remarkable differences in the ionization mechanism, and care must be taken when identifying protomer ions across conventional and plasma post ionization sources. The protomer ratios were skewed to the keto forms upon introduction of methanol vapor near the plasma source and conversely, acetonitrile vapor skewed the ratios to the amino protonated forms. This highlights that solvent doping in plasma sources is a potent strategy to tune protomer populations before mass spectrometric interrogation.

Keywords: Ionization mechanisms, protomers, isomerization, Ion molecule reactions, Fluoroquinolones

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Structure and Function of Cryptophanes and their Cationic Inclusion Complexes by High Resolution Ion Mobility Mass Spectrometry

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Understanding microscopic intramolecular interactions is key to effectively harnessing the power of molecular recognition and molecular complementarity for designer applications. Water soluble cryptophanes can selectively encapsulate a variety of cations, leading to a range of promising applications from medical imaging to environmental water remediation. To gain detailed insight into the processes that underpin the molecular recognition of specific cations by water soluble cryptophanes, a series of 7 cryptophanes with modified structures, (cryptophane-222(OH)6, cryptophane-223$(OH)_6$, cryptophane-233$(OH)_6$, cryptophane-224$(OH)_6$, cryptophane-233$(OH)_7$, cryptophane-222$(COOH)_3(OH)_3$ and cryptophane-222$(COOH)_6$) and their complexes with alkali metals (Li, Na, K, Rb, Cs) or ammonium cations were examined with multistage mass spectrometry and ion-mobility mass spectrometry. The periodic relationship between cryptophane-cation host guest structures revealed an initially large structure with Li, followed by a reduction in size with larger alkali metals. The significant effect of the cation on the structure of the host-guest complex was detected using both conventional TWIMS and cyclic TWIMS.

Keywords: TWIMS, host–guest chemistry, ESI-MS, molecular recognition, collisional cross section

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Electrostatically tuning the low-lying excited states of Acetophenone using simple cations (H+, Na+, Li+, K+) in the gas phase.

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Control over the photon energies that activate molecular photoinitiators is a developing area of research – the typical desire is to red-shift the activation wavelengths to reduce damage to co-located molecules and thus to more selectively target the photoinitiator amongst the matrix. The ability to red-shift the action spectrum is desirable for applications including in the curing of surface coatings, paints and protective coatings. In this fundamental research we target the effects of simple cations bound to a common chromophore, acetophenone, to probe the shift in the action spectra and rationalise the outcomes using quantum chemical calculations and electric-field computation.

These dimers were investigated using a linear quadrupole mass spectrometer coupled with a ns pulse-width Nd:YAG laser to measure dissociation between wavelengths 225-350 nm. Experimental results have been coupled with computational methods (TDDFT and SCS-CC2) to further develop modelling of the effect oriented electric fields offer regarding dissociation tuning.

The low lying-excited states of the acetophenone-cation dimers noticeably shifted when compared to neutral acetophenone. It was seen that significantly shifting these excited states led to altering the preferred dissociative pathway, potentially shutting off the desirable α-cleavage mechanism. Action spectra were obtained for each dimer, and the maximum wavelength of dissociation was compared against the electric field strength offered by the bound cation. The shift in the $^{1}\pi\pi^{*}$ excitation energy (TDDFT) and experimental results vs electric field strength offered by the cation followed a linear trend, where the stronger the electric field, the more red-shifted the excitation and dissociation wavelengths. The electric field effect of protonation resulted in the inability of the molecule to undergo α-cleavage, resulting in a variety of different photoproducts.

Keyword: -

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Unlocking Bioactive Peptides: Enhancing Mammalian Milk Proteome Analysis with Liquid Chromatography-Tandem Mass Spectrometry

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Marsupial milk has a unique proteome that changes over lactation to match the requirements of the joey. Recent publications show koala-specific genes expressed in the mammary gland have anti-microbial properties, and in other marsupials, there is evidence for gut- and lung-specific growth factors delivered to joeys through milk. Several types of milk formula are commercially available for hand-rearing joeys, though these are invariably made from cow milk as the base ingredient and do not contain koala-specific components. If key proteins are missing from milk used to hand-rear joeys, then joey development might be improved by identifying these proteins in koala milk, and reintroducing them to milk formula to aid conservation efforts.

To study the proteome and peptidome of such unique samples, sample preparation procedures for liquid chromatography-tandem mass spectrometry must be optimised using commercially available raw milk sources. The study will seek to optimize the methods used for proteome analysis by testing the efficacy of filtration-assisted sample preparation and in-solution digestion. Likewise, protein precipitation, solvent-solvent extraction, and filtration procedures will be experimented with to determine the most effective approach for peptidomic analysis. We present data on raw jersey cow, and raw camel milk which like marsupial milk is uniquely low in lactose. This raw data and existing koala genome datasets are explored for previously-identified bioactive peptides. This insight will serve as the template for ranking sample preparation methods in milk proteomics and peptidomics. We will ultimately use these methods in our efforts to explore koala milk proteome which will aid their conservation.

Keywords: Koala, proteome, peptidome, bioactive peptides

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Global lipidomics analysis reveals metabolic changes from treatment with antipsychotics and subsequent cannabidiol administration in healthy volunteers.

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Randomised clinical trials support the use of cannabidiol (CBD) as a treatment for schizophrenia, with clinical evidence that CBD ameliorates both positive and negative symptoms, without adverse-effects commonly experienced with antipsychotics. While yet to be clarified, CBD's mechanism is well-understood to elicit metabolic response in lipid-derived agonists of the endocannabinoid system, suggesting CBD may exert its therapeutic properties, in part, through lipid signalling or modifications to lipid homeostasis.

To investigate, we analysed lipid profiles of healthy volunteers in a drug-integration study of CBD with second-generation antipsychotics. Volunteers were administered with amisulpride (150 mg, n = 7), olanzapine (10 mg, n = 8), quetiapine (300 mg, n = 6), risperidone (3 mg, n = 10), or placebo (n = 7) for ten days, with adjunctive CBD (800 mg) from day 4 till study completion. Untargeted liquid chromatography-tandem mass spectrometry quantified the relative expression of more than 2500 lipids belonging to 20 lipid species in serum collected at baseline (day 0), post-antipsychotic (day 3) and drug-combination treatment (day 9).

Multivariate analysis (sPLS-DA, OPLS-DA) revealed 19 lipids to be significantly disrupted following antipsychotics and CBD administration, belonging to four lipid species (ceramide, phosphatidylcholine, sphingomyelin, and triglyceride) commonly associated with schizophrenia pathology, with regulatory trends suggesting a potential influence of fatty-acid chain length on triglyceride regulation. Notably, a further refined list of three lipid candidates exhibited excellent ability to distinguish post-antipsychotic and combination therapy (area under the receiver operating curve > 0.8), with CBD effectively restoring these putative lipid markers back to baseline levels following their disruption with antipsychotics.

To our best knowledge, this is the first reported examination of human lipids following CBD, post-antipsychotic treatment. Our findings indicate that assessment of relatively accessible fluid lipids could support future diagnostics for monitoring treatment in schizophrenia and enhance understanding of molecular mechanisms behind CBD's efficacy.

Keywords: Lipidomics, high-resolution mass spectrometry (LC-MS/MS), cannabidiol, antipsychotics, schizophrenia and mental health.

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Identification of the lipids on the skin we're in

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Sebum is produced by sebaceous glands and released as an oily secretion onto the surface of human skin. Fatty acids are abundant and diverse in sebum; both as free fatty acids and esterified to complex lipids. Sebum fatty acids exhibit a range of unusual structural features including site(s) of unsaturation and chain branching that are not observed in other aspects of the human lipidome. Challenges in identifying these structures mean that the precise composition and function of sebum remains to be determined. Despite these challenges, recent studies have suggested that the skin lipidome may provide biomarkers for neurodegenerative diseases such as Alzheimer's and Parkinson's disease. These discoveries motivate an urgent need to unravel the complexity of skin lipids and better understand this crucial component of the skin we're in.

Sebum samples were first collected by swabbing the nose and forehead. Swabs were extracted with methanol, hydrolysed to release esterified fatty acids and derivatized using 1-(4-(aminomethyl)-3-iodophenyl) pyridin-1-ium. Thus derivatised, samples were subjected to parallel workflows utilising C_{18} reversed-phase chromatography hyphenated with (i) photodissociation mass spectrometry and (ii) ozone-induced dissociation mass spectrometry. Analysis of sebum extracts using these approaches revealed upwards of 400 distinct fatty acids with carbon chain lengths ranging from 9-38 carbons with degrees of unsaturation ranging from 1-4 double bonds. Saturated fatty acids exhibited a high degree of monomethyl-chain branching with even-numbered carbon chains exhibiting a preference for branching at the penultimate position (*iso*) compared with odd-numbered chains where substitution at the antepenultimate position (*anteiso*). In addition, other non-canonical methyl branched points were identified along with unusual dimethyl-branched chain structures. The most prevalent double bond position in unsaturated fatty acids was found to be *n*-10 with examples of branched-chain unsaturated and branched-chain polyunsaturated lipids described here for the first-time (e.g., *iso*-FA 18:1*n*-10 and *anteiso*-FA 19:2*n*-11,14). These discoveries suggest potentially new lipid metabolism that may be responsive to age, environment or disease state.

Keywords: sebum, fatty acids, photodissociation, ozone-induced dissociation

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LipidQuan -Targeted Lipid Profiling solution

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Lipid metabolism is complex and involves a large number of metabolic reactions resulting in an enormous number and variety of actual lipid entities within living cells. LIPID MAPS (http://www.lipidmaps.org/) currently stores more than 40000 lipid structures. The situation is further complicated by the wide dynamic range of lipid concentrations which can vary by 106 or more (from nanomolar fatty acids to attomolar eicosanoid lipid mediators). The level of precision of most systems-wide measurements is not yet sufficient to detail specific levels or concentrations of cellular components.

In order to compare lipidomic data across laboratories absolute quantitative data must be used since relative values can vary widely not only between laboratories but also between instruments due on various factors including analyst errors, sample preparation method differences (e.g. extraction methods) and ion suppression effects when using ESI MS etc. The lack of accurate characterisation of the lipid species also severely hinders interpretation of disruptions to the lipid metabolism associated with disease and physiological states.

The proposed platform is an integrated high throughput analytical tool for accurate and robust measurement (>1500 injections) of a carefully selected set of lipid species (~currently 500) from sample preparation through to data handling and pathway elucidation. The platform can also be used for more in depth targeting of specific class of lipids of interest. Validation of the chromatographic method was performed at multiple sites (Wilmslow, Beverly and Duke University) by different analysts to show robustness and ease of method transfer.

The calibration, system suitability and QC standards used in this platform will be sourced pre-mixed from commercial vendors (Avanti Lipids). SymphonyTM Software will be used to automate the entire workflow and integrated with Skyline (MacCoss Lab Software (https://skyline.ms/project/home/begin.view) for data processing to enhance efficiency and flexibility. Once quantitative data has been generated and processed, pathway mapping tools and discovery tools by XCMS/METLIN can also be confidently applied to determine biological relevance of changes in concentration and make data comparisons between laboratories.

Keywords: Lipidomics, High-throughput, targeted, profiling, inter-laboratory.

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Fragment screening by native mass spectrometry to find novel inhibitors of virulence in *Burkholderia pseudomallei*

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With no targeted treatments available, the rare tropical disease melioidosis continues to infect and kill. With an average of 10 cases in Australia annually, it is most often diagnosed at a late stage of infection where antibiotics are no longer effective, resulting in a mortality rate of 20%. The bacterium responsible; *Burkholderia pseuodmallei;* presents a challenge in developing new targeted anti-microbials due to its innate drug-resistance. Drugs that target virulence rather than bacterial viability offer an alternative mechanism of action to classical bactericidal antibiotics, that may limit the development of acquired drug-resistance.

Cyclophilins PpiA and PpiB enzymes produced by *Burkholderia pseudomallei* have been identified as essential for virulence. Their deletion results in a non-virulent phenotype due to their function in the cis/trans isomerization of proline residues in newly translated virulence factors. Exploring PpiA and PpiB as drug targets could lead to a targeted therapy, whereby their inhibition disarms the bacterium, allowing the hosts immune system to elicit a more effective response.

Fragment-based drug discovery (FBDD) is a fast-growing innovation that allows novel drug leads to be uncovered. Combining this with the medium throughput screening technique, native mass spectrometry as a preliminary fragment screening method, allows a large area of chemical space to be explored, leading to a diverse range of potential drug leads.

This poster will discuss the utility of native mass spectrometry (nMS) in our FBDD approach, and the features of FT-ICR and nanoESI that facilitate mass analysis under native conditions. Preliminary results from the study of PpiA and PpiB protein-fragment interactions will also be discussed, including crystallography trials, nMS screening trials and intentions to screen both proteins against a 720-member fragment library.

Keywords: Fragment-based drug discovery, native mass spectrometry, burkholderia pseudomallei, anti-virulence targets

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Development of highly sensitive mass spectrometry methods for phosphorylated protein analysis

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Alzheimer's Disease (AD) is by far the most common cause of dementia, which makes developing an effective diagnosis method of AD essential. The overarching aim for this project is to develop an effective mass spectrometry-based analysis workflow for detection of salivary phosphorylated tau protein as a biomarker in the early diagnostic stage of Alzheimer's Disease. Such investment is important to overcome current limits in detecting low abundance salivary AD biomarkers and contributing to the development of AD screening in clinical trials. The approach to build up a mass spectrometry (MS)-based clinical diagnosis method involves utilising mass-tag enrichment and drift time differences in ion-mobility MS to improve sensitivity for phosphorylated peptide analysis.

Our approach exploits development of a novel ion mobility mass spectrometry-based shift reagent. Here we incorporate features for both enrichment and enhanced analytical identification. The masstag we have chosen for enrichment is a derivative of the commercial Phos-tag, a metal complex containing two bis(pyridylmethyl)amine moieties chelating with dinuclear Zinc(II) cations, with additional disulfide linkage connecting one pyridine group and biotin. The mass-tag is activated by solid phase streptavidin-biotin interaction. Phosphorylated peptides are enriched with this mass-tag under solid phase conditions, where oxygen atoms of phosphate and the pair of zinc cations are coordinated tightly. The tagged phosphorylated peptides are readily cleaved from biotin through reduction of disulfide bonds. Improvement of MS sensitivity is observed through phosphorylated peptide enrichment when compared to methods using TiO2. A distinguishing shift in drift time vs m/z from un-tagged phosphopeptide to tagged phosphopeptide was observed, assisting in rapid identification and characterisation.

Keywords: Alzheimer's disease, p-Tau, phosphoproteomics, mass-tag, IM-MS

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Australian Proteome Analysis Facility: Supporting proteomics research in Australia for over 25 years

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The Australian Proteome Analysis Facility (APAF), hosted at Macquarie University, has been providing state-of-the-art proteomics and mass spectrometry services for Australian researchers since it's foundation over 25 years ago. APAF is supported by NCRIS through BioPlatforms Australia and is proudly NATA accredited for proteomics analyses to the ISO 17025 standard. We have a strong track record in delivering high quality results for our research and industry partners and collaborators.

APAF houses a wide range of mass spectrometers to cater for a variety of different MS based proteomics experiments. Our team of highly experienced scientists are ready to support your research with advice on experimental design, performing sample preparation and data acquisition, and reporting, statistics and bioinformatic analysis. We support most MS-based proteomics techniques including protein identification from intact proteins or digests, quantitative proteomics (label free DIA and DDA, or labelled experiments), protein-protein interaction analysis (by crosslinking MS, co-immunoprecipitations/pulldowns and other methods), post-translational modification analysis (including phosphorylation, methylation, redox and glycan analysis), and targeted MS assays. Our team is also experienced in handling difficult sample types and analysis of non-model organisms. APAF can further analyse proteins using non-MS based techniques such as amino acid analysis, or emerging techniques such as mass photometry.

Here, we highlight our expanding capability for protein analysis, including cutting edge instrumentation and advances in method development, and highlight success in collaboration with researchers and industry from across Australia and the world.

Keywords: proteins; proteomics; mass spectrometry; native; top-down; bottom-up;

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Proteomics Profiling of *Burkholderia cenocepacia* K56-2 Infection in THP-1 Macrophage Cells

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Burkholderia cenocepacia is a bacterial pathogen that commonly infects cystic fibrosis (CF) patients. It's innate antimicrobial resistance and ability to replicate within macrophages results in persistent lung infections. Conventional proteomics profiling of infected macrophages is challenging due to the low infection index of *B. cenocepacia*, resulting in only a few cells infected within *in vitro* models, limiting the fields' ability to understand the pathways subverted during intracellular replication. To improve proteomic studies on the impact of *B. cenocepacia* on macrophages we have explored approaches to increase the uptake of *B. cenocepacia* K56-2 using opsonization with polyclonal anti-*Burkholderia* serum and THP-1 macrophage cells. Opsonization increases bacterial uptake at 3hrs post-infection accompanied by a significant increase in viable intracellular bacteria. Proteomics analysis reveals opsonization improves the detection of *B. cenocepacia* within infected macrophages with three times increase in quantity of bacterial proteins detected across replicates compared to unopsonized infections. Importantly, opsonization does not drive drastic effects on the host proteome compared to uninfected controls with few proteome alterations detectible at 3 hrs. In contrast, at 24hrs post-infection, we observed pronounced differences in the proteomic profiles of infected THP-1 macrophages compared to uninfected controls revealing bacteria driven proteome changes. These findings demonstrate more than 100 host proteins were differentially expressed at 24hrs post-infection and functional enrichment analysis identified several cellular processes associated with *B. cenocepacia* infections including cytokine and interferon-mediated signalling pathways. Within opsonized *B. cenocepacia* infections, the ability to improve the detection of the *B. cenocepacia* proteome confirms the expression of known virulence factors during intracellular replication including T6SS and T2SS-associated proteins. Altogether, these results support opsonization coupled to proteomic analysis provides a mechanism to begin to dissect the impact of *B. cenocepacia* on macrophage cells addressing the issue of low infectivity.

Keywords: Proteomics, *B. cenocepacia*, Infection, Macrophages

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Proteome profiling of potato and brewers spent grain waste informing commercial outcomes

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Food waste in Australia is an ever-present economic and environmental issue, costing the Australian economy $37 billion annually with 7.6 million tonnes of food wasted per year and contributing up to 3% to Australia's greenhouse-gas emissions. Transforming these waste streams to new viable products is therefore vital. Herein, we have utilised bottom-up proteomic approaches to identify the composition of potential bioactives in potato and brewers spent grain waste. In addition, we also profiled the bioactivity (primarily neuroprotective, antioxidant and antimicrobial properties) of potato and brewers spent grain waste. Together, the proteome identification and bioactive profiling of these waste streams will greatly inform and guide the production and transform agricultural and food waste into commercial and biomedical products.

Keywords: proteomics, bioactive profiling, food waste.

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Mapping of a CNS Copper-Delivering Agent and its CYP-450 Metabolites in Mouse Brain Regions

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Transition metals such as copper (Cu) are essential for the health and function of neurons; regional dyshomeostasis of Cu is a major hallmark of central nervous system (CNS) conditions including amyotrophic lateral sclerosis (ALS) and Parkinson's Disease (PD). Thus, established or emerging therapies for these disorders aim to restore CNS Cu levels from pathological levels (high or low). Treatment with Cu-delivering agents (e.g., Cu(II)-ATSM) for ALS and PD patients addresses the localised Cu-deficiency observed in the Cu-proteome within the CNS. Yet, there is no direct evidence for the Cu-delivery agent or related metabolites to localise in central nervous tissues, or if and how these enter the cell. There is only indirect evidence of their effect through detected changes of transition metal concentrations within CNS sections, e.g., through laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS).

This project aimed to (1) determine the phase I and II metabolites of Cu(II)-ATSM via a liver microsome assay followed by MS-led discovery metabolomics and (2) employ a targeted metabolomics approach using high-resolution mass spectrometry (HR-MS) and HR-MS imaging to determine the spatial distribution of neuroprotective metal-delivering agents and their metabolic derivatives in individual mouse brain sections derived from a treatment pilot study. In the microsome assay (0-60 min incubation), six compounds have been identified as phase I and II metabolites. These metabolites were mapped by targeted MS/MS in dissected tissues, with MS imaging to occur next. Downstream, the disease-relevant transition metals will be mapped in the same tissues by LA-ICP-MS to validate the mechanistic effects of the identified compounds in the tissues. The scoped work will provide complementary data and direct insights into molecular mechanisms underpinning effects of a Cudelivery agent in the CNS.

Keywords: mass spectrometry imaging, metal dyshomeostasis, copper

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Interactive Design and Application of MassQL Queries after Preprocessing for the Annotation of PFAS in LC-TIMS-PASEF data

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We present a new workflow for the design and application of MassQL queries within an interactive data exploration software for untargeted metabolomics. The Mass Spec Query Langauge (MassQL) is a domain specific language meant to be a succinct way to express a query in a mass spectrometry centric fashion. Here it is applied to data after its untargeted preprocessing. Due to the full integration into the MetaboScape software, the workflow provides multiple tools for the identification and validation of characteristic rules for compound classes of interest. These rules can then be formalized as MassQL queries, and subsequently be applied to reveal other, potentially unknown compounds fulfilling these rules.

Starting with 48 PFAS that could be uniquely annotated using a target list, characteristic fragments and neutral losses have been identified. To this end, SmartFormula3D and MetFrag in-silico fragmentation have been applied, revealing fragments like C2F5- or C3F7-, C4F9- from homologous rows, but also neutral losses like C3F6, CO2 to be common for certain groups of PFAS. Compounds grouped by such common features were then evaluated to find their ion mobility ranges. These rules (fragments, neutral losses and ion mobility ranges) have then been formalized into MassQL queries, which then were used to filter the same feature table. One example is the following, which comprises the above mentioned homologous row and further narrows down to the ion mobility range of the previously known PFAS:

QUERY scaninfo(MS2DATA) WHERE MS2PROD=118.987:TOLERANCEMZ=0.005 AND MS2PROD=168.987:TOLERANCEMZ=0.005 AND MS2PROD=218.986:TOLERANCEMZ=0.005 AND MOBILITY = range(min=120,max=210). This exposed yet unidentified compounds featuring the common characteristics and similar Kendrick Mass Defects. For some of them their generated molecular formulas helped finding structure candidates in the ChemSpider database, validated using MetFrag in-silico fragmentation.

Keywords: VIP-HESI, massQL, Environmental, timsTOF

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Microflow 4D-Proteomics for robust, high-throughput sample analysis

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The potential amount of information obtained from a single sample is increased at lower flowrates due to a concomitant increase in ionization efficiency. However, nano-flow chromatography suffers from overhead times, and relatively low reliability particularly for samples prone to aggregation due to narrow inner diameter capillaries, columns, and emitters. Advancements in ion transmission, desolvatization, and detection technology continuously improve the sensitivity of mass spectrometers, providing the means to apply higher flowrates for peptide separation while retaining high sensitivity at low sample amounts. In this context, we evaluated the Shimadzu LC with microflow capability with the newly developed microflow sprayer for the VIP-HESI source with respect to gradient performance, chromatographic peak shape and width, retention time stability, and overall proteomics performance.

An analytical flow pump / microflow pump setup was used for direct as well as trap-and-elute injections (Shimadzu LC-40B X3 and LC-Mikros JP). For peptide separation, a ReproSIL Saphir 100 C18 column (150 x 1 mm, Dr. Maisch HPLC GmbH) was applied. For improved ionization, 3% DMSO was added to the solvents. Eluting peptides were detected with a timsTOF HT mass spectrometer. The mass spectrometer was equipped with a VIP-HESI source with a 50 µm inner diameter ESI metal capillary inserted.

Chromatographic separation performance and retention time stability over hundreds of injections were confirmed with a spiked-in mixture of 40 synthetic peptides (PROCAL, JPT Peptide Technologies GmbH). An ESI needle with 50 µm inner diameter provided efficient ionization at long lifetime. PASEF and dia-PASEF acquisitions were optimized to provide accurate quantitation and analytical depths.

Keywords: VIP-HESI, microflow, proteomics, timsTOF

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Exploring Brain Extracellular Vesicles through Discovery Driven Proteomics Uncovers Singluar Molecular Clues Regarding The Elevated Occurrence of Dementia in Individuals with psycosis

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Schizophrenia (SCHZ), a mental disorder, is linked to various co-morbidities, including a higher prevalence of age-associated dementia. While extracellular vesicles (EVs) have been implicated in brain pathology, neurodegeneration, and dementia, their specific role(s) in SCHZ remains largely unknown. Furthermore, the impact of brain EVs on the development and progression of dementia in individuals with schizophrenia is yet to be defined.

In this study, discovery-driven proteomics was employed to examine the proteome compositions of brain extracellular vesicles (EVs) in post-mortem brain tissues (prefrontal cortex, BA9). The subjects included individuals with schizophrenia (SCHZ) and those with preclinical Alzheimer's disease (n=40). The PROSPR method was used to isolate brain EVs, and advanced bioinformatics analysis was conducted to identify shared dysregulated molecular pathways between these two disease conditions.

Brain extracellular vesicles (EVs) exhibit remarkable consistency in the regulation patterns of proteins previously implicated in important neurodegenerative processes associated with aging. Proteins such as Glial Fibrillary Acidic Protein (GFAP), Immunoglobulins, myelin basic protein (MBP), and the microtubule protein TAU show strikingly consistent patterns of regulation in brain EVs across both schizophrenia and Alzheimer's disease.

The data obtained from our study offers novel insights into the potential role(s) of brain extracellular vesicles (EVs) in the increased occurrence of aging-associated dementia among individuals with psychosis. These findings have the potential to contribute towards the identification of novel molecular targets for the disease.

Keywords: -

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Exploring connectome imbalances in Schizophrenia through the in-depth characterization of brain extracellular vesicles

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Neuroimaging studies examining resting state parameters have revealed the presence of a chaotic connectome in individuals with schizophrenia (SCHZ). However, the molecular mechanisms underlying these network imbalances remain poorly understood. Likewise, while extracellular vesicles (EVs) are known to facilitate intercellular communication in the brain during both healthy and diseased states, their specific role(s) in the context of the brain connectome in schizophrenia have yet to be explored.

Brain extracellular vesicles (EVs) were collected from 30 individuals using the PROSPR method from three key brain regions that are known to play a central role in the imbalances observed in the resting state connectome in schizophrenia (SCHZ). These brain regions include the prefrontal cortex, caudate, and hippocampus. Following the collection, we employed a discovery-driven next-generation proteomics approach to examine and characterize the proteomes of the brain EVs, as we previously described. Subsequently, we conducted parametric and non-parametric variance and correlation analyses ($p<0.05$) to analyze the proteomics data obtained.

Our findings reveal disrupted regulation of distinct synaptic proteins associated with brain extracellular vesicles in individuals with Schizophrenia (SCHZ). Additionally, we identified a network of molecular exchange, mediated by EVs, in the caudate region, involving myelin basic protein (MBP), Collapsin Response Mediator Protein 2 (DPYSL2), and glial fibrillary acidic protein (GFAP). This network exhibited significant imbalances (Fischer's correlation coefficient $p<0.001$) specifically in SCHZ.

Our research suggests that brain extracellular vesicles (EVs) play a role in dynamic intercellular molecular exchange networks, which could serve as indicators of connectome imbalances associated with brain pathology and Schizophrenia (SCHZ). Moreover, our Systems Biology proteomics data strengthens the idea that the molecular underpinnings of psychotic mental disorders can be identified, emphasizing the need for further exploration to benefit individuals within these clinical populations.

Keywords: -

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Unveiling the Plasma and Tissue Lipid Profiles of a Murine Model of Endometriosis

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Endometriosis is a chronic, benign disease, characterised by endometrium-like tissues growing outside the uterus. The gold standard for diagnosis involves surgery, however there is a multi-year delay to diagnosis and treatment. Despite the urgent need, little progress has been made in discovery and validation of minimally-invasive diagnostic biomarkers for endometriosis. In this regard, animal models may contribute to the investigation of endometriosis pathogenesis and biomarker discovery. Using a preclinical murine model of endometriosis, the first aim of this study was to utilise mass spectrometry (MS) to investigate and compare lipidomic profiles of plasma from endometriosis and sham mice. The second aim was to employ MS imaging to spatially characterise endometriotic lesions from mice, and to identify if lipids and metabolites found in lesions correlate with those found in plasma.

Comparative analysis of plasma lipids found 57 features to be associated with endometriosis. These included a range of fatty acids, di- and triacylglycerols, lysophospholipids, phosphatidylcholines, phosphatidylethanolamines, phosphatidylserines, and sphingolipids. MSI analysis identified 32 discriminatory features between endometriotic lesions and fat tissues. The main lipids identified included phosphatidylcholines and phosphatidylethanolamines. One lipid, PC 34:2, was conserved in both plasma and endometriotic lesions from endometriosis mice, making it a potential candidate as a minimally-invasive diagnostic biomarkers of endometriosis.

This investigation demonstrated the utility and limitations of murine endometriosis models and MS in identification of aberrant metabolic profiles between cases and controls, providing information on how lipids and metabolites may be contributing to disease pathophysiology. It also highlighted the potential of MSI for direct characterisation of endometrial tissue and endometriotic lesions. While a biomarker candidate was identified in this study, further research with larger cohort sizes and validation in humans with endometriosis is warranted. The information generated provides information on how lipids and metabolites may be contributing to disease pathophysiology.

Keywords: endometriosis, minimally-invasive diagnostic biomarkers, preclinical murine model, lipidomic profiles, mass spectrometry (MS) and MS imaging

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HiPLEX-IHC MALDI Imaging of FFPE Kidney tissue at 5µm utilizing microGRID on timsTOF fleX MALDI-2

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Matrix assisted laser desorption ionization (MALDI) imaging is a widely accepted methodology for determining spatial localization of analytes on tissue. Intact protein has always been of interest; however, ionization efficiencies and resolution of the images have been a challenge and trade off when imaging intact proteins. As such, methodologies have been developed to overcome this ionization limitation, such as the MALDI HiPLEX IHC workflow, and in conjunction with new spatial resolution technologies on the timsTOF fleX platforms (microGRID), high spatial resolution profiling of intact proteins in a multiplexed fashion becomes possible.

FFPE human kidney tissues were first prepared using the standard MALDI HiPLEX IHC workflow. Briefly, three antibodies with photocleavable peptide tags (Vimentin, Histone H2A, and ATPase-1A1) were bound to tissue overnight. Peptide tags were then released using UV light and CHCA matrix applied. Matrix recrystallization was performed, followed by analysis on a timsTOF fleX MALDI-2 at 5µm using microGRID.

The MALDI HiPLEX-IHC workflow was evaluated on FFPE kidney tissues at spatial resolution of 20µm, then 5µm. The three peptides associated with the antibodies were at 1222.79 m/z for ATPase-1A1, 1230.84 m/z for Vimentin, and 1226.82 m/z for Histone H2A. Overlay of the three corresponding masses showed significant localization of the peptides to areas predicted to be rich in the protein of interest (glomeruli for Vimentin, etc.). A secondary higher multiplexed experiment included the original three antibodies plus CD8α (1350.76 m/z), CD68 (1216.75 m/z), HER2 (1210.74 m/z), and Collagen 1A1 (1234.87 m/z). Both imaging runs successfully localized and identified all antibodies used while containing minimal to no artifacts at 5µm resolution.

This work demonstrates the highly desirable capabilities MALDI HiPLEX IHC coupled with the high spatial 5µm resolution from microGRID.

Keywords: MALDI imaging, microGRID, FFPE, timsTOF

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P-45 has been withdrawn.

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Fetal Plasma Metabolite Analysis through Lipidomics and Isotope-Ratio

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Maternal diet has a major impact on the intrauterine environment and consequently influences fetal cardiac development, leading to long-term impact on cardiac function and structure. The potential of exposure to monosaccharide fructose and disaccharide sucrose (table sugar) during pregnancy on fetal heart structure remains elusive. Consequently, it is crucial to fully understand the exact nature of deleterious effects of saccharides and the consequences of nutrient displacement during pregnancy to establish optimal dietary guidelines to support healthy fetal cardiac development. The aim of this study is to understand the effects of high saccharide diets (found in highly processed carbohydrates) and dietary lipids on the fetal heart structure.

Plasma samples and cardiac ultrasound images were taken from pregnant mothers enrolled in the landmark BABY1000 study at the 36th gestational week. Plasma metabolites were analysed using Q Exactive Orbitrap Mass Spectrometer (MS) coupled with UHPLC for untargeted lipid profiling. Levels of carbohydrate processing and animal protein origin were determined using an elemental analyser isotope ratio MS (EA-IRMS) analysis of $\delta^{13}C$ (ratio of ^{13}C to ^{12}C isotopes) and $\delta^{15}N$ (ratio of ^{15}N to ^{14}N isotopes) respectively.

In this study, we measured 794 plasma lipids from our untargeted lipidomic profiling. Among these lipids, 88 lipid species—encompassing triglycerides (TGs), phosphatidylethanolamine, sphingomyelins (SMs), phosphatidylcholines—exhibited significant upregulation ($p < 0.05$) in response to dietary sugar and protein consumption. Several lipid species—TGs, SMs, ceramides–were also found to be responsible for various changes in fetal heart structure, especially the right ventricular size and the interventricular septum diameter.

The results suggest a profound change in cardiac lipids across many classes that are responsible for diverse function like cell signalling in response to a highly processed and animal protein content of maternal diet. Further studies with a larger sample cohort—through the Barcelona IMPACT study—are currently underway to confirm the relationships we have found.

Keywords: -

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