Abstract Book

Oral Presentations

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IMSC Plenary Lecture 1, Plenary 3, August 18, 2024, 17:00 - 18:00

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Professor Dame Carol Robinson[1]
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Membrane protein complexes – from recombinant complexes to regions of the brain

Membrane proteins are the gateways to cells and as such play critical roles in physiology, health and disease. Their important also makes them critical drug targets. Mass spectrometry is now contributing to these areas of research.

Methods

To distinguish membrane protein drug targets unequivocally new methods have been developed involving analysis directly from membranes and top-down IRMPD for sequencing and location of PTMs. Further recent developments will be presented.

Novel Aspect

The ability to sequence membrane proteins directly from membranes

Preliminary Data or Plenary Speaker Abstract

Early analysis of membrane proteins using mass spectrometry revealed challenges due to lipid and cofactor binding, incomplete detergent micelle removal, and multiple post-translational modifications. To address these complexities, we have been developing innovative strategies over the past decade or so. These efforts have enabled us to gain new insights into the relationship between lipid binding and post-translational modification. A key aspect of our approach has involved using an infrared laser to activate and dissociate membrane protein complexes.

In this presentation, I will discuss the evolution of our research, starting from bacterial membrane proteins such as ABC transporters, progressing to pivotal G-protein coupled receptors, and most recently expanding to study specific regions of the human brain. I will highlight the instrumental advancements necessary for our success at each stage and the valuable information we have uncovered as we progress.

Looking ahead, the ability to release complexes from natural membranes, vesicles, and tissues, coupled with advancements in soft landing techniques, suggests that mass spectrometry will play an increasingly crucial role in structural biology, healthcare, and understanding diseases.

Plenary - IMSF Thomson Medal Award Lectures, Plenary 3, August 19, 2024, 08:30 - 09:30

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Professor Richard Yost[1]
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Advances and Applications of Ultraviolet Photodissociation for Characterization of Biological Molecules

Ultraviolet photodissociation (UVPD) is an ion activation mode that results in extensive fragmentation of many classes of molecules, often more than obtained from most other MS/MS methods. Ion activation/dissociation can be accomplished using a single 5 ns laser pulse from a UV excimer laser. UVPD offers a versatile MS/MS technology for characterization of lipids, nucleic acids, peptides and proteins, including mapping modifications and localizing ligand binding sites.

Methods

For this work, ultraviolet photodissociation was implemented on several Orbitrap mass spectrometers outfitted with an optical window and using a 193 nm excimer laser. Ions were irradiated with one or more laser pulses in a trapping cell, and the resulting fragment ions were transferred to an Orbitrap analyzer for detection with high resolution and high mass accuracy. All ions were generated by electrospray ionization.

Novel Aspect

UVPD results in production of unique types of fragment ions that aid localization of modifications and offer structural insight.

Preliminary Data or Plenary Speaker Abstract

Advances in mass spectrometry instrumentation and experimental design have led to significant inroads in the characterization of biological molecules, thus translating to new applications in the field of lipidomics, biotherapeutics, proteomics and structural biology. For example, there has been growing interest in employing MS/MS strategies to examine native protein structures by disassembling the complexes and sequencing the constituent proteins in the gas phase. In the context of protein-ligand complexes, the relative abundances of fragment ions generated by UVPD correlate with variations in the intramolecular and intermolecular interactions that stabilize particular regions of the proteins. Owing to the fast, high energy deposition of UV photoactivation, products retaining non-covalently bound ligands are formed and reveal binding site information. For multimeric protein complexes, UVPD disassembles the complexes to reflect aspects of sub-unit architecture as well as generating sequence ions that identify the proteins. UVPD has also proven useful for elucidation of key structural details of lipids. This presentation will highlight the capabilities of UVPD for characterization of biological molecules and cover new strategies to improve the performance of UVPD.

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Professor Jennifer Brodbelt[1]
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The Role of Tandem (Hyphenated) Methods for Mixture Analysis

Tandem or hyphenated techniques such as LC/MS/MS have become the workhorses for trace analysis (the detection and/or quantitation of analytes present in a mixture at low levels). To understand the central role of these instrumental approaches in trace analysis, it is helpful to consider the concepts underlying tandem techniques, particularly as they relate to trace analysis. Key concepts include the role of sensitivity and selectivity, the increase in "informing power" provided by tandem techniques, and how these concepts can permit us to compare tandem techniques such as GC/MS to the individual techniques (GC or MS) when performed at their highest resolution. These concepts are well illustrated by many examples of tandem techniques for trace analysis, including biomedical and environmental analysis.

Methods

The triple quadrupole mass spectrometer, typically in combination with a gas or liquid chromatograph (GC/MS/MS and LC/MS/MS), is perhaps the most iconic example today of a tandem analytical instrument. Several characteristics of the triple quadrupole have made it the "laboratory workhorse" for trace analysis, including the remarkable efficiency of the low-energy collision-induced dissociation (CID) process in an RF-only multipole collision cell, the ease of computer control, and the capability for rapid scanning, rapid switching from mass to mass, and high transmission efficiency, enabling a wide variety of MS/MS scans. The efficiency of selected reaction monitoring means that triple quadrupoles dominate MS/MS for detection and quantitation of targeted compounds in trace mixture analysis.

Novel Aspect

Tandem analytical methods employing mass spectrometry have revolutionized trace mixture analysis over the past four decades.

Preliminary Data or Plenary Speaker Abstract

I will introduce the concepts underlying tandem or hyphenated analytical techniques and their importance for trace analysis and illustrate these concepts with examples of tandem techniques such as GC/MS/MS, LC/MS/MS, and ion mobility/MS/MS. I will then provide a personal perspective on the conceptualization and development of the triple quadrupole mass spectrometer, illustrating the roles of innovation, serendipity and persistence.
The triple quadrupole mass spectrometer has become the most common mass spectrometer in the world today, with sales of over $1 billion per year. It is the gold standard for quantitative analysis in metabolomics, clinical analysis, drug discovery/development, and environmental analysis. But when I proposed the triple quadrupole as the "ultimate computerized analytical instrument" as a new PhD student in Chris Enke's research group at Michigan State University in 1975, the NSF reviews were uniformly negative, with experts in the field unanimous that the proposed instrument would never work. Fortunately, the Office of Naval Research funded the proposal, and the instrument did work! A chance conversation with Jim Morrison at the 1977 ASMS Conference revealed that he had built (but not yet published) a triple quadrupole instrument for optical studies of ions; Jim invited me to visit his lab in Melbourne to take the first MS/MS data using low-energy CID. In the 45 years since, tandem mass spectrometry has evolved into a widely accessible, practical analytical technique. And

low-energy CID in an RF collision cell, which reviewers had considered impossible, is the basis of every modern MS/MS instrument.

One can hardly name a significant advancement in science that was not made possible by the invention and development of new tools to see something or measure something, from litmus paper to telescopes to tandem mass spectrometers. And common to these inventions and developments have been innovation, serendipity and persistence.

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Professor Yu Xia[1]
[1]Tsinghua University

Empowering Structural Lipidomics with Isomer-Resolved Mass Spectrometry

Keynote: Professor Yu Xia Tsinghua University 114 - Empowering Structural Lipidomics with Isomer-Resolved Mass Spectrometry, Plenary 3, August 19, 2024, 10:00 - 10:25

Structural lipidomics has emerged as a fast-developing research field, aiming to resolve lipid isomers undetected by traditional analysis methods, discover uncanonical lipid structures, and provide new insights into altered lipid metabolism linked to health and disease. Isomer-resolved mass spectrometry is central to structural lipidomics. Due to the lack of authentic standards of lipid isomers, isomer-resolved mass spectrometry must independently identify and quantify lipid isomers from complex mixtures. In this presentation, I will showcase the latest development of tandem mass spectrometry (MS/MS) methods which are capable of lipid analysis at detailed structural levels, including the C=C location, C=C geometry, sn-position, methyl branching, etc.

Methods

Offline Paternò-Büchi (PB) derivatization was coupled with liquid chromatography – tandem mass spectrometry (LC-MS). The PB reagents, lipid standards, and other chemicals were obtained commercially. A low-pressure mercury lamp (254 nm, BHK, Inc., Ontario, CA, USA) was utilized to initiate the PB reactions. MS data were collected on an X500R QTOF mass spectrometer (SCIEX, Toronto, CA) and a timsTOF mass spectrometer (Bruker Daltonics, Germany).

Novel Aspect

The development of isomer-resolved MS methods and lipidomic workflows for lipid analysis.

Preliminary Data or Plenary Speaker Abstract

Our group has developed a series of PB reagents for the derivatization of the C=C in various classes of lipids. The charge-tagging PB reagents can significantly enhance the detection limit for neutral lipids. The photo-catalysis PB reactions can achieve ~90% derivatization efficiency under visible light irradiation. A distinct advantage of PB-MS/MS lies in its high precision in the compositional analysis of C=C location isomers, which remains unaffected by variations in reaction yield or MS/MS conditions. To date, PB-MS/MS has been developed for 18 classes of lipids, including glycosphingolipids, glycerolipids, glycerophospholipids, among others, offering a detection limit in the nM range. In addition to C=C, we have also developed MS/MS methods based on radical-induced dissociation (RDD) for the analysis of sn-position and chain modifications in lipids. This is accomplished by derivatizing the lipid with a radical precursor. Upon collisional activation, the radical precursor undergoes homolytic cleavage, releasing the radical for subsequent RDD. These new MS/MS methods have been incorporated into various lipidomic workflows, such as shotgun analysis, liquid chromatography-MS, MS imaging, and single-cell analysis, revealing an unexpected diversity of lipid molecules across diverse biological systems. Furthermore, the compositional analysis of lipid isomers is less prone to interpersonal variations, enabling a more sensitive detection of differential lipid species compared to traditional lipid quantitation at the sum composition level. This advantage significantly enhances lipidomic phenotyping in a broad range of biomedical applications.

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Ondrej Peterka[1], Professor Peter Meikle[2], Dr Anne Bendt[3], Dr. Tucker Hallmark[4], Dr. Clay Davis[5], **Prof. Michal Holčapek**[1]
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CLIG interlaboratory study on the harmonization of lipid concentrations in human plasma

Michal Holčapek 347 - CLIG interlaboratory study on the harmonization of lipid concentrations in human plasma, Plenary 3, August 19, 2024, 10:25 - 10:44

The field of lipidomics is one of the most rapidly growing omics technologies today because many researchers have recognized the importance of lipidomic data in their research. However, there are also some bottlenecks that limit the faster spread of lipidomic analysis, such as the lack of experience of newcomers (solvable by proper training), lack of standardized protocols (one of the key efforts of the International Lipidomic Society, ILS), and the problems related to the fact that different laboratories report slightly different values of molar concentrations for identical samples, which complicates interlaboratory studies. The presented work is the community effort of the Clinical Lipidomics Interest Group (CLIG) in ILS on how to handle and overcome these difficulties.

Methods

Instructions for measurements of molar concentrations of 157 lipid species from 11 lipid subclasses were prepared for individual workflows based on mass spectrometry (MS), liquid chromatography – MS (LC/MS), or supercritical fluid chromatography – MS (SFC/MS). The list of lipid species and measurement instructions were prepared as a consensus of a series of online CLIG meetings. All groups determined molar concentrations in 4 human plasma samples (NIST SRM 1950, Diabetic, High triacylglycerol, and Young African American) provided by NIST. 37 internal standards (IS) from 11 lipid subclasses were prepared for this study by Avanti Polar Lipids. Typically, 4 IS for the majority of classes were used with at least 2 deuterated IS.

Novel Aspect

The goal of this interlaboratory study is the harmonization of determined concentrations of lipid species using different workflows.

Preliminary Data or Plenary Speaker Abstract

This interlaboratory ring trial is organized by the CLIG with the goal of harmonizing molar concentrations reported by individual laboratories using different workflows. This comparison contains 32 data sets from 25 research groups from four continents for four basic workflows: a/ lipid species separation using reversed-phase (RP)-LC/MS, b/ direct infusion MS, c) lipid class separation using SFC, and d) lipid class separation using hydrophilic interaction liquid chromatography (HILIC). The effects of various parameters on the determined concentrations are studied. All concentrations are determined by all available IS. Lipids are extracted using a unified double Folch protocol performed by all participating laboratories and compared with the centrally prepared extract by the same protocol and optionally with their own extraction protocol. The effect of data normalization using determined NIST SRM 1950 concentrations by individual laboratories for the normalization of data for the other 3 plasma samples is visualized to illustrate possible improvements for interlaboratory cohorts. The results for some sphingolipid and phospholipid classes are highly consistent among individual laboratories, but there are also lipid classes with significant differences. The most pronounced problem is the class of cholesteryl esters, where the in-source fragmentation could seriously complicate the quantitation of individual lipid species. The results are compared with previously reported concentrations of lipids in human plasma (J. Lipid Res. 58 (2017) 2275 and J. Lipid Res. 51 (2010) 3299). This interlaboratory study allows the visualization of various effects, which can

be generalized and used for future improvements in the experimental design of large-scale, multi-laboratory studies.

M.H. and O.P. acknowledge the support of ERC Adv grant No. 101095860 sponsored by the European Research Council.

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Prof Anthony Don[1], Mr Jun Yup Lee[1], Dr Jonathan Teo[1]
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Quantifying brain lipid synthesis and turnover through deuterium labelling of endogenous brain lipids in vivo

Anthony Don 396 - Quantifying brain lipid synthesis and turnover through deuterium labelling of endogenous brain lipids in vivo, Plenary 3, August 19, 2024, 10:44 - 11:03

The dominant genetic risk factor for Alzheimer's disease is inheritance of the E4 allele of the APOE gene, which encodes the lipid transport protein Apolipoprotein E (ApoE). Although this has been known for over 30 years, the biochemical mechanisms through which APOE gene variants modify the risk of developing Alzheimer's disease have remained elusive due to a paucity of methods for tracking brain lipid metabolism in vivo. Lipidomic analysis quantifies brain lipids at a snapshot in time, but cannot follow dynamic lipid synthesis and turnover. Here, we have used deuterium oxide (D2O) administration and high resolution LC-MS/MS to follow the synthesis and turnover of endogenous brain lipids, and to test the requirement for ApoE in physiological brain lipid turnover.

Methods

Wild-type (WT) C57BL6 and ApoE knockout (Apoe-/-) mice were administered D2O in their drinking water from embryonic day 14 until 2 months of age, then returned to 100% water. A parallel cohort received only 100% H2O. Groups of 6 mice were sacrificed at 2, 4, and 8 months of age, and lipids were extracted from dissected brain tissue. Lipids were quantified by LC-MS/MS on a Q-Exactive HF-X, using data dependent acquisition in positive and negative ion mode. Lipids were first identified in samples from unlabelled mice based on precursor and product ions. High accuracy precursor ion m/z and precise elution times were then used to identify and quantify peaks for deuterated isotopologues in mice given D2O, correcting for naturally-occurring isotopes.

Novel Aspect

A novel in vivo deuterium labelling and data analysis workflow demonstrates that ApoE is required for physiological myelin lipid turnover.

Preliminary Data or Plenary Speaker Abstract

From 537 identified lipids, levels of 85 were significantly increased (false discovery rate-adjusted $P < 0.05$), and one was reduced, in the hippocampus of 9-month-old unlabelled Apoe-/- compared to WT mice (i.e. mice given only H2O). Only 6 and 10 lipids, respectively, were significantly increased in the cortex and cerebellum of Apoe-/- compared to WT mice, and no lipids were reduced. More than half of the lipids that were higher in Apoe-/- hippocampus were myelin-specific galactosylceramide and sulfatide species. Higher levels of these lipids in Apoe-/- mice suggested either abnormal synthesis or impaired turnover.

In mice given D2O, a bell-shaped distribution of deuterated isotopologues was observed for each lipid, with >95% of measured lipids containing one or more deuterium atoms at the cessation of D2O administration (2 months of age). The mean deuteration state for all lipids declined over time, as deuterated lipids were replaced with non-deuterated counterparts. Lipid turnover was quantified as the change in mean deuteration state of each lipid over time, and was fastest for phosphatidylcholine species and slowest for cholesterol and sulfatides. The mean deuteration state for all lipids measured was highly consistent between mice within each group, with coefficients of variation of 2-4% at 2 months, 4-9% at 4 months, and 8-17% at 8 months of age (6 mice per group). Turnover (decline in the mean deuteration state) of cholesterol, the major sulfatide species d18:1/24:0 and d18:1/24:1, and galactosylceramide d18:1/24:0-OH was significantly reduced in all four brain regions (hippocampus, corpus callosum, cortex, and cerebellum) of Apoe-/- compared to WT mice. This impaired turnover could explain the higher overall levels of these lipids in the brains of

Apoe-/- compared to WT mice, illustrating the capacity for in vivo stable isotope labelling to generate new insights into the molecular basis for neurodegenerative diseases.

Amy Liang[1,2], Dr. Corey Giles[1,3,4], Tilly Van Buuren-Milne[1], Alexandra Faulkner[1], Michelle Cinel[1], Thy Duong[1], Ms Nat Mellett[1], Dr Kevin Huynh[1,3,4], Professor Peter Meikle[1,3,4,5]
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Automated retention time calibration for complex, targeted reverse phase chromatography based lipidomics.

Amy Liang 452 - Automated retention time calibration for complex, targeted reverse phase chromatography based lipidomics, Plenary 3, August 19, 2024, 11:03 - 11:22

Reverse phase chromatography provides separation that complements targeted lipidomics, increasing the coverage of measurable lipids. The diversity of isomeric and isobaric lipids in a sample result in multiple lipid species to be measured in a single targeted scan. Scheduled multiple reaction monitoring (MRM) increases the quality and quantity of measured lipids by only scanning for lipids at their expected retention time (RT). However, variation in solvent composition and column quality can result in significant shifts in RT for each lipid. This often necessitates manual recalibration for methods with many MRM transitions, which can be time-consuming and prone to error. We have developed a statistical approach to enable recalibration of RT that is applicable across all reverse phase conditions.

Methods

Our targeted lipidomics platform utilises an Agilent 1290 Infinity II HPLC with a 6495C QqQ mass spectrometer. Chromatographic conditions include a ZORBAX eclipse plus C18 column (2.1 x 100mm, 1.8µm, Agilent) with a solvent composition comprising of water, acetonitrile, and isopropanol with ammonium formate and medronic acid. Model predictors included RTs from 152 MRM transitions that correspond to single, highly abundant peaks – that can be automatically obtained using the peak apex in our pooled quality control plasma samples. The RT of these transitions were used to estimate the RTs of other MRM transitions in a penalised regression model (LASSO, n = 35 previously curated RT calibrations) within a 3-fold cross validation framework.

Novel Aspect

We developed a workflow for automatic recalibration of RT that is applicable to all targeted LC-MS methods with MRM windows.

Preliminary Data or Plenary Speaker Abstract

We utilized our targeted lipidomics methodology, comprising 688 MRM transitions over a 16-minute gradient, with the busiest region scanning 131 concurrent MRMs. This method features retention time windows ranging from 0.4 minutes to 7.0 minutes (mean: 1.0 ± 0.83 minutes), depending on the number of closely eluting isomeric and isobaric lipid species . In general, retention times for MRM transitions measuring multiple lipid isomers varied enough to require frequent recalibration. For example, PE P-16:0/22:5, [M+H]+ 750.5 / 387.3 m/z, appears with two isomers and shows deviations in RT ranging from 7.28 minutes to 7.89 minutes (from 35 RT calibrations across 3 instruments). Identification of ideal reference lipids for modelling resulted in a list of 152 MRM transitions spanning 31 classes and subclasses, including several of which were non-physiological internal standards. Automatic retention time for the 152 lipid species is obtained by identifying the time at the apex of the chromatographic peak. Using data from the 35 available manually curated retention time datasets, LASSO was used to predict the RT of all the MRM windows, one at a time. Examination of each model revealed that each model utilized an average of 29 MRM transitions (SD = 43, range = 0 - 118) . Where the models utilized 0 transitions, the MRM had stable retention times, that did not require recalibration, or utilized large window sizes, thus only requiring the previously assigned RT.

Overall, we observed robust, cross-validated prediction across 3 instruments running the same chromatographic gradient. This workflow is routinely used in our targeted lipidomics platform when preparing new batches of solvent, columns, or samples and it takes much less time compared to the usual manual recalibration.

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Probing fatty acid alterations linked to cholesterol dysregulation in Niemann-Pick Type C Disease

Stephanie Cologna 168 - Probing fatty acid alterations linked to cholesterol dysregulation in Niemann-Pick Type C Disease, Plenary 3, August 19, 2024, 11:22 - 11:41

Advances in the field of mass spectrometry-based measurements have broadened our understanding of the dysregulated pathways in a number of human diseases. One example is Niemann-Pick Disease Type C (NPC), a fatal, lysosomal cholesterol storage disorder with no FDA-approved therapy. While much effort has been focused on mass spectrometry-based proteomic analysis in NPC, lipidomics has been less studied. At the core of many metabolic disorders including NPC is lipid dysregulation, and the link between cholesterol storage in NPC and fatty acid dysregulation, for example, is not fully understood. Our focus herein is combining lipidomic strategies with biochemical and molecular tools towards understanding the role of fatty acids in cell survival and death in the context of NPC.

Methods

A time course study was performed using a knock-in human NPC1 I1061T murine model and controls. The cerebellum and cortex were investigated at 4, 9, and 15 weeks. Primary skin fibroblasts from individuals with NPC as well as a HeLa cell line harboring the I1061T mutation and controls were also studied. Targeted LC-MS assays utilized authenticated standards, retention time matching and were obtained using the positive reflectron mode. Untargeted lipidomics was performed using both positive and negative mode data collection with the iterative MS workflow. Cytotoxicity assays, fluorescence imaging of cholesterol by filipin, and quantitative western blots were performed using established protocols.

Novel Aspect

This study revealed an interrelationship between fatty acid and cholesterol dysregulation integral to Niemann-Pick Type C disease.

Preliminary Data or Plenary Speaker Abstract

Distinct lipidome changes were observed in the cortex and cerebellum when comparing wild type and I1061T mutant mice. In the cortex, eight diglyceride lipids significantly increased at 15 weeks while on the other hand 16 Ether-PE plasmologens were reduced in the NPC mouse. A contrasting trend was noted for lysophosphatidylethanolamines (LPEs) which was reduced in the cortex at this terminal age but elevated in the cerebellum. Importantly, we report an interesting transition in the cerebellar abundance of 62 ceramides and 52 triglyceride species. Levels of these lipid classes, which are implicated in neuronal apoptosis, were initially lower in 4 week mutant animals compared to wild type but eventually increased in symptomatic mice beginning at 9 weeks. The same trend was observed with free and total PUFAs, suggesting that fatty acid levels could possibly correlate initially with cell survival strategies followed by neurodegeneration. In proof-of-concept studies, we observe fatty acid levels can be normalized in NPC cell models using the fatty acid desaturase 2 (Fads2) pharmacological inhibitor, CP24879. Relative amounts of PUFAs were shown to go down alongside triglycerides, cardiolipins, ceramides, oxidized phosphatidylinositides, and fatty acid esters of hydroxy fatty acids (FAHFAs). Surprisingly, we report that the expression of the mutant NPC1 cholesterol transporter, the protein defective in NPC disease, is reduced by PUFA enrichment and increased by fatty acid depletion suggesting that fatty acid levels play a role in the regulation of this protein. Interestingly, the effect observed for wildtype cells is the opposite. Ongoing work concentrates on identifying nuclear receptor targets of fatty acids that are specifically involved in NPC. These results allude to a crucial interplay of cholesterol storage and fatty acid homeostasis and highlight the potential of lipid-centered therapies for Niemann-Pick Type C.

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Lipidomic Alterations in the Retina of a mouse model of Zellweger Spectrum Disorder Investigated by Mass Spectrometry Imaging

Rachel Pryce 176 - Lipidomic Alterations in the Retina of a mouse model of Zellweger Spectrum Disorder Investigated by Mass Spectrometry Imaging, Plenary 3, August 19, 2024, 11:41 - 12:00

Zellweger spectrum disorder (ZSD), caused by mutations in PEX genes, occurs in about 1 in 50,000 births and involves peroxisome dysfunction. Peroxisomes perform β-oxidation of very long chain fatty acids (VLCFA), synthesis of ether phospholipids and docosahexaenoic acid (DHA) and regulate reactive oxygen species. ZSD is a multi-system progressive disorder, in which childhood blindness occurs in nearly all patients. The PEX1-G843D mutation occurs most frequently, in 30% of ZSD cases. Mice with the PEX1-G844D mutation mimic ZSD and show retinal degeneration, but the resulting lipid changes have not yet been investigated. We sought to monitor the distribution and relative abundance of FA and phospholipids in the retina of wild type and PEX1-G844D mice by mass spectrometry imaging (MSI).

Methods

Retina tissue from 30-day and 90-day wild type (WT) and PEX1-G844D mice was compared to investigate lipidomic changes. Flatmount and neuroretina sections were thaw-mounted on indium-tin-oxide coated microscope slides for analysis. Samples were analyzed in triplicate using both matrix-assisted laser desorption ionization (MALDI) and silver-assisted laser desorption ionization (LDI) mass spectrometry imaging (MSI) using a MALDI-TOF MS system (Bruker Daltonics) at a spatial resolution of 75 µm. Dual polarity MALDI imaging with 1,5-diaminonapthlene was used to target a wide range of phospholipids whereas silver-assisted LDI was used to target unsaturated free fatty acids. MSI data was processed and analysed using FlexImaging 4.1 (Bruker Daltonics) and R 4.3.2.

Novel Aspect

MALDI and AgLDI MSI were used to investigate fatty acid and associated phospholipid dysregulation in Zellweger disorder.

Preliminary Data or Plenary Speaker Abstract

In PEX1-G844D mice, VLCFA levels were increased significantly reflecting the underlying peroxisome dysfunction. FA32:5, for example, was 11x and 35x more abundant in the 30-day and 90-day mice, respectively. Some changes were regional, such as FA30:7, which increases twice as much in the ventral region. The long chain fatty acids, generally trended down, decreasing between 25-75%.

DHA (FA22:6) displayed a 3x (30-day) and 12x (90-day) decrease in the PEX1 mice. DHA-associated changes in phospholipid abundance were also observed. Ether phospholipids were reduced, including PE(P-16:0/20:4), which decreased 2-fold in the PEX1-G844D mice. These changes are also often localized, such as with PA O-42:1, where the signal is reduced 2.5-fold in the ventral retina and only 1.4-fold in the dorsal at 90-days

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Associate Professor Ronda Greaves[1]
[1]Victorian Clinical Genetics Services

Mass spectrometry's contribution to neonatal endocrinology and metabolism – where to from here?

Keynote: Associate Prof. Ronda Greaves Murdoch Children's Research Institute 762 - Mass spectrometry's contribution to neonatal endocrinology and metabolism – where to from here?
Meeting Room 5, August 19, 2024, 10:00 10:25

Preliminary Data or Plenary Speaker Abstract

Children, and particularly neonates, are not miniature adults and this brings with it testing complexities for many analytes such as steroids, amino acids, organic acids and acyl carnitines. It is now over 50 years since mass spectrometry (MS), by gas chromatography – MS, was used as part of the differential diagnosis of inborn errors of metabolism and some 30 years since liquid chromatography – MS/MS revolutionised newborn bloodspot screening. This has resulted in a significant reduction in mortality and morbidity for neonates in countries that have the specialised infrastructure, but unfortunately in other countries MS is still an emerging technology. Even in developed countries, MS has not truly been embraced by the routine paediatric laboratory despite the clinical need, and steroid analysis serves as a clear example.

Starting from cholesterol, steroidogenesis involves a cascade of enzyme processes to form intermediate and biologically active (and metabolic products of) glucocorticoids, mineralocorticoids, progestins, androgens and estrogens. An understanding of the steroid pathway requires an appreciation of the relative enzyme activity and regulation. This is particularly relevant for the interpretation of preterm versus full term neonatal steroid profiles because the foetal adrenal zone persists until at least the equivalent of term despite early delivery. In daily practice, one of the most common reasons for measuring steroids in neonates is for the investigation of disorders of sex development, such as congenital adrenal hyperplasia (CAH) and "normal" preterm steroid patterns include a mixture of 15 and 16-hydroxylated and sulphated steroids. The intricacies of the steroid pathway clearly place the need to have routine MS-based testing. Future directions for routine steroid analysis should include considerations for the smart laboratory, where Lean processes such as random access and full automation are used, to remove the current bottlenecks that inhibit MS use for large scale first-tier testing.

This presentation aims to provide an insight into the role of clinical MS for the analysis of steroids and inborn errors of metabolism and will include a practical example of the challenges associated with the introduction of newborn screening of CAH.

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Dr. Stefani Thomas[1], Carly Twigg[1], Joohyun Ryu[1]
[1]University Of Minnesota

Adopting fundamental principles from the clinical laboratory to accelerate the clinical translation of targeted mass spectrometry-based proteomic assays

Stefani Thomas 590 - Adopting fundamental principles from the clinical laboratory to accelerate the clinical translation of targeted mass spectrometrybased proteomic assays, August 19, 2024, 10:25 - 10:44

In vitro diagnostic testing is an indispensable tool in clinical practice for disease prognosis, diagnosis, monitoring, and treatment response prediction. More than 70% of clinical decisions rely on laboratory tests. Mass spectrometry (MS)-based assays that measure proteins are not widely used in clinical practice. As such, there is a significant opportunity for MS-based proteomic assays to support and improve patient care to realize the potential of precision medicine. With the availability of consensus guidelines, the path is clearer towards the expanded implementation of these assays in the clinical lab. Here, we present the practical aspects of developing and validating MS-based proteomic assays in a clinical research setting using plasma fibrinogen – a critical indicator of hemostasis – as an exemplar.

Methods

Stable isotope-labeled Fibrinogen gamma (UniProt accession # P02679) peptides (13C6,15N2-Lys; 122YLQEIYNSNNQK134 and 13C6,15N4-Arg; 135YEASILTHDSSIR146) and the corresponding unlabeled crude peptides (>70% purity) were synthesized by Synpeptide. The parallel reaction monitoring (PRM) fibrinogen assay was developed using a Q Exactive Plus mass spectrometer coupled with a Vanquish HPLC system. The scheduled PRM method was 10 min. The Tier 2 PRM assay was developed and validated according to the National Cancer Institute's Clinical Proteomic Tumor Analysis Consortium (CPTAC) consensus guidelines. The EDTA plasma samples were digested at 70°C for 120 min using SMART Digest Trypsin magnetic beads, and the samples were de-salted using 96-well SOLA Solid Phase Extraction plates prior to vacuum concentration and PRM analysis. Data analysis was conducted using Skyline.

Novel Aspect

Developed and validated plasma fibrinogen PRM assay with a throughput of 90 samples/24 hours supporting a large-scale clinical research study.

Preliminary Data or Plenary Speaker Abstract

The fibrinogen PRM assay calibration curves were generated in triplicate using 11 calibrators from 0.375 – 4,800 fmol to determine the limit of detection (LOD), limit of quantification (LOQ) and linearity. The calibration curves were prepared by diluting the unlabeled ("light") peptides in pooled digested plasma matrix spiked with a constant amount of "heavy" internal standard peptide. The LOD of each peptide (mean + 3 x standard deviation of the blank matrix with an accuracy of 80 – 120%) was ~2.3 fmol, and the LOQ (3 x LOD with CV <20%) was ~7 fmol. Intra-, inter-, and total assay CVs were determined by analyzing 3 levels of quality control (QC) samples in triplicate across 5 days as a proxy for the assay repeatability. For all 3 QC levels of both peptides, intra-assay CV was <2.5%, inter-assay CV was <3.7%, and total assay CV was <3.9%. Assay stability and selectivity were also assessed.

The EDTA plasma samples were processed in batches of ~90. A pooled plasma sample prepared from 100 randomly selected samples was processed with each batch and was used as a processing QC. Protein concentration was determined using a NanoDrop spectrophotometer (A280nm). Aliquots of 200 µg protein were used for a 120 min digestion at 70°C with vortexing (1500 rpm) using 5 µL of SMART Digest Trypsin magnetic beads in a final volume of 30 µL added into SureSTART V-bottom 96-

well plates. After desalting and drying, the peptides were reconstituted in LC mobile phase A containing an equimolar mixture of both heavy fibrinogen peptides. The calibration curve was prepared and run weekly, and the 3 QC samples were prepared in weekly batches and they were run daily. The throughput of the entire workflow was ~90 samples/24 hours. Fibrinogen protein concentrations were calculated using the mean concentration of both peptides.

Priscilla Yeung[1,2], Yajing Liu[1], Samuel Yang[1], Ashley Ruan[1], Christina Kerr[1], Carolyn Wong[2], Run-Zhang Shi[1,2], David Iberri[3], Ruben Luo[1,2]
[1]Department of Pathology, Stanford University, [2]Clinical Laboratories, Stanford Health Care, [3]Department of Medicine, Division of Hematology

Clonality Determination by Detecting Unmodified Monoclonal Serum Free Light Chains Using On-Probe Extraction Coupled with Liquid Chromatography-High-Resolution Mass Spectrometry

Priscilla Yeung 92 - Clonality Determination by Detecting Unmodified Monoclonal Serum Free Light Chains Using On-Probe Extraction Coupled with Liquid Chromatography-High-Resolution Mass Spectrometry, Meeting Room 105, August 19, 2024, 10:44 - 11:03

An essential clinical biomarker of plasma cell neoplasms is serum free light chains (FLC), which are circulating antibody light chains that are unbound to heavy chain. The current widely-used immunoassay method quantifies total serum FLCs, including the polyclonal background. Although a skewed kappa/lambda (K/L) FLC ratio is often used as a proxy for clonality, some patients, such as those with chronic kidney disease, can have elevated total FLCs that lead to ambiguous results that can benefit from a direct measurement of monoclonal proteins. The purpose of this study was to develop a method that coupled an on-probe extraction (OPEX) immunocapture step with liquid chromatography-high-resolution mass spectrometry (LC-HR-MS), abbreviated as OPEX-MS, to determine the clonality of serum free light chains.

Methods

Remnant patient samples with serum FLC immunoassay results from the Stanford Clinical Chemistry Laboratory were collected and processed according to Institutional Review Board protocols approved by Stanford Health Care. OPEX Immunocapture was performed using microprobes pre-coated with streptavidin linked to biotinylated anti-kappa light chain and anti-lambda light chain antibodies. Captured proteins were separated by reverse-phase LC and analyzed using HR-MS.

Novel Aspect

The OPEX-MS method can serve as a complementary approach to directly determine clonality in patients with difficult-to-interpret FLC immunoassay results.

Preliminary Data or Plenary Speaker Abstract

Four cohorts of samples from unique patients were tested based on immunoassay FLC results: negative (n = 50), kappa elevated (n = 49), lambda elevated (n = 45), and dual elevated (n = 100). In the negative, kappa elevated, and lambda elevated cohorts, OPEX-MS showed good overall concordance with the immunoassay method and identified clonal FLCs in a subset of the dual elevated cohort with a normal kappa/lambda ratio. In positive samples, this method provides a unique retention time, accurate mass, and light glycosylation status.

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Ms Keziah Liebenberg[1], Ms Erin Craig[2], Ms Meredith Spradlin[1], Dr Michael Keating[1], Dr Robert Tibshirani[2], Dr Livia Eberlin[1]
[1]Baylor College Of Medicine, [2]Stanford University

Alterations in glutaminolysis detected by direct mass spectrometry techniques enable diagnosis and molecular subtyping of breast and ovarian cancers

Keziah Liebenberg 569 - Alterations in glutaminolysis detected by direct mass spectrometry techniques enable diagnosis and molecular subtyping of breast and ovarian cancers, Meeting Room 105, August 19, 2024, 11:03 - 11:22

Glutamine metabolism is a critical energy source for a variety of cancer types. Evidence suggests a correlation between cancer invasiveness and glutaminolysis dysregulation. For example, distinct molecular breast cancer subtypes may dysregulate glutaminolysis to varying degrees, potentially contributing to their prognoses and treatment response differences. We hypothesize that detection of glutamine and related metabolites by direct mass spectrometry techniques can provide the basis for diagnosing and subtyping of cancer, particularly hormone-driven cancers including breast and ovarian. Here we employed desorption electrospray ionization mass spectrometry imaging and the MasSpec Pen to investigate glutamine to glutamate ratios in different breast and ovarian cancer subtypes. The results indicate that these methods could potentially serve as valuable tools for diagnosing and subtyping these cancers.

Methods

A retrospective statistical analysis was conducted on datasets collected with DESI-MSI (breast: n= 122; ovarian: n = 78) and the MasSpec Pen (breast: n = 159). The log-ratio of intensities of glutamate to glutamine (GGR) was calculated for normal breast tissues and the four major breast cancer molecular subtypes (luminal A, luminal B, HER-2, and triple negative), similarly for normal ovarian tissues and the two major grade subtypes (high and low). A permutation test was developed to determine statistical significance between the logGGR values of different classes. The mean logGGR values underwent 100,000 permutations between tissue types, with their differences calculated following each iteration. The resulting p-values were adjusted for multiple comparisons using the Benjamini-Hochberg method.

Novel Aspect

Direct mass spectrometry techniques reveal valuable insights into energy dysregulations in breast and ovarian cancers towards their detection and subtyping.

Preliminary Data or Plenary Speaker Abstract

During glutaminolysis, glutamine is converted to glutamate by the enzyme glutaminase. A metric for the dysregulation of glutaminolysis can be provided by calculating the GGR, for which a higher GGR value correlates to an upregulation of glutaminolysis. In our study, statistical analysis of the DESI-MSI dataset revealed that the logGGR was significantly increased in breast cancer (mean = 0.85) compared to normal breast tissue (mean = -0.07; p-value < 1e-6). Similarly, the logGGR value was found to be significantly increased in breast cancer (mean = 0.47) compared to normal breast tissue (mean = -0.58; p-value 2e-6) in the MasSpec pen dataset. These results reveal that glutaminolysis is potentially upregulated in breast cancer compared to normal breast tissue. Interestingly, the logGGR values of each breast cancer subtype was significantly increased when compared to normal breast tissue in the DESI-MSI dataset. TNBC, the most aggressive breast cancer subtype, exhibited the greatest logGGR value (mean = 1.53; p-value <1e-6). Luminal A, the least aggressive breast cancer subtype, displayed the lowest logGGR value (mean = 0.64; p-value = 8e-4). These findings highlight the relationship between dysregulated glutaminolysis and breast cancer invasiveness. Preliminary immunofluorescence experiments have revealed a correlation between an increased glutaminase

expression and breast cancer invasion, where invasive ductal carcinoma tissue revealed a greater expression of glutaminase compared to ductal carcinoma in situ. Similarly, statistical analyses revealed a significant difference between the logGGR values calculated for normal ovarian tissues, and high and low grade ovarian cancers. Interesting, statistical analyses revealed that the logGGR values were significantly decreased in ovarian cancer tissues compared to normal ovarian tissue, an opposite trend to that seen for breast cancer. These results display the utility of mass spectrometry techniques to probe the energy dysregulations that drive different cancers and their invasiveness.

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Dr Dan Lane[1,2], Mr Borislav Lazarov[2], Dr Colleen Maxwell[1], Prof Leong L Ng[1], Prof Donald JL Jones[1], Dr Pankaj Gupta[1,2]
[1]University Of Leicester, [2]University Hospitals of Leicester

The Validation Processor: the development of a novel tool that that automates, standardises, and accelerates mass spectrometric assay validation

Dan Lane 87 - The Validation Processor: the development of a novel tool that that automates, standardises, and accelerates mass spectrometric assay validation, Meeting Room 105, August 19, 2024, 11:22 - 11:41

To construct clinically available mass spectrometry assays, laboratories must perform assay validation – a set of rigorous experiments to ensure accuracy, precision, and robustness. Data analysis/reporting of analytical validation is slow: current processes are greatly heterogenous across laboratories and can take months per test, relying on cumbersome manipulation of mass spectrometry output files into Excel workbooks and manual preparation of accreditation-ready validation reports.

Thousands of biomarkers with potential clinical value are discovered each year, but this slow development and validation of clinically viable assays represents a rate limiting step in translation of new assays from research to the clinic. An interactive, automated tool was developed to process mass spectrometric validation data and knit accreditation-ready reports in under 5 minutes.

Methods

Leveraging the R coding language, we built an interactive graphical user interface allowing users to upload mass spectrometry output files (.csv, .txt, or .xlsx) from validation batches. We included functionality to examine calibration plots, apply different response function models, explore bias and precision across batches, plot stability data, and set acceptance criteria (e.g., ≤25% intra-batch precision), among other aspects. The default criteria are ANSI/ASB Standard 036 for Method Validation. The interface includes a report generator which knits the data into an accreditation-ready summarised report, outlining which analytes passed/failed.

To test the tool, a quantitative assay for 16 hypertension medications was developed in plasma (10 µL, precipitated with 125 µL acetonitrile) and underwent analytical validation using a Waters Xevo TQ-XS.

Novel Aspect

The Validation Processor is a novel tool that could standardise, automate, and accelerate mass spectrometry biomarker translation across clinical/research/industrial laboratories.

Preliminary Data or Plenary Speaker Abstract

Three validation batches were analysed and exported (.txt files), each containing a set of experiments required to test assay suitability (calibration model, bias/precision, carryover, interference, ionisation suppression, limit of detection/quantification, stability, and dilution integrity). Using the file explorer function to locate the export files, data were imported and the batches were compiled. The processor was designed to automate data review based on standard validation criteria, hence default values were applied ($R2 > 0.95$, bias/precision <20%, etc.).

Multiple calibration models were evaluated (linear, quadratic, various weightings) and the best model was applied to each analyte based on the highest R2 (N.B., specific models can be selected with user input). Each analyte was measured against the default parameters of each experiment,

where individual graphics (e.g., boxplots, stability change line graphs) and tables (e.g., results of dilution integrity) could be exported.

The validation report (.docx) was then generated, compiling together a summary of the experiments, the validation criteria used, and a detailed breakdown of the results. System information (e.g., dates, user input, etc.) was printed within the report too – an important function for traceability. In respect of clinical laboratories, the report was built and is populated in accordance with GCLP guidelines.

From data import to exporting the fully populated validation report, the processing time was <1 minute (AMD Ryzen 7 4700U, 8 GB RAM), potentially saving approx. £2000 per validation (compared to manual review).

In summary of the antihypertensive assay tested through the validation tool, the mean R2 (0.972, SD: 0.024), limit of detection (30.0 ng/mL, SD: 4.6), bias (-3.5%, SD: 8.1), intra-batch precision (15.9%, SD: 3.5), and inter-batch precision (11.6%, SD: 3.3) were reported. This was a simple protein precipitation method with a <1 minute chromatographic gradient that may be of clinical applicability in therapeutic drug monitoring and adherence testing.

Assistant Professor Ruben Luo[1,2], Christopher Pfaffroth[2], Samuel Yang[1], Priscilla Yeung[1,2], James Zehnder[1,2], Run-Zhang Shi[1,2]
[1]Stanford University, [2]Stanford Health Care

Microprobe-Capture In-Emitter Elution Coupled with Mass Spectrometry for Structural Elucidation and Clinical Testing of β2-Transferrin

Ruben Luo 12 - Microprobe-Capture In-Emitter Elution Coupled with Mass Spectrometry for Structural Elucidation and Clinical Testing of β2-Transferrin, Meeting Room 105, August 19, 2024, 11:41 - 12:00

Cerebrospinal fluid (CSF) leak may lead to potentially life-threatening meningitis if left untreated. CSF leak can be diagnosed by detecting a diagnostic marker β2-transferrin (β2-Tf) in secretion samples. β2-Tf is a proteoform of human transferrin (Tf) mainly present in CSF and barely detectable in other body fluids. The clinical utility and diagnostic value of β2-Tf in CSF leak have been demonstrated. β2-Tf, together with the typical Tf proteoform in serum β1-Tf, were named after their electrophoretic mobility in gel electrophoresis. However, the structures of β1-Tf and β2-Tf have not been elucidated. A novel affinity capture sample preparation technique, microprobe-capture in-emitter elution (MPIE), was combined with mass spectrometry (MS) to study the structures of β1-Tf and β2-Tf.

Methods

As a sample preparation technique, MPIE utilizes the analytical power of a label-free optical sensing technology (next-generation biolayer interferometry, BLI) to overcome the challenge of lack of process monitoring in the conventional affinity capture-MS analysis. To implement MPIE, an analyte is first captured on the surface of a microprobe, and subsequently eluted from the microprobe inside an electrospray emitter. The analyte is immediately ionized via electrospray ionization (ESI) for MS analysis, achieving the direct coupling between MPIE and MS. The capture process is monitored in real-time via BLI. By this means, BLI and MS are combined in the form of MPIE-ESI-MS, which is readily deployed to analyze the Tf glycoforms and elucidate the structures of β1-Tf and β2-Tf.

Novel Aspect

The MPIE-ESI-MS method successfully elucidated the structures of β1-Tf and β2-Tf. It is a novel clinical test for CSF leak.

Preliminary Data or Plenary Speaker Abstract

The Tf glycoforms in CSF samples, serum samples, and secretion samples from patients suspected of CSF leak were analyzed using MPIE-ESI-MS. To verify the structures of β1-Tf and β2-Tf, the Tf glycoforms were separated by gel electrophoresis and analyzed using MPIE-ESI-MS.

Based on the MPIE-ESI-MS results of serum, CSF, and secretion samples, the structures of β1-Tf and β2-Tf were elucidated. As Tf glycoforms, β1-Tf and β2-Tf share the amino acid sequence and intramolecular disulfide bonds, but have varying N-glycans. β1-Tf, the major serum-type Tf, has two G2S2 N-glycans on Asn413 and Asn611, while β2-Tf, the major brain-type Tf, has an M5 N-glycan on Asn413 and a G0FB N-glycan on Asn611.

A set of 11 secretion samples from patients suspected of CSF leak were analyzed using the MPIE-ESI-MS method, among which 5 samples were positive for β2-Tf and the rest were negative as measured by IFE test and clinical manifestations. The MS peak at 78008 Da was observed in the MPIE-ESI-MS results of the 5 positive samples but not found in those of the 6 negative samples, which further verified that β2-Tf was indeed the major brain-type Tf. In addition, the analytical sensitivity of the MPIE-ESI-MS method for CSF and secretion samples was explored. A pooled CSF sample was mixed with water at 1:1, 1:4, 1:9, and 1:19 ratios to prepare a dilution series for analysis. The peak intensities decreased with the pooled CSF sample dilution, and it was demonstrated that the MPIE-ESI-MS method was able to detect β2-Tf in at least 10-fold diluted CSF (1:9 pooled CSF : water

mixture). When testing clinical specimens, i.e., secretion samples from patients suspected of CSF leak, the MPIE-ESI-MS method has advantages, especially for those with ambiguous agarose gel immunofixation electrophoresis (the conventional method) test results.

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Dr. Gili Ben-Nissan[1], Dr. David Morgenstern[1], Mr. Yegor Leushkin[1], **Prof. Michal Sharon**[1]
[1]Weizmann Institute Of Science

Mass Spectrometry Analysis in Near-Physiological Conditions

Keynote: Professor Michal Sharon Weizmann Institute 85 - Mass Spectrometry Analysis in NearPhysiological Conditions, Meeting Room 106, August 19, 2024, 10:00 - 10:25

Proteasomes, known for their intracellular protein degradation role, have recently emerged as key players in extracellular proteostasis, particularly in blood. While the functions of intracellular proteasomes are well studied, extracellular proteasomes remain enigmatic. This study focuses on unraveling the mysteries surrounding circulating blood proteasomes, employing advanced mass spectrometry (MS) techniques. By exploring the unique features of these proteasomes, we aim to bridge the gap in understanding between intracellular and extracellular proteasomes. This research not only sheds light on their fundamental biology but also offers insights into their potential roles in health and disease, with implications for novel therapeutic approaches targeting protein degradation pathways.

Methods

To study circulating blood proteasomes, we generated a mouse strain with a chromosomal C-terminal FLAG-tag on the PSMB2 subunit of the 20S proteasome. This tag allowed for the purification of intact 20S proteasomes from mouse tissues. Biochemical analyses were performed to assess the activity of the proteasomes, while native and denaturing MS techniques were utilized to examine their integrity, stability, and composition. MS2 analyses, coupled with HCD activation and pseudo-MS3 dissociation, were conducted on two types of Orbitrap instruments, including a modified EMR and a UHMR, to characterize the overall integrity, stability and subunit composition of the two-subtypes of proteasomes. Reversed-phase separation and denaturing top-down sequencing analysis were used to characterize their composing subunits and pinpoint their unique post-translational modifications.

Novel Aspect

Combining MS strategies revealed specialized, active 20S proteasomes circulating in the bloodstream, underscoring their biological relevance and complexity.

Preliminary Data or Plenary Speaker Abstract

Our study revealed significant differences in the activities of intra- and extracellular proteasomes. The extracellular proteasome showed reduced trypsin and chymotrypsin activities but increased caspase activity, indicating its specialization for extracellular functions. Both proteasome forms remained intact, weighing approximately 720 kDa, yet extracellular proteasomes had a higher prevalence of half proteasomes, suggesting lower stability. Using advanced mass spectrometry techniques, including front-end activation and MS2 with HCD activation, we identified unique modifications in blood 20S proteasomes not found in their intracellular counterparts. These modifications, such as cysteinylation and glutathionylation, were specific to certain alpha and beta subunits. Remarkably, treating liver proteasomes with cysteine or glutathione resulted in synthetic labeling of these subunits, indicating their role in altering the extracellular proteasome particle activity. Moreover, changes in physiological conditions significantly increased c20S proteasome levels in mice, mirroring human observations and providing a valuable model for further research.

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Mr Cameron Fairweather[1,2], Dr Xin Zhang[1,2], Dr Daniel Garama[3], Prof Patrick Sexton[1,2], Prof Denise Wootten[1,2], Dr Tracy Josephs[1,2]
[1]Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, [2]ARC Centre for Cryo-electron Microscopy of Membrane Proteins, [3]Hudson Institute of Medical Research

RAMP it up! Exploring conformational dynamics of the amylin receptors using HDX-MS

Cameron Fairweather 426 - RAMP it up! Exploring conformational dynamics of the amylin receptors using HDX-MS, Meeting Room 106, August 19, 2024, 10:25 - 10:44

Amylin is a peptide hormone expressed in the pancreas, serving as a key satiation signal that moderates gastric emptying and enhances energy expenditure. The amylin 3 receptor (AMY3R) represents one of the primary sensors for the amylin peptide and is, therefore, a focal point for therapeutic interventions in metabolic conditions such as diabetes and obesity. AMY3R is a heterodimer composed of the class B GPCR calcitonin receptor (CTR) and receptor activity-modifying protein 3 (RAMP3). While recent cryoEM structures of AMY3R and other amylin receptor subtypes have illuminated details of peptide and G protein engagement, a comprehensive understanding of how distinct receptor subtypes and peptide agonists manifest distinct pharmacological properties remains elusive.

Methods

To gain deeper insights into the molecular mechanisms governing AMY3R pharmacology, we leveraged hydrogen deuterium exchange mass spectrometry (HDX-MS) to investigate the conformational dynamics of AMY3R (CTR:RAMP3) in various states. Receptor components were expressed in Tni insect cells, detergent solubilised and purified by affinity and size-exclusion chromatography. We purified AMY3R in apo (unbound) conformation, as well as bound to two peptide agonists: rat amylin (rAmy) and salmon calcitonin (sCT). HDX-MS was performed using a LEAP automated liquid handling system, an ACQUITY UPLC M-Class system and a SYNAPT G2 SI HDMS mass spectrometer (Waters). Data was processed using ProteinLynx Global Server and DynamX HDX Data Analysis software.

Novel Aspect

Our study provides insights into the activation of AMY3R and a structural basis for the pharmacological profiles of two agonists.

Preliminary Data or Plenary Speaker Abstract

We identified changes in AMY3R dynamics caused by the binding of two peptide agonists. Both peptide agonists appeared to stabilise the AMY3R extracellular domain, including at regions distal to the agonist binding site, suggesting an allosteric mechanism of stabilisation at this domain. Conversely, we observed that both agonists destabilised the CTR:RAMP3 interface in the transmembrane region of the receptor, possibly caused by a rearrangment of the CTR transmembrane helices upon agonist binding. We also noted key differences in the two peptide-bound states themselves. rAmy had a more pronounced effect in altering the dynamics at the RAMP3 C-terminus, a region shown to directly interact with the CTR intracellular loop 2 (ICL2) to produce the AMYR phenotype. We also noted that sCT restricted the dynamics of the CTR TM6 region to a greater degree than rAmy, perhaps due to the slower dissociation rate of sCT.

Professor Tara Pukala[1], Ms Chia-De Ruth Wang[1], Mr Lewis McFarlane[1]
[1]Department of Chemistry, University Of Adelaide

Extending the molecular view of snake venoms to higher order structure

Tara Pukala 597 - Extending the molecular view of snake venoms to higher order structure, Meeting Room 106, August 19, 2024, 10:44 - 11:03

Venomous snakes possess a sophisticated system for biological warfare, comprising a multifaceted cocktail of pharmacologically active proteins. However, our current molecular view of snake venoms is critically limited, with the full diversity of protein structures significantly underappreciated. Advancing high-throughput structure determination of venom components is therefore of great importance for improved envenomation treatment, new leads for unique biotechnology research tools, and informing on mechanisms that shape venom evolution.

'Omics' platforms have enabled rapid identification of proteins to compile a rich catalogue of snake venom components at the primary sequence level. While knowledge of protein families and abundances is informative, the venomics field has largely ignored proteoforms and higher order protein structures, the ultimate determinant of protein stability and function.

Methods

This presentation will describe ongoing research aimed at developing top-down and native mass spectrometry-based methodologies for venomics, reliant on capabilities ion mobility mass spectrometry and protein derivatisation.

Venom fractionation is first achieved using methods that retain non-covalent assemblies (such as size exclusion and anion exchange chromatography). Using a venom-gland transcriptomics guided approach, we combine traditional bottom-up venomics strategies with top-down protein analysis, augmented by a range of fragmentation modes and ion mobility resolution, to reveal new isoforms of venom proteins. A further combination of intact mass analysis under reducing and non-reducing conditions reveals covalent assemblies, while native mass spectrometry analysis identifies non-covalent interactions between venom components.

Novel Aspect

New knowledge of snake venom components beyond the primary sequence, utilising a multifaceted mass spectrometry based approach.

Preliminary Data or Plenary Speaker Abstract

Implementing a complex-centric proteomic profiling platform that enables enhanced detection, sequencing and quantification of intact proteins and proteoforms, we describe venom protein interactomes for selected snakes of medical significance with greater detail and throughput.

Separation of venom proteins by chromatography coupled to intact mass spectrometry analysis allows for broad profiling of the venom fractions and reveals a diverse repertoire of proteoforms in various toxin families. Transcriptomic and proteomic analysis has shown diverse expression of toxin genes, with ion mobility improving the sequence information available from top-down experiments.

Importantly, we have identified among the first examples of both covalent (disulfide linked) and non-covalent quaternary structures for a range of venom protein families. Examples show enhanced bioactivity compared to their monomeric counterparts, and therefore this work also emphasises the importance of understanding higher-order protein interactions in venom toxicity through assessment of bioactivity assays.

The diverse composition of snake venoms presents a complex challenge, characterised by variations within and between species, a range of protein forms, and numerous post-translational modifications. This study introduces a comprehensive strategy merging omics, intact mass spectrometry, and other biophysical methods to provide groundwork for future research into snake venom characterisation.

Weijing Liu[1], Wilson Phung[2], Wendy Sandoval[2], Dr. Joanne Ford[3], Rosa Viner[1]
[1]Thermo Fisher Scientific, [2]Genentech, [3]Thermo Fisher Scientific

Automated molecular glues screening using native mass spectrometry

Weijing Liu 266 - Automated molecular glues screening using native mass spectrometry, Meeting Room 106, August 19, 2024, 11:03 - 11:22

Many proteins involved in diseases are considered "undruggable" using traditional small-molecule inhibitors due to their lack of well-defined binding pockets. Molecular glue (MG), by strengthening weak intrinsic interaction between target and E3 ligase, enabling the targeted protein degradation via the ubiquitin-proteasome system. Native mass spectrometry (nMS) preserving the noncovalent interactions allows the identification of E3-MG-target ternary complex. However, offline manual sample preparation and direct infusion limits the throughput. A newly designed integrated liquid chromatography system coupled to native MS streamlines the rapid online buffer exchange, parallel compound binding, and direct nMS analysis of complexes. The novel workflow has been applied for MG screening to assess their efficacy by directly detecting the ternary complexes.

Methods

The Thermo Fisher Scientific Vanquish LC equipped with a UV detector, fraction collector, dual-injection autosampler, and dual Flex pumps was used. Proteins were online buffer exchanged into ammonium acetate using a NativePac OBE-1 column and fractionally collected into a 96-well plate at the rate of 1.5 min/run. Each well of the 96-well plate was preloaded with ligands. The fraction collector chamber was set at desired binding temperature. Upon mixing proteins with ligands, incubation began while the remaining wells awaited desalted proteins. After all the wells were filled with desalted proteins, samples from each well were directly infused into the either Q-Exactive UHMR or OT Ascend using 15µm bullet emitter on the Easy Spray source.

Novel Aspect

Automate ligand binding assessment and protein degrader screening via high-throughput native mass spectrometry benefiting drug discovery

Preliminary Data or Plenary Speaker Abstract

We initially utilized Carbonic Anhydrase (CA) as the target to evaluate ligand binding efficacy of the proposed workflow. Initial tests employed 8 ligands with varying Kd values, ranging from <100nM to >10µM. The wells were preloaded with 100 µM of ligands respectively. The online buffer exchanged CA was fractionally collected into the ligand preloaded wells at 1.5-min/run and thus the final ligand concentration was 25 µM. Subsequently, the incubated samples were directly injected into MS at 5µl/min flow rate and sprayed at 1.6-1.8 kV using a 15 µm bullet emitter. We observed an increase in the fractional abundance of the CA-ligand complex with decreasing ligand Kd values. This surpassed conventional on-column desalting of CA-ligand complex, limited to Kd <1µM. It results from the post-column binding and low spray voltage minimizing the dissociation of weakly bound complexes.
To apply such workflow for molecular glue (MG) screening of potential protein degraders, we have identified a system involving weak intrinsic interaction between protein target and E3 ligase strengthened by MG. The preliminary study comprised of basal affinity measurement, four MGs screening, and one negative control measurement in which ligand binds only to E3 ligase but not to target protein. Without MG, the relative abundance of E3-target complex was 0.24%. When incubated with various MGs (MG-A, MG-B, MG-C, MG-D), the relative abundances of E3-MG-target complex were 15.6%, 100%, 100%, and 1% respectively. The inclusion of a negative control compound resulted in a decrease of the E3-target complex abundance to 0%. The apparent Kd ranking of MGs and controls, MG-C<MG-B<MG-A<MG-D<control<negative control, correlated well with measurements obtained from other biophysical assays. Overnight incubation revealed the instability of the E3-MG-target complex, emphasizing the necessity for rapid nMS characterization.

Future plan involves expanding this workflow to include broader MG libraries for more extensive screening.

Dr. Duong Bui[1], Dr. Elena Kitova[1], Professor Lara Mahal[1], Professor John Klassen[1]
[1]University Of Alberta

Deciphering Mechanisms and Thermodynamics of Protein Assembly using native mass spectrometry

Duong Bui 50 - Deciphering Mechanisms and Thermodynamics of Protein Assembly using native mass spectrometry, Meeting Room 106, August 19, 2024, 11:22 - 11:41

Most of the naturally occurring proteins exist as oligomers (homo or hetero complexes). Protein oligomerization is integral to structural stability, gene expression, signal transduction, and the regulation of various cellular processes. Conversely, protein aggregation can lead to biological dysfunction or human diseases, most notably neurodegenerative disorders, such as Alzheimer's disease and Parkinson's disease. Characterizing the thermodynamics of protein oligomerization is essential to understanding normal cellular processes and to guide development of therapeutics that target diseases where aberrant oligomerization is a key factor. Here, we describe for the first time the implementation of slow mixing mode native mass spectrometry (SLOMO-nMS) for quantifying the dissociation constants (Kd) of coupled equilibria and demonstrate the application of the assay to multi-step protein association reactions.

Methods

The SLOMO-nMS measurements were performed using Q Exactive Classic and Ultra High Mass Range Orbitrap mass spectrometers equipped with modified nanoESI sources. To implement SLOMO-nMS, two solutions of different compositions were loaded, in a layered fashion, into a nanoESI emitter and ion signal was monitored continuously. For heterocomplexes, the concentrations of interacting species in the solutions differed; for protein homo-oligomerization, the concentrations of interacting species were the same, but at different pH. The relative response factors (RFs) of the interacting species were obtained by solving a system of equations. To demonstrate reliability and versatility, SLOMO-nMS was used to measure the oligomerization of concanavalin A (ConA) and insulin (In) and association of SARS-CoV-2 spike protein with angiotensin converting enzyme 2 (ACE2).

Novel Aspect

First demonstration of SLOMO-nMS for quantifying coupled equilibria.

Preliminary Data or Plenary Speaker Abstract

Concanavalin A, a plant lectin, is used extensively in lectin arrays and tissue staining and is a potent anti-cancer agent. At neutral pH, the protein exists predominantly as homodimer (ConA2) and homotetramer (ConA4), with a Kd,tetramer-dimer of 24 μM. There is no reported Kd,dimer-monomer. At pH 7, nMS analysis revealed signal corresponding to ConA, ConA2 and ConA4. Application of SLOMO-nMS at increasing pH results in a decrease in the abundance of ConA2 and an increase in ConA and ConA4. Analysis of the changes in abundance yields the relative RFs, from which Kd,dimer-monomer of 1 μM and Kd,tetramer-dimer of 18 μM were calculated. Notably, the latter Kd agrees well with the literature value.
Insulin, a small protein that regulates blood glucose levels, exists as a hexamer in the pancreas and disassembles into monomer upon release into the bloodstream. The oligomerization of In at neutral pH is commonly described as proceeding through coupled equilibria involving monomer-dimer-hexamer (In-In2-In6), with Kd of 7 μM and 3 μM, respectively. However, nMS analysis at pH 7.4 identified signal corresponding to monomer through hexamer, indicating that oligomerization proceeds through the sequential monomer binding. The relative RFs determined from the SLOMO-nMS time-dependent changes in abundance enabled the Kd value for the five coupled equilibria to be determiend. Notably, Kd,dimer-monomer agrees with the literature value. Moreover, treating the higher order oligomers as hexamer gives an apparent Kd of 5 μM for the dimer-hexamer equilibrium, in agreement with the reported value.

Finally, to illustrate the power of SLOMO-nMS for uncovering and quantifying biologically important interactions, it was applied to the sequential association of recombinant SARS-CoV-2 spike protein and ACE2. Notably, the relative RFs deviate significantly from 1.0 and the Kd determined by SLOMO-nMS are as much as two orders of magnitude larger than values recently measured by nMS.

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Ryan Julian[1], Evan Hubbard[1], Brielle Van Orman[1], Thomas Shoff[1]
[1]Uc Riverside

Isomerization of tau provides mechanistic insight into the underlying causes of Alzheimer's disease

Ryan Julian 76 - Isomerization of tau provides mechanistic insight into the underlying causes of Alzheimer's disease, Meeting Room 106, August 19, 2024, 11:41 - 12:00

Amino acid isomerization occurs spontaneously in long-lived protein under physiological conditions, and isomerization of a single residue can impact protein structure, function, and turnover. Because isomerization exists and occurs largely outside the realm of biological control, it provides a baseline chemical diagnostic for evaluating proteostasis as a collective process. Defects in autophagy that lead to protein aggregation are independent of those that allow isomerization, making isomerization an orthogonal measure of brain health and cognitive status. Fortunately, recent developments in mass spectrometry have enabled rapid and sensitive means for identifying and quantifying isomerization in proteins in the brain.

Methods

Several methods were used to quantify isomerization including data-dependent, data-independent, and targeted approaches in combination with liquid chromatography. The key requirements for successful characterization regardless of the data acquisition paradigm include separation of the isomeric forms followed by repeated MS/MS acquisition for identical m/z targets. This is contradictory to typical proteomics approaches where repeated characterization is avoided but necessary to quantify and identify peptides containing isomeric residues. Quantitation is easily afforded by comparison of ion intensity as isomerization has little effect on ionization or ion abundance.

Novel Aspect

Asp isomerization reveals information about the underlying causes of neurodegeneration in human brains

Preliminary Data or Plenary Speaker Abstract

Of 6000 proteins examined from human brain samples, relatively few (~100) contain isomerized amino acid residues. Further examination reveals that the extent of isomerization correlates with disease status for even fewer proteins, two. Therefore, isomerization is rare, which potentially enables its occurrence to be highly diagnostic. Interestingly, tau is both susceptible to isomerization and is strongly associated with many forms of neurodegeneration. In autosomal dominant Alzheimer's Disease (AD), isomerization of tau is strongly associated with defects in presenilin that cause autophagic disruption. In control brains, including those with abundant protein aggregation, isomerization of tau is minimal. In sporadic AD, a full range of isomerization is observed, including cases with minimal isomerization. However, closer examination of the isomer types between sporadic AD and control reveals reproducible differences. In control brains, D-isoAsp is the dominant isomer while L-isoAsp is most abundant in sporadic AD. This suggests that isomerization reveals variation in the brain biology that is not correlated with or detectable by examination of amyloid plaque or tau fibrillation. Furthermore, comparison of tau isomerization in sporadic AD with MMSE scores reveals a surprisingly strong correlation where lower isomerization corresponds to better cognitive performance. This suggests that defects enabling isomerization are likely to negatively impact brain function.

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Professor Roger Summons[1], Dr Diana Dumit[1], Professor Xiaolei Liu[2]
[1]Department of Earth, Atmospheric and Planetary Sciences, Massachusetts Institute of Technology, [2]School of Geosciences, University of Oklahoma

Carotenoid pigments as environmental proxies for ancient, low oxygen environments.

Keynote: Professor Roger Summons Massachusetts Institute of Technology 596 - Carotenoid pigments as environmental proxies for ancient, low oxygen environments, Meeting Room 109, August 19, 2024, 10:00 - 10:25

Mass spectrometry is and has been the primary tool used to identify and catalog photosynthetic pigments preserved in sedimentary rocks over geological time. This report examines the how the compositions of carotenoids in environmental samples can inform what we find sedimentary rocks and, thereby, better understand their environmental and geological significance.

Methods

Intact pigments are typically identified by liquid chromatography–mass spectrometry (LC-MS) performed on Agilent high performance LC systems coupled to a quadrupole time-of-flight mass spectrometer through an electrospray ionization interface. Compound separations are achieved with a reverse phase gradient and ZORBAX SB-C18 columns. Molecular structures are identified based on accurate masses of the molecular species and MS2 spectra. Geologically reserved carbon skeletons are typically examined using GC-QQQ-MS methods that utilize reaction monitoring chromatograms and product ion mass spectra.

Novel Aspect

Rapid carotenoid production and sequestration in a small lagoon on Cape Cod mimics processes commonly observed in the geologic record.

Preliminary Data or Plenary Speaker Abstract

The carotenoid inventories of modern environmental samples and the pathways by which carotenoids are preserved are intimately dependent by the prevailing redox conditions. Oxygen and high light levels lead to rapid degradation of carotenoids whereas hydrogen sulfide enhances production of particular compounds as well as aiding their preservation via rapid sulfurization and sequestration in sulfur-bound macromolecules. Chemical deconstruction of this sulfur-bound component is key to elucidating the carotenoid inventories in environments that serve as modern analogues for their ancient counterparts.

The geological record of carotenoid pigments typically includes a particular suite of saturated hydrocarbons dominated by carotanes together with mono- and diaromatic hydrocarbons produced, in the main, by cyanobacteria as well as green and purple sulfur bacteria. Their chemical structures and carbon isotope compositions enable some to be directly attributed to particular source organisms which, in turn, provide clues about the prevailing environmental conditions under which the sediments were deposited. Carotenoids tend to occur in elevated abundances in environments where oxygen is scarce or absent or during periods of time when the oceans experienced oxygen depletion known as oceanic anoxic events (OAEs).

This presentation addresses ways that we can use data from a contemporary low-oxygen environment to better understand ancient counterparts.

Mr. Hyeon-Woo Lee[1], Mr. Hoon Lee[1]
[1]Korea Institute of Nuclear Safety

Study on the Determination Methods of the Natural Radionuclides(U-238, Th-232) in Building Materials and Consumer Products Using ED-XRF and ICP-MS

Hyeon-Woo Lee 56 -Study on the Determination Methods of the Natural Radionuclides (U-238, Th-232) in Building Materials and Consumer Products Using ED-XRF and ICP-MS, Meeting Room 109, August 19, 2024, 10:25 - 10:44

In Korea, a large number of consumer products are produced for the purpose of health promotion. In some of these products that advertise the benefits of weak radiation, such as negative ions and far infrared, naturally occurring radioactive materials (NORMs) such as monazite and zircon may be mixed in the manufacturing process. To protect the public from unreasonable radiation exposure, the regulation of raw materials and products has been continuously emphasized, and it is essential to use accurate radioactivity data and develop validated analytical methods for exposure dose assessment. In this study, analytical methods were validated for the determination of radionuclides (U-238, Th-232) in building materials and consumer products using ED-XRF and ICP-MS.

Methods

The 94 samples used to analyze natural radionuclides include building materials such as tiles, cement, paint and plasterboard, and consumer products such as ceramics, filter, mask, patch, bracelets, and necklaces. Prior to sample analysis, the method was validated using five certified reference materials. ED-XRF (Spectro) was used for screening and qualitative analysis of uranium, thorium, and other elements in powder samples. ICP-MS (Thermofisher) was used for quantitative analysis of U-238 and Th-232, and samples were prepared by alkali-fusion for decomposition and Fe co-precipitation for simplification of complex matrices. The calibration standard solutions for ICP-MS were prepared by diluting and mixing the uranium and thorium standard solutions, and Re-187 was added as an internal standard.

Novel Aspect

The analytical method validated in this study is expected to be useful for uranium and thorium analysis in consumer products.

Preliminary Data or Plenary Speaker Abstract

ED-XRF results for uranium and thorium of five certified reference materials showed accuracy ((experimental value-certified value)/certified value, %) within 30% and precision (standard deviation/mean of experimental value, %) within 10%. ICP-MS results showed accuracy and precision within 8% for both U-238 and Th-232. The results confirm that alkali-fusion can completely decompose environmental samples such as soil, sediment, coal fly ash, and raw materials such as bauxite and zircon, which are difficult to decompose by acid digestion. In addition, ICP-MS has been shown to provide precise measurements at low concentration levels and to have excellent selectivity and sensitivity, resulting in analytical applicability in various mediums. Using the same analytical method, building materials and consumer products that advertise weak radiation benefits were analyzed, and the building materials showed environmental-level radioactivity concentrations. On the other hand, the consumer products showed that 6 out of 47 products exceeded the raw material registration level of 1.0 Bq/g defined by the [Act on Protective Action Guidelines Against Radiation in the Natural Environment]. Specifically, bath salts, health patch, eye patch, and belt showed relatively high Th-232 radioactivity concentrations ranging from 1.4 to 8.2 Bq/g, and U-238 was highest in bath salts at 0.7 Bq/g. Samples above the raw material registration level of 1.0 Bq/g had higher thorium concentrations than uranium, suggesting the use of raw materials such as monazite, which has relatively high thorium concentrations. The process of rapid screening using ED-XRF to identify

products that exceed registration levels, and precise analysis using ICP-MS are considered to be efficient and useful for investigating a wide range of consumer products.

500

Dr Samir Damare[1]
[1]CSIR National Institute Of Oceanography

Mass spectrometry as a tool for understanding biological processes in Oceans

Samir Damare 500 - Mass spectrometry as a tool for understanding biological processes in Oceans, Meeting Room 109, August 19, 2024, 10:44 - 11:03

Life in Oceans has always intrigued humanity, and the quest to explore diversity is increasing. In the last two and half decades, new technologies have emerged in sequencing and mass spectrometry which are being targeted to understand life in Oceans. Proteomics is one of the omic technologies used to analyse the functional genome of marine organisms. For the biological processes to be understood, genetic studies should be complemented with proteomics studies.
In a project titled TraceBioMe, it was envisaged to carry out proteome mapping of the Indian Ocean, in terms of the peptides present in the vertical water column as well as upper biologically active seafloor sediment.

Methods

The water and sediment samples were collected starting from the Bay of Bengal, North-South transect along the 90E Ridge, up to the horse latitude in the southern Indian Ocean, in the Indian Contract Region in the Central Indian Basin and ending in the Arabian Sea.
The samples were processed onboard and later analysed using LCMS QToF. The peptide list was generated using Spectrum Mill software and further analyses with regard to functional assignment were carried out using UniPept and Galaxy Platform.

Novel Aspect

This study will form a good background for establishing the proteomic map in the Indian Ocean.

Preliminary Data or Plenary Speaker Abstract

More than 80 % of the peptides belonged to the Bacteria while only a small fraction was found to match with the archaeal groups. UniPept categorized the classes alpha-, beta- and gamma-proteobacteria under the phylum Pseudomonadota. The biological processes to which the peptides showed higher similarity were proteolysis, transmembrane transport, cell division, methylation, DNA repair and DNA replication.

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Prof. Dr. Clemens Walther[1], Dr. Grec Balco[2], Dr. Brett Isselhardt[2], Mr. Aaron Lehnert[1], Ms. Laura Leifermann[1], Mr. Paul Hanemann[1], Dr. Darcy van Eerten[1], Dr. Mike Savina[2], Mr. Tobias Weissenborn[1], Prof. Dr. Klaus Wendt[3]
[1]Leibniz University Hannover, [2]Lawrence Livermore National Laboratory, [3]University Mainz

Nuclear forensics on single micrometer sized particles: recent developments of secondary neutral mass spectrometry for ultra-trace isotope analysis

Clemens Walther 589 - Nuclear forensics on single micrometer sized particles: recent developments of secondary neutral mass spectrometry for ultra-trace isotope analysis, Meeting Room 109, August 19, 2024, 11:03 - 11:22

Micrometer-sized radioactive particles are of highest concern in environmental and life sciences, cosmochemistry and forensics. From their composition, detailed information on the origin is obtained including peaceful uses like a nuclear power reactor, medical isotope production or gamma ray sources for technical applications. Sometimes, the sample might hint at illegal trafficking or military use. Recent radioactive contamination in the Ukraine might originate from the Chornobyl accident. But what if the radioactivity was released by damage of one of the recent reactors such as Sapoirischschja. Potential risks to human health is assessed. Long-term weathering of the particle in the environment leads to release of radionuclides previously bound inside the particle. Hot particles from different environmental media are investigated.

Methods

Single particles are extracted from environmental media, fixed onto W-needles and introduced into a Time-of-Flight Secondary Ion Mass Spectrometer. In addition to operating in SIMS mode, secondary neutral atoms can be post ionized by resonant Laser Ionization (rL-SNMS) using two 10kHz nanosecond Nd-YAG lasers pumping up to five tunable Ti:Sa lasers. This highly element-selective ionization suppresses isobaric interferences such as U-238/Pu-238 and Pu-241/Am-241 by up to five orders of magnitude without need for chemical sample preparation. Actinide elements and fission products are analyzed at the below femtogram level, allowing isotope selective imaging with spatial resolution better than 100nm. In contrast to dynamic SIMS, only negligible mass is consumed, leaving the particle intact for further studies.

Novel Aspect

Isotope distribution in single µm-particles is imaged at 70 nm spatial resolution with isobar suppression consuming only attograms of sample.

Preliminary Data or Plenary Speaker Abstract

Detection and localization of ultra-trace concentrations of radioactive actinides has been demonstrated on hot particles of uranium oxide fuel with sub femtogram sample consumption. SEM analysis as well as TOF-SIMS and rL-SNMS analysis were combined, keeping the original particle sample intact for further analyses. Miniscule amounts of isotopes contained within the particle were unambiguously identified and distributions within the hot particles were imaged in ion maps. Without any sample preparation, minor trace isotopes like Pu-238, Am-242m or Cm-244 were detected with lowest isobaric interferences. From the isotope patterns, conclusions are drawn on origin (e.g. reactor type) and history of the particle (e.g. fuel enrichment, recycling and burn-up). The measurement of fission products Sr, Cs, Ba, Eu allows to further detail these information and furthermore allows dating of particle age. Up to five elements are measured within one working day, making this a fast method for emergency response.
We demonstrated, that the fission gases Kr and Xe are still confined in particles from the Chernobyl accident in spite of the very high temperatures during their formation. Kr and Xe isotope ratios reveal

information on Pu / U fission ratios and other reactor operation details. Furthermore, ratios of actinides and fission products are indicative of leaching processes due to decade long weathering of particles in the environment.
Since SNMS is quasi non-destructive, particles are still available after isotopic characterization for further investigation like chemical leaching: Selected individual particles were sequentially leached in stepwise more aggressive chemicals. The solutions are then measured via gamma spectrometry and ICP-MS to analyze their uranium content and the amounts of leached fission and breeding products. From these data, conclusions on potential risks to human health and environment are drawn.
The technique is applicable to many more elements and opens up new scientific applications beyond the nuclear field.

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Professor Oliver Jones[1]
[1]RMIT University

Combining contaminants of emerging concern with environmental isotopes to distinguish wastewater and agricultural impacts on groundwater systems

Oliver Jones 477 - Combining contaminants of emerging concern with environmental isotopes to distinguish wastewater and agricultural impacts on groundwater systems, Meeting Room 109, August 19, 2024, 11:22 - 11:41

Wastewater treatment plants are used worldwide to purify domestic and industrial wastewater before it is returned to the environment. Even after treatment, wastewater may contain a variety of contaminants, including nutrients (usually nitrogen and phosphorus) and organic pollutants (e.g. endocrine disruptors and pesticides). These compounds can leak into groundwater via old and/or damaged infrastructure, leaching from biosolids storage areas, and/or release of effluents. Remediating such pollution is complicated by the fact that similar contaminants and impacts can come from other sources, such as agriculture. It is often difficult to distinguish the true source of such contamination, especially where wastewater treatment plants are located in agricultural areas. Novel methods are therefore needed to characterise impacts where there are multiple potential sources.

Methods

This study analysed stable and radioactive isotopes via accelerator mass spectrometry and multiple Contaminants of Emerging Concern using liquid chromatography triple quadrupole mass spectrometry (LC–QQQ–MS) to generate unique fingerprints of pollution. These were then combined with hydrogeological and nutrient measurements to distinguish the impact on the local groundwater of a Wastewater treatment plant in southeast Victoria (Australia) from that of local agriculture.

Novel Aspect

Using ratios of environmental contaminants as tracers provides a means to better characterise the nature of impacts on groundwater systems.

Preliminary Data or Plenary Speaker Abstract

The radioactive isotope tritium provided a sensitive indicator of recent (post-1990s) leakage, suggesting WWTP infrastructure had at some point treated or partially treated effluent to the underlying groundwater system. This was corroborated by water stable isotopes, which showed clear demarcation between background groundwater and on-site wastewater. The CECs, particularly the carbamazepine:simazine ratio, provided a means to further distinguish wastewater impacts from other sources, with groundwater down-gradient of the plant reporting elevated ratios compared to those up-gradient. Distinctive CEC ratios in impacted groundwater close to the WWTP and further down-gradient are interpreted to represent a change in composition over time (i.e., recent vs. legacy contamination), consistent with the site development timeline and possible changes in effluent composition resulting from infrastructure upgrades. The data indicate a complex set of co-mingled plumes, reflecting different inputs (in terms of both quantity and concentration) over time. The results also suggest that groundwater up-gradient of the plant has been impacted by surrounding agricultural land uses, while groundwater adjacent to and down-gradient of the plant exhibits elevated nutrient concentrations characteristic of wastewater derived impacts

Mr. Robert Kirkby[1], Dr. Johannes Friedl, Dr. Daniele De Rosa, Prof. Timothy Clough, Prof. David Rowlings, Prof. Peter Grace
[1]Queensland University Of Technology, [2]Boku Life Sceinces University , [3]University of Basilicata, [4]Lincoln University

Using an automated soil incubation system coupled to online IRMS to resolve N2 and N2O emission pathways from agricultural soils

Robert Kirkby 634 - Using an automated soil incubation system coupled to online IRMS to resolve N2 and N2O emission pathways from agricultural soils, Meeting Room 109, August 19, 2024, 11:41 - 12:00

Acid-sulphate sugarcane soils in the subtropics are known hot-spots for environmentally significant nitrous oxide (N_2O) emissions. However the reduction of reactive N_2O to non-reactive dinitrogen (N_2) via specific pathways remains a major uncertainty for nitrogen (N) cycling and losses from these soils due to methodological constraints in measuring N_2 against the 78% atmospheric background. This study investigated the magnitude and the N_2O:N_2 partitioning of N_2O and N_2 losses from a subtropical acid-sulphate soil under sugarcane production using a fully automated soil incubation system coupled to an Isotope Ratio Mass Spectrometer (IRMS) and emissions of N_2O:N_2 resolved using ^{15}N gas flux methods. These results established the contribution of hybrid (co- and chemo-denitrification) and heterotrophic denitrification to N_2O and N_2 losses.

Methods

A fully automated laboratory based soil incubation system was coupled to an isotope-ratio mass-spectrometer enabling real time ^{15}N gas flux analysis of $^{15}N_2O$ and $^{15}N_2$ at sub-daily resolution for continuous monitoring of soil N_2O and N_2 fluxes over 30 days. Complementary measurements of soil O_2, CO_2 emissions, pH, temperature, nitrate and ammonium concentration, and soil moisture data were also collected to determine drivers of measured emissions via generalised additive models. Contributions of hybrid pathways for N_2O and N_2 production were resolved with calculations from Clough (2001), assuming hybrid pathways of N gas production could result in increases in m/z 29 only following the addition of a ^{15}N label to unlabelled soil.

Novel Aspect

High resolution $^{15}N_2O$+$^{15}N_2$ methods enable quantification of both environmentally significant gasses at sub-diel resolution- a methodological constraint hindering N budgeting.

Preliminary Data or Plenary Speaker Abstract

The ^{15}N soil incubation system captured two N_2O and N_2 fluxes day^{-1} from each of eight soil chambers, based on the linear increase of N_2O and N_2 over three successive measurements of the isotopologues of N_2O and N_2 via IRMS. The deviation (%) of a single N_2 flux from the daily average flux estimate deviated by as much as 97% from the daily average, equal to > 2 kg N_2-N ha^{-1} day^{-1}, highlighting the need for sub-daily measurements. The precision of the IRMS for N_2 (N_2O+N_2) at 95% confidence intervals (n=48) for Δ29R was 9.5 × 10−7 and 2.8 × 10−6 for Δ30R. This equated to a method detection limit (MDL) for N_2 of 18.24 g N ha^{-1} day^{-1} with aD assumed at 0.6. The in-batch DL for N_2 ranged from 5.5 × 10−7 to 2.1 × 10−6 for Δ29R, and from 2.1 × 10−6 to 3.2 × 10−6 for Δ30R, with 95% of all fluxes exceeding the respective daily DL. The DL for N_2O was 1.3 × 10−4 for 45R and 6.3 × 10−5 for 46R. Given the high enrichment of N_2O measured against a low background, all measurements exceeded the DL. The DL for the concentration of N_2O was 0.11 ppm, which equates to a DL at a 95% confidence interval of 1.5 ppm, resulting in a MDL for N_2O of 1.5 g N ha−1 day−1. Peak losses of N_2O and N_2 reached 6.5 kg N ha^{-1} day^{-1}, totaling > 50 kg of N_2O+N_2-N ha^{-1}. Emissions were dominated by N_2, accounting for more than 57% of N_2O+N_2 losses, demonstrating that the reduction of N_2O to N_2 proceeded even under highly acidic conditions. Over 40% of N_2O, but only 2% of N_2 emissions, were produced via hybrid pathways.

748

Professor Evan Bieske[1]
[1]University of Melbourne

Spectroscopic studies of bare and hydrogenated carbon cluster cations

Keynote: Professor Evan Bieske University of Melbourne 748 - Spectroscopic studies of bare and hydrogenated carbon cluster cations, Meeting Room 110, August 19, 2024, 10:25 - 10:44

Preliminary Data or Plenary Speaker Abstract

Carbon's bonding propensities are reflected in an array of different carbon allotropes, including linear chains, cyclocarbons, fullerenes, graphene, nanotubes, diamond and amorphous carbon. Whereas some of these allotropes have been thoroughly studied, others have proved more elusive to generate, capture and characterize. To learn more about the structures and properties of small carbon clusters, we have spectroscopically interrogated positively charged clusters containing between 10 and 40 carbon atoms. With increasing size, these clusters progressively adopt cyclic, bi-cyclic, graphenic and fullerene structures, with all four isomers coexisting for some sizes (e.g. C32+-C40+). The coexistence of isobaric isomers presents severe challenges for measuring unambiguous electronic or infrared spectra that we have addressed by marrying ion-mobility mass spectrometry and laser spectroscopy. This has allowed us to obtain electronic spectra of mobility-selected cyclocarbons and show that they have sharp absorptions spanning the visible and near infrared spectral regions. We have also spectroscopically interrogated singly hydrogenated carbon clusters (CnH+). The smaller clusters tend to be linear, but with increasing size, are gradually supplanted by monocyclic isomers. Again, depending on size and structure, spectra of the hydrogenated carbon clusters have sharp bands in the visible and near UV range. Recently, we have commenced spectroscopic studies in the infrared region, measuring spectra of hydrocarbon cations across the 5.5-18 µm range. Infrared spectra should eventually help elucidate the role of hydrocarbon cations in space, and their possible relevance to unidentified infrared emission bands at 6.2, 7.7, 8.6, 11.2, and 12.7 µm.

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Minsu Kim[1], Kyeongmin Nam[1], Minwook Hwang[1], Prof. Jongcheol Seo[1], Kyunghwan Kim[1]
[1]Postech

Research on size dependent molecular behavior of ESI-generated charged droplets by using X-ray scattering

Minsu Kim 491 - Research on size dependent molecular behavior of ESI-generated charged droplets by using X-ray scattering, Meeting Room 110, August 19, 2024, 10:25 - 10:44

Electrospray ionization (ESI) is a method to make a solution sample into gaseous ions by inducing Coulombic explosion of the sample droplets under strong electric field. While the droplets are getting smaller, unique molecular behaviors different from bulk solution appear, for instance, formation of unique molecular cluster or drastic concentration increase of analytes in the droplets. However, since the nanodroplets are tiny and rapid, the detailed mechanism of these intriguing properties are difficult to observe. Therefore, we have introduced ultra-short and super-bright X-ray Free Electron Laser (XFEL) to elucidate no-mans-land of the electrospray-ion-formation mechanism and its diverse unique properties.

Methods

We have developed a custom vacuum-ESI/X-ray scattering chamber to measure wide-angle X-ray scattering (WAXS) and small-angle X-ray scattering (SAXS) observe nanodroplets in Angstrom and sub-micrometer scale simultaneously. Then, we utilized ultra-short and super-bright X-ray pulse from PAL-XFEL due to low sample density and high speed and small size of the nanodroplets.
Using this setup, we have been trying to obtain 1) size distribution of the nanodroplets and 2) unique molecular behaviors depending on the size of the nanodroplets by collecting WAXS and SAXS patterns simultaneously. We have successfully estimated the size of the nanodroplets and trying to find clues of the molecular behaviors related to the size of the nanodroplets

Novel Aspect

The size distribution of ESI-nanodroplets were observed during the spray process for the first time.

Preliminary Data or Plenary Speaker Abstract

Methanol (MeOH) was chosen as a solvent since water was impossible to spray in vacuum without IR heating laser.
Phosphotungstic acid hydrate (PTA) and Lithium chloride (LiCl) were chosen as spray agents.

The droplet size was monitored by using SAXS, which measures electron density contrast of the target. Also, WAXS was simultaneously measured for 1) augementing SAXS information by providing volume information of the proved droplets, 2) monitoring unique behaviors of the analytes in the droplets.
The SAXS and WAXS patterns of the droplets were successfully obtained, thus proved that droplet size distribution could be elucidated by using X-ray scattering with X-FEL.

PTA and LiCl was compared since high-atomic-number atoms produce much more intense signal that is proportional to atomic number squared. The opening cone angle of the PTA and LiCl was carefully adjusted, so that they can have simillar droplet size distribution. LiCl spray was used as blank signal of the WAXS by doing so, and will be used for distinguishing PTA signal buried in the data.

Unique molecular behaviors from WAXS have yet to be analyzed, however, scattering pattern of the nanodroplets was different from each other depending on which sample is used.

In the future, the behaviors of the nanodroplets related to the polarity of the applied voltage, flow rate of the solution, or the shape of the emitter will be investigated.

Dr Xianglei Kong[1]
[1]Nankai University

Generation and Identification of Free Radical Cations Using a UV/IR Double-Beam Laser System Combined with an FT ICR Mass Spectrometer

Xianglei Kong 594 - Generation and Identification of Free Radical Cations Using a UV/IR Double-Beam Laser System Combined with an FT ICR Mass Spectrometer, Meeting Room 110, August 19, 2024, 10:44 - 11:03

The high reactivity, extremely short lifespan, and instability of radicals make it challenging to study their structures. To address this, here we develop a mass-spectrometry-based method to generate radical cations and to study their structures in the gas phase.

Methods

Our research delves into the properties of biomolecular radical ions through the use of the UV/IR dual-beam photodissociation method in mass spectrometry and spectroscopy. we utilized UV irradiation to generate various radical cations in the gas phase. These were then isolated in the cell of an FT ICR MS and studied further with tandem mass spectrometry and IR photodissociation (IRPD) spectrometry and spectroscopy.

Novel Aspect

Our findings underscore the potential of the UV/IR dual-beam photodissociation method for researching free radicals and related species.

Preliminary Data or Plenary Speaker Abstract

Intriguingly, we observed different IRPD spectra of radical cations of cytidine generated by UV lasers at different wavelengths, indicating their different structures. Our theoretical calculations revealed that changing the energy of a single UV photon can selectively generate radical isomers due to the energy barrier of the primary radical ions' hydrogen migration generated by the cleavage of the C-I bond in the precursor ions. Additionally, we identified and studied important intracluster bonding reactions through IRPD spectroscopy.

708

Shibdas Banerjee[1]
[1]Indian Institute of Science Education and Research Tirupati

Stabilizing Reactive Intermediates in Aqueous Microdroplets

Shibdas Banerjee 708 - Stabilizing Reactive Intermediates in Aqueous Microdroplets, Meeting Room 110, August 19, 2024, 11:03 - 11:22

Water becomes a wonder chemical when it forms microdroplets. Such microdroplets are ubiquitous on Earth. Recent advances in microdroplet chemistry hinted at the mysterious nature of water microdroplets, which is strikingly different than the corresponding bulk phase. Chemical reactions often proceed through the intermediacy of unstable species. The lifetime of such reactive species could be so fleeting (e.g., nanoseconds to picoseconds) that they remain undetected. Kinetics and product studies sometimes indirectly support the existence of such intermediates. This talk will provide an overview of the state-of-the-art microdroplet technologies currently being exploited in our laboratory to intercept fleeting chemical and biological intermediates, which are otherwise difficult to study.

Methods

We made water microdroplets behave like superacids to capture and stabilize extremely short-lived carbocations (lifetime ~ nanoseconds to picoseconds) formed as elusive intermediates in chemical reactions. Likewise, we detected the spontaneous transformation of phenols into phenyl carbocations in water microdroplets, which we have utilized to perform the ipso-substitution reaction of phenol in a semi-preparative scale. We also tamed aqueous microdroplets to intercept reactive carbanion species in real-time from the reaction aliquot, probing their intermediacy. While many intensive attempts in the past either failed to capture or underestimated the co-populated intermediate conformers from the protein folding/unfolding reaction, we succeeded with microdroplet techniques to kinetically trap, resolve, and quantify protein conformers in equilibrium that evolve during unfolding in solution.

Novel Aspect

Overall, this presentation will address our developments with the mysterious power of water microdroplets to investigate chemical and biochemical transformation.

Preliminary Data or Plenary Speaker Abstract

How the unique nature of microdroplets renders reactive intermediate stabilization and nitrogen fixation in the environment and offers an opportunity to separate water isotopologues will also be highlighted.

514

Prof Peter O'Connor[1]
[1]University Of Warwick

Advances in Two-Dimensional Mass Spectrometry

Peter O'Connor 514 Advances in Two-Dimensional Mass Spectrometry, Meeting Room 110, August 19, 2024, 11:22 - 11:41

Two-dimensional mass spectrometry (2DMS) allows acquisition of fragment information from all precursors simultaneously by modulation of a trapped ion packet position through a fragmentation zone. The advantages of Two-dimensional Mass Spectrometry (2DMS) are several. 2DMS does not require precursor ion isolation prior to fragmentation, so resolution in the precursor ion dimension is only limited by the number of scan lines chosen, and the ion beam stability. Having all fragments from all precursors allows the user to avoid any ion selection biases (either by the user or by the DDA algorithm). Having all fragments from all precursors also allows interesting chemical correlations to be revealed in the spectra.

Methods

The fragmentation zone for 2DMS is best created using either an electron beam (ExD methods) or by using a laser (IRMPD or UVPD). The modulation is created using a classic Gaumann pulse sequence (a low amplitude frequency sweep pulse (P1), an iterated delay (T), and a second sweep pulse (P2 =P1) or by using the SWIFT/SWIM pulse sequences from Ross and Marshall. In either case, all ions are modulated at their own cyclotron frequencies, through the fragmentation zone and fragments are born (or not) at the frequency of the precursor ions, so an FT of the peak intensities of all peaks will extract the modulation frequencies of all ions, thereby correlating fragments with their precursor ions, even in complex mixtures.

Novel Aspect

We will discuss the state of the art in 2DMS, and attempt to project the longer-term utility of these techniques.

Preliminary Data or Plenary Speaker Abstract

Over the past 10 years, our group has been working to implement this methodology and have done so using all the fragmentation methods above. We have also implemented an MS/2DMS methodology and a TIMS/2DMS methodology as well. Furthermore, we have shown the ability to differentiate fragments at greater than 4 sigma) from two different precursors which are only 19 mDa apart.

We have also found that 2DMS is particularly useful in all cases where complex mixtrues of overlapping species are present, so we are currently using it for polymer structural differentiation, top-down proteomics, lipidomics, and glycan analysis. The goal, eventually, is to try to do this method on extremely complex mixtures such as petroleum or similarly complex environmental extracts.

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Prof Adam Trevitt[1], Dr Oisin Shiels[1], Dr Boris Ucur[1], Professor Stephen Blanksby[2], Dr Samuel Marlton[1]
[1]School of Chemistry and Molecular Bioscience, University of Wollongong, [2]School of Chemistry and Physics, Queensland University of Technology

Laser photodissociation and ion reactivity of selected protonation-site isomers

Adam Trevitt 667 - Laser photodissociation and ion reactivity of selected protonation-site isomers, Meeting Room 110, August 19, 2024, 11:41 - 12:00

The separation and assignment of protonation isomers (protomers) is a challenge for mass spectrometry and requires a combination of techniques. Once separated though, the effect of the protonation site on the photostability properties of these ions is accessible for study. Also, with varying proton position, the influence of the internal electric field – and its orientation – can also be clearly evaluated and characterised. In this presentation, we examine this in the context of two gas-phase protomer situations combining laser photo-initiation and ion-trap mass spectrometry: (i) photodissociation action spectroscopy and (ii) radical kinetics of distonic radical ions.

Methods

A combination of room temperature ion-trap mass spectrometry, ion-molecule kinetics, and a tunable UV-Vis pulsed-laser photodissociation is used to measure the photodissociation and reactivity of m/z selected ions formed from electrospray ionisation. Ion filtering with FAIMS is also utilised. Computational results for excited-states and ground-state kinetic modelling assist in the assignment of spectra and rationalising the formation of product ions.

Novel Aspect

Combination of ion filtering, lasers and ion-molecule kinetics provide insights into the effect of ion internal electric fields

Preliminary Data or Plenary Speaker Abstract

In our research, ion trap mass spectrometry of electrosprayed ions is performed using LTQ Velos and XL platforms and these can be equipped with the FAIMS (high field asymmetric waveform ion mobility spectrometry). These systems are modified to allow selected laser pulses (nanosecond, tunable wavelength) to pass down the long axis of the linear ion trap. Photoproduct yields can be recorded as a function of photon energy to build an action spectrum of m/z selected ions.

We first will show how FAIMS ion filtering can separate small protonation isomers and this is confirmed using UV PD spectroscopy with careful comparison of the vibronic details within the spectrum. This is important to (i) unequivocally validate the protomer separation and, in turn, (ii) validate the electronic structure calculations of chromophores that are different only by the site of protonation.

Using this strategy, protonation isomer-selected radical ions are isolated and separated and this allows detailed analysis of the electric field effect, imparted by protonation, on the radical kinetics. This provides unprecedented detail to evaluate and benchmark calculations of these reactions and thus can guide/validate quantum methods. For ion kinetics, we show the popular DFT methods do not adequately describe the long-range interactions between these ion-radical + neutral reactions. And although relative weak, these long-range forces give rise to pre-reactive complexes are the gatekeepers of reactive. Furthermore, the influence of the charge appears to act through-space rather than through-bond.

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Dr Anne Bendt[1]
[1]National University Of Singapore

Clinical Translation of Lipidomics – a case study

Keynote: Dr Anne K. Bendt National University of Singapore 112 - Clinical Translation of Lipidomics – a case study, Plenary 3, August 19, 2024, 15:00 - 15:25

Lipid metabolites beyond triglycerides and cholesterol have vast potential for applications in clinical diagnostics, with substantial societal and economical value. To successfully evolve from the current research-grade analytical methods to assays suitable for routine clinical applications, a harmonization - if not standardization - of these mass spectrometry-based workflows is necessary. I will use the ceramides as a case study for successful clinical translation of lipidomic findings into routine assays. Additionally, this presentation is intended to provide an overview of international efforts to tackle the issues of workflow harmonization, and to serve as an open invitation for others to join this growing community. Specifically, past and planned interlaboratory ring trials for select lipids with potential for clinical diagnostics will be discussed.

Breanna Dixon[1,2], Dr Waqar Ahmed[1], Dr Drupad Trivedi[1], Prof Stephen Fowler[1], Dr Tim Felton[1,2]
[1]University Of Manchester, [2]Centre for Precision Approaches to Combatting Antimicrobial Resistance

Uncovering the resistance phenotype of carbapenemase-producing Enterobacteriaceae (CPE) using metabolomics

Breanna Dixon 48 - Uncovering the resistance phenotype ofcarbapenemase-producing Enterobacteriaceae (CPE) using metabolomics, Plenary 3, August 19, 2024, 15:25 - 15:44

Antimicrobial resistance is a growing public health challenge. One subset of particular concern are carbapenemase-producing Enterobacteriaceae (CPE). Conventional clinical methods for detecting CPE typically involve culture-based techniques with lengthy incubation steps, and the sensitivity afforded by these approaches is poor. There is a need to develop rapid and accurate methods for the detection of resistance. In this study we sought to characterise the metabolome of bacterial isolates belonging to CPE and non-CPE groups using high resolution LC-MS. With cellular phenotype closely linked to the metabolome, we hypothesised that the acquisition of resistance would result in fundamental differences in microbial metabolism. We aimed to uncover discriminative metabolites with diagnostic potential for the prediction and timely detection of CPE.

Methods

Three independent replicates were inoculated from CPE (n = 18) and non-CPE (n = 18) precultures and grown for 6h. Metabolism was quenched with cold methanol and intracellular metabolites were extracted using a modified Folch method. Polar and non-polar metabolites were analysed using HILIC and RP-LC-MS, respectively, on an Orbitrap QExactive Plus.
Spectra were preprocessed with XCMS and feature selection was performed using machine learning models and stringent selection criteria. MS/MS data were acquired for the resulting inclusion list and putative annotation was performed using spectral library matching. Annotations were confirmed to MS1 level using standard compounds where possible.
Receiver operating characteristic curve analysis was used to ascertain the predictive performance of the obtained metabolites.

Novel Aspect

We believe this to be the first study to utilise LC-MS for the investigation of the CPE metabolome.

Preliminary Data or Plenary Speaker Abstract

Application of Bonferroni-correction to Welch's t-test p-values revealed 116 and 274 differential features ($p \leq 0.05$) between CPE and non-CPE groups in HILIC ESI+ and ESI- mode, respectively. For RP, 13 differential features ($p \leq 0.05$) were seen in ESI+ mode, whilst no features met the criteria in ESI- mode. The intracellular HILIC datasets were further refined according to variable contributions towards each ML model for the comparison of CPE and non-CPE (ESI+ n=16, ESI- n=42).
Targeted single ion monitoring with ddMS2 was performed on precursor ions belonging to the reduced feature lists. With respect to the intracellular samples, we were able to putatively annotate seven unique metabolites from HILIC ESI+ data, four unique metabolites from HILIC ESI- data, and two from RP ESI+ data. We were able to confirm eight annotations to MS1 level.
Combining these 13 intracellular metabolites into a single model for the prediction of CPE demonstrated excellent performance metrics across all three machine learning algorithms, with PLS-DA displaying overall superiority for AUC (AUC = 0.967) but was otherwise equivalent with knn for specificity (83.3%), sensitivity (100%) and accuracy (92.6%).

We have demonstrated the ability to model CPE based on the metabolome using high resolution LC-MS/MS and have unveiled potential metabolite biomarkers for the acquisition of carbapenem resistance with high predictive performance. Future studies should seek to validate these compounds and explore their diagnostic potential in the clinical setting for the detection of carbapenem resistance in a more timely manner.

Mr. Prabhu Rangabashyam[1], Dr. Lukáš Cudlman[2,3], Miss Qiyu (Monica) Liu[1], Dr. Venkateswara R. Narreddula[1], Dr Berwyck Poad[1], Dr. Josef Cvačka[2,3], Professor Stephen Blanksby[1]
[1]School of Chemistry and Physics and the Central Analytical Research Facility, Queensland University of Technology, [2] Institute of Organic Chemistry and Biochemistry of the CAS, [3]Charles University, Faculty of Science

Elucidating novel fatty acid structures in vernix caseosa by combining ultraviolet photodissociation and ozone-induced dissociation mass spectrometry

Prabhu Rangabashyam 438 - Elucidating novel fatty acid structures invernix caseosa by combining ultraviolet photodissociation and ozone-induced dissociation mass spectrometry, Plenary 3, August 19, 2024, 15:44 - 16:03

Vernix caseosa is a lipid rich biofilm that protects newborn babies and is comprised of a diverse array of lipid classes. The lipidome complexity and structural diversity presents a significant challenge to conventional chromatography-mass spectrometry methods in providing unambiguous identification of known lipids and structural elucidation of novel fatty acids. Here we describe the application of reversed-phase liquid chromatography (LC) in conjunction with ultraviolet photodissociation (UVPD) and ozone-induced dissociation (OzID) mass spectrometry (MS) to surmount this challenge and enable robust de novo identification of the fatty acids (FAs), the building blocks of the vernix lipidome.

Methods

A pooled vernix caseosa lipid extract from ten individuals was hydrolysed and derivatised with 1-(4-(aminomethyl)-3-iodophenyl)pyridin-1-ium (4-I-AMPP). Thus derivatized, fatty acids were subjected to reversed-phase LC-MS on three platforms: (i) a linear ion-trap mass spectrometer (Thermo Scientific, LTQ-XL) modified to enable UVPD at 266 nm; (ii) a tribrid Orbitrap platform (Thermo Scientific, Fusion Lumos) fitted with a 213 nm laser; and (iii) an ion-mobility-enabled quadrupole time-of-flight mass spectrometer (Waters SYNAPT G2-Si) modified for ozone-induced dissociation.

Novel Aspect

Chromatographic alignment of UVPD and OzID mass spectra identify hitherto unreported unsaturated methyl branched-chain fatty acids in vernix caseosa

Preliminary Data or Plenary Speaker Abstract

The preliminary analysis of this pooled fatty acyl sample found evidence for over 400 distinct FAs spanning carbon-chain numbers ranging from 9 to 40 and degrees of unsaturation ranging from 0 up to 6. Consistent with prior reports, examination of the UVPD mass spectra found evidence for large numbers of abundant branched-chain fatty acids, with direct evidence for 140 branched-chain FAs among the 176 saturated FAs identified and comprising nearly 27 mol% of this fraction. Moreover, UVPD data were used to assign site(s) of methyl-branching and revealed a broad trend of even carbon-number exhibiting methyl-branching at the penultimate position (iso), while odd carbon number variants were predominantly branched at the antepenultimate position (anteiso). Notably, evidence is presented here for the methyl-branching at other non-canonical positions, including the 4-Me FAs (with respect to the carboxylic acid) spanning carbon chains from 9-to-18 carbons, as well as doubly and triply methylated chains.
Unsaturated FAs were found to comprise almost 42 mol% of fatty acids in vernix caseosa, ranging in carbon-chain length from 10–40 and up to 6 degrees of unsaturation. Leveraging the advantages of LC-MS for fatty acid analysis, ultra-long chain fatty acids are observed for the first time and are characterised by the presence of polyunsaturation (e.g., FA 38:4) and also branched-chain monounsaturated FAs. Chromatographic alignment of UVPD and OzID mass spectra enabled the explicit assignment of both positions of unsaturation and chain branching. Taken together, these

data show evidence for ultra-long chain monounsaturated with unusual sites of unsaturation (e.g., FA 36:1n-10), alongside branched-chain isomers that have been subject to desaturation by different enzyme systems (e.g., 34Me-FA 35:1n-9).

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Mr. Sk Ramiz Islam[1,2], Dr. Soumen Kanti Manna[1,2]
[1]Saha Institute of Nuclear Physics, [2]Homi Bhabha National Institute

Identification of novel glucose-independent, and reversible metabolic pathways associated with anti-proliferative effect of metformin in HepG2 cells

Sk Ramiz Islam 205 - Identification of novel glucoseindependent, and reversible metabolic pathways associated with anti-proliferative effect of metformin in HepG2 cells, Plenary 3, August 19, 2024, 16:03 - 16:22

Despite metformin's success in reducing HCC incidence, its mechanism against this prevalent cancer remains elusive. While AMPK activation and its effects on central carbon metabolism are often implicated, its impact on the glucose-deprived microenvironment of tumor core remains unclear. Thus, investigating its impact under both glucose-replete and deprived conditions is crucial for a comprehensive understanding of its potential. This would be essential to rationalize its anti-cancer utility and develop novel therapeutic strategies using metformin or its analogues. Furthermore, the impact of metformin withdrawal on cancer cells has never been examined. A detailed analysis of cellular response upon metformin withdrawal is warranted to formulate effective preventive and therapeutic strategies for HCC.

Methods

In this study, the dose- and time-dependent effects of metformin on viability and proliferation of liver cancer (HepG2) cells were examined. This was followed by examination of time-dependent (3 - 48 hr) effects of a sub-lethal dose of metformin treatment on cell viability, cytotoxicity, proliferation as well as associated changes in cellular metabolome with or without glucose deprivation. To examine the reversibility of the effect of metformin and glucose deprivation on metabolism, gene and protein expression and phenotypes, cells were treated with metformin under glucose deprivation for 24 hrs followed by withdrawal of metformin and/or replenishment of glucose. Combined analysis of transcriptomic and metabolomic signatures was performed to identify pathways of interest and elucidate the underlying mechanism.

Novel Aspect

Identification of novel metabolic targets of metformin and their reversibility in cancer could redefine its scope in HCC management.

Preliminary Data or Plenary Speaker Abstract

The evolution of metabolic signatures was examined to identify early metabolic events. Even sub-lethal metformin treatment was found to dramatically reduce proliferation in glucose-deprived cells that otherwise didn’t show any significant reduction in proliferation. Metabolomic analysis revealed that apart from mitochondrial impairment, derangement of fatty acid desaturation, one-carbon, glutathione and polyamine metabolism were associated with metformin treatment irrespective of glucose supplementation. Depletion of pantothenic acid, downregulation of essential amino acid uptake and metabolism alongside purine salvage were identified as novel glucose-independent effects of metformin. These were significantly correlated with cMyc expression and reduction in proliferation.Overall, a strong metabotype-phenotype correlation irrespective of glucose supplementation was observed. While effects of metformin on suppression of EAA uptake and metabolism, pantothenate level and purine salvage pathways are novel in the context of cancer, the alterations in fatty acid, one-carbon and polyamine metabolism are novel with respect to liver cancer. These indicate potential synergism between metformin and agents targeting orthogonal pathways and/or intermittent fasting. Withdrawal of metformin restored both viability and proliferation irrespective of glucose supplementation. The comparison of metabolic signatures showed upregulation or downregulation of same metabolic pathways that were found to be,

respectively, downregulated or upregulated upon metformin treatment indicating reversal of these derangements. The rescue experiment not only established reversibility of the phenotype, metabotype and cMyc expression but also indicated a coordinate relationship between cMyc downregulation, metabolic derangements associated with the anti-proliferative effect of metformin. The complete reversal of changes in central carbon metabolites and fatty acids indicated that the observed mitochondrial toxicity was also reversible indicating potential implication of stopping metformin treatment in people with or at risk of HCC. Correlation analysis in reversibility experiments also indicated that metabolic signatures more accurately reflect the phenotype compared to the gene expression.

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Mr Agustinus Thomas Soerianto[1], Dr Vinzenz Hofferek[1], Dr Eleanor Saunders[1], Dr Fleur Sernee[1], Professor Malcolm McConville[1]
[1]University Of Melbourne

Utilizing high resolution mass spectrometry and cheminformatic approaches to delineate the full metabolic capability of the parasitic protist Leishmania mexicana.

Agustinus Thomas Soerianto 445 - Utilizing high resolution mass spectrometry and cheminformatic approaches to delineate the full metabolic capability of the parasitic protist Leishmania Mexicana, Plenary 3, August 19, 2024, 16:22 - 16:41

Parasitic protists, such as sandfly-transmitted Leishmania spp, cause a range of chronic and acute diseases that impose a massive toll on human health globally. There has been limited success in developing vaccines against any of these diseases and current drug therapies are inadequate. The development of new and urgently needed therapeutics for these pathogens has been hampered by the lack of knowledge on the full metabolic potential and relevance of specific pathways. In this study, high-resolution accurate mass (HRAM) mass spectrometry coupled with advanced cheminformatic and ^{13}C-stable isotope labelling approaches has been used to significantly expand coverage of the metabolome and operation of unique metabolic pathways in these divergent eukaryotes, with the view of identifying new drug targets.

Methods

Different developmental stages of Leishmania mexicana were cultured in completely defined media and extracted in methyl tert-butyl ether (MTBE):methanol:water. Polar fractions were analyzed by both hydrophilic interaction chromatography (HILIC) and anion exchange chromatography (IC) coupled to an Orbitrap™ IQ-X™ Tribrid™ mass spectrometer.
MS features were initially annotated using authentic standards and MS/MS database matching. Unidentified MS/MS features were further annotated using in silico MS/MS fragmentation modelling (CFM-ID) supplemented with structure predictive algorithms (SIRIUS), and molecular networking based on spectral similarity (GNPS). In a complementary approach, stable isotope labelling was used to validate and characterize the de novo synthesis of unanticipated metabolites, including metabolites that did not have annotation at the MS/MS level due to spectra chimerism or poor fragmentation.

Novel Aspect

First attempt to define the metabolome of divergent, non-model eukaryotic cells beyond canonical metabolic pathways and genome predictions.

Preliminary Data or Plenary Speaker Abstract

Initial annotations using standard methodologies produced ~500 metabolite annotations at the MS/MS level out of 2400 total MS/MS features; of which 325 were confidently annotated based on RT matching with authentic standards (level 1 annotation). Analysis of unannotated features to in silico generated MS/MS spectra of putative molecular structures generated an additional 160 putative annotations, including novel metabolites that were not predicted from current genome annotations. MS/MS structure predictive algorithms resulted in the assignment of compound classes to 1800/2400 MS/MS features, as well as putative annotations which corroborate most of the metabolite annotations analysed using other methods. Finally, ^{13}C-stable isotope labelling with a panel of ten ^{13}C-labeled carbon sources and amino acids, was used to confirm precursor-product relationships and the operation of predicted pathways in different developmental stages. These analyses confirm that arginine is converted to arginosuccinate, as well as intermediates in a novel pathway leading to GABA synthesis. Both pathways lead to the synthesis of ‘dead-end’ metabolites that might be secreted and further metabolized by enzymes in the host cells. Additional

unanticipated pathways included a threonine synthetic pathway from aspartate, a proline synthetic pathway from arginine and glutamine catabolism into central carbon metabolites, as well as synthesis of an unusual methylated pentose sugar. Overall, our results revealed previously uncharacterized metabolic pathways that could aid in drug development and demonstrate the value in utilizing a multifaceted approach in analysing metabolomic datasets.

Patrik Španěl[1], Kseniya Dryahina[1]
[1]J. Heyrovský Institute of Physical Chemistry

Gas Phase Reactions of O-, OH-, O2- and NO2-with Volatile Fatty Acids for Quantitative SIFT-MS Breath Analyses

Patrik Španěl 86 - Gas Phase Reactions of O- , OH- , O2 - and NO2 - with Volatile Fatty Acids for Quantitative SIFT-MS Breath Analyses, Plenary 3, August 19, 2024, 16:41 - 17:00

Real-time Selected Ion Flow Tube Mass Spectrometry (SIFT-MS) analysis of volatile fatty acids (VFAs) in human breath is compromised by ketone and alcohol interferences when using positive reagent ions. Given the significance of VFAs as biomarkers for diseases like Gastroesophageal Reflux Disease (GERD), cystic fibrosis (CF), inflammatory bowel disease (IBD), and Non-Alcoholic Fatty Liver Disease (NAFLD), this study investigates the application of negative reagent ions in SIFT-MS for breath analyses. This approach aims to enhance VFA quantification specificity and accuracy by addressing the challenge of product ion overlap, thus paving the way for improved disease monitoring and diagnosis.

Methods

SIFT-MS instrument was used to generate negative reagent ions O-, OH-, O2- and NO2- and positive reagent ions H3O+, NO+ and O2+ and switch them in a rapid cycle while introducing a constant flow of air containing variable amounts of VFA vapors at different humidities. Reaction kinetics data were obtained to allow accurate quantification of trace levels of VFAs in breath. Formic, acetic, propionic, butyric, pentanoic, and hexanoic acids were studied with and without interfering substances such as acetone and ethanol, commonly found in human breath. Leveraging the unique reactivity of these negative ions with VFAs, real-time, non-invasive methods are developed that reduce false positives and enhance analytical precision.

Novel Aspect

SIFT-MS with negative ions uniquely quantifies VFAs in breath, enabling precise, real-time analysis while avoiding metabolic interference.

Preliminary Data or Plenary Speaker Abstract

The data characterize the interactions between the chosen negative reagent ions and VFAs in SIFT-MS. Rate coefficients and product ion branching ratios were obtained for each VFA, in general indicating that the O-, OH- and O2- all react by proton abstraction forming (M-1)- product anion. The main outcome is the development of analytical strategies to distinguish VFAs from overlapping signals of isotopologues of acetone and ethanol, enhancing the accuracy and robustness of breath analysis. The data shows that using reagent anions reduces spectral interferences, which allows for more accurate identification and quantification of VFAs. Comparative analyses between breath samples from NAFLD patients and healthy controls demonstrate the potential of this method in clinical diagnostics. Quantitative discrepancies in acetic acid concentrations between these groups were observed, illustrating the SIFT-MS capability to contribute meaningfully to diagnosing and monitoring metabolic disorders.

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Professor Ying Ge[1]
[1]University of Wisconsin

New Strategies to Address the Challenges in Top-down Proteomics

Keynote: Professor Ying Ge University of Wisconsin 755 - New Strategies to Address the Challenges in Top-down Proteomics

Preliminary Data or Plenary Speaker Abstract

Proteoforms - encompassing the diverse protein products arising from alternative splice isoforms, genetic variations, and posttranslational modifications (PTMs) originating from a single gene - are fundamental drivers in biology. Top-down mass spectrometry (MS)-based proteomics (TDP), analyzing whole proteins without digestion, offers a comprehensive perspective of proteoforms, which is invaluable in deciphering proteoform function, uncovering disease mechanisms, and advancing precision medicine. However, there are major challenges in TDP including protein solubility, proteome complexity and dynamic range, analytical sensitivity, and data analysis. To address these challenges, we are developing a multi-pronged approach to address the challenges in TDP in a comprehensive manner by developing new MS-compatible surfactants for protein solubilization, novel materials and new strategies for multi-dimensional chromatography separation of proteins, novel nanomaterials for enrichment of low-abundance proteins. Recently, we have developed high sensitivity LC-MS methods for analysis of proteoforms from a small amount of samples. We developed a highly sensitive, functionally integrated LC-MS-based TDP method for comprehensively analyzing proteoforms from single cells. This method reveals heterogeneity in large proteoforms within single muscle cells, underscoring the potential of TDP in uncovering the molecular underpinnings of cell-to-cell variation in complex systems. Moreover, we have developed small-scale serial size exclusion chromatography (s3SEC) to separate proteoforms based on size from minimal sample amounts and coupled it with high sensitivity capillary RPLC-MS/MS for comprehensive proteoform coverage in complex mixtures. We are now developing new LC-MS strategies for native TDP. Moreover, we developed a native nanoproteomics strategy for the enrichment and subsequent native top-down mass spectrometry analysis of endogenous cardiac troponin complex directly from human heart tissue. Additionally, we have developed a new comprehensive user-friendly software package for both denatured and native TDP.

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Mr Jack Bennett[1,2], Dr Corinne Lutomski[1,2], Dr Tarick El-Baba[1,2], Mr Konstantin Zouboulis[1,2], Dr Carla Kirschbaum[1,2], Dr Joshua Hinkle[3], Christopher Mullen[3], Dr John Syka[3], Professor Dame Carol Robinson[1,2]
[1]Kavli Institute for Nanoscience Discovery, University Of Oxford, [2]Department of Chemistry, University Of Oxford, [3]Thermo Fisher Scientific

Uncovering hidden protein modifications with native top-down mass spectrometry

Jack Bennett 559 - Uncovering hidden protein modifications with native top-down mass spectrometry, Meeting Room 105, August 19, 2024, 15:25 - 15:44

Post-translational modifications (PTMs) tightly regulate biomolecular function by modulating protein structure and interactions. Native top-down mass spectrometry (nTDMS) enables the structural effects of PTMs to be directly observed. However, such measurements have proven challenging due to numerous methodological hurdles including: 1) isolating specific endogenous protein complexes in sufficient quantities and purities for native mass spectrometry; 2) effectively transmitting, activating, and detecting ions over a wide m/z range almost simultaneously; and 3) interpreting the resulting fragmentation data to discover novel biomolecular modifications. Overcoming these obstacles will enable in-depth profiling of endogenous proteoform complexes using nTDMS, unlocking a deep understanding of the molecular mechanisms employed by the cell to regulate protein function.

Methods

Native top-down mass spectrometry measurements were acquired using Orbitrap Eclipse and Orbitrap Ascend hybrid mass spectrometers. The Orbitrap Ascend was modified to 1) enable infrared multiple photon dissociation (IRMPD) for ion desolvation and activation, and 2) efficiently isolate large ions. Native protein ions were fragmented using either HCD or IRMPD. The resulting spectra were analysed using recently developed approaches that can identify novel protein modifications from fragmentation spectra alone.
Endogenous membrane preparations were disrupted by sonication and directly analysed. Recombinant mammalian proteins were stably expressed in HEK293 GnTI- cells (ATCC) following lentiviral transduction. Proteins were purified by a single round of affinity purification prior to nTDMS analysis. Endogenous membrane proteins were isolated from brains under gentle conditions using nanobody-enabled immunoprecipitations

Novel Aspect

We developed nTDMS-compatible immunoprecipitations, advanced instrumentation, and novel spectral analysis approaches to facilitate direct analysis of endogenous membrane protein complexes.

Preliminary Data or Plenary Speaker Abstract

Native mass spectrometry enables the direct observation of protein complexes within heterogenous biomolecular systems, uncovering quantitative insights into their biophysical and compositional properties. When combined with intact protein fragmentation, nTDMS can be employed to directly probe the effects of PTMs and sequence variants on protein structure, providing mechanistic explanations into the functional effects of individual modifications. In this talk, I will describe our recent work focused on developing nTDMS for the analysis of endogenous membrane protein complexes. We have developed an IRMPD-enabled ‘Tribrid’ mass spectrometer, capable of quadrupole selection up to m/z 8,000. This instrument, in combination with a powerful new ‘discovery-mode’ approach to nTDMS spectral analysis, has enabled us to elucidate novel and unexpected protein modifications across a variety of complex biological systems. Specifically, applying our nTDMS workflow to the analysis of bovine rod outer segment membranes has led us to uncover distinct effectors in the G protein-coupled receptor (GPCR) / G protein-mediated signalling

cascade, providing novel insights into the processing of both the Gα subunit and the Gβγ complex. Furthermore, we have recently begun to analyse glycosylated membrane proteins isolated from cells and tissue. To isolate such systems, we have developed a nTDMS-compatible co-immunoprecipitation method that enables the purification of specific transmembrane proteins and associated biomolecules from individual mice brains. Due to the inherent heterogeneity of the isolated protein complexes, it is challenging to resolve fine structural details from native mass spectra alone. However, by applying our discovery-mode nTDMS approach, we were able to identify distinctive structural features including GPCR heterodimerisation. We believe that together, our advances provide a general pathway towards endogenous protein complex analysis using nTDMS, enabling the discovery of functionally-important protein modifications and their structural effects.

Dr. Dimitris Papanastasiou[1], **Dr Athanasios Smyrnakis**[1], Ioannis Orfanopoulos[1], Ilias Panagiotopoulos[1], Rafail Gioves[1], Nikolaos Manolis[1], Mariangela Kosmopoulou[1], Mr Alexandros Lekkas[1], Dr. Eduardo Carrascosa[2], Stuart Pengelley[2], Michael Krause[2], Niels Goedecke[2], Oliver Raether[2]
[1]Fasmatech, [2]Bruker Daltonics GmbH

A new adaptation of the Omnitrap platform integrated into a trapped ion mobility time-of-flight mass spectrometer

Athanasios Smyrnakis 624 - A new adaptation of the Omnitrap platform integrated into a trapped ion mobility time-of-flight mass spectrometer, Meeting Room 105, August 19, 2024, 15:44 - 16:03

The Omnitrap™ platform offers new capabilities for multi-stage tandem processing of ions in the gas phase using a broad range of ion activation-dissociation methods. Here, we present the next generation omnitrap platform integrated into a trapped ion mobility time-of-flight mass spectrometer. Numerous improvements are implemented in this latest flavor of the hardware, enabling superior ExD performance, enhanced robustness and MSn workflows performed at high repetition rate. The coupling of the Omnitrap platform to a trapped ion mobility analyzer offers a new powerful tool for in-depth characterization of biomolecular ions. The design and capabilities of the new instrument are presented using a series of top-down MSn experiments and a wide range of proteins.

Methods

Proteins are prepared both under native and denaturing conditions and subsequently sprayed using low-microflow and nanoflow electrospray ionization sources. Top-down data are processed by a new software configured with a new set of algorithms addressing the high spectral complexity typically observed in top-down mass spectra. A new instrument control software is developed to synchronize the omnitrap platform and the timsTOF mass spectrometer. Improvements are introduced in the design of the electron source using simulations and experimentation. New MSn experiments are performed in the Omnitrap platform with intact proteins and novel DDA workflows are implemented for the analysis of complex samples using variable electron and collisional activation energies.

Novel Aspect

A new instrument configuration combining the omnitrap platform and trapped ion mobility time-of-flight mass spectrometry for in-depth characterization of proteins.

Preliminary Data or Plenary Speaker Abstract

The new time-of-flight mass spectrometer accommodating the Omnitrap platform and a trapped ion mobility analyzer was first evaluated in terms of ion transmission and space charge capacity over a wide mass range. It is demonstrated experimentally that at least up to 10M charges can be processed in a single scan cycle leading to enhanced signal-to-noise fragmentation spectra with a dynamic range of >4 orders of magnitude.
A new set of electron optics is developed and improved ECD performance is reported whereby the abundant formation of b- and x-type fragments is no longer observed in the fragmentation spectra, in contrast to experiments performed with the original version of the Omnitrap platform. Electron currents in excess of 1 μA are measured across segment Q5, leading to faster ExD reaction times. Fine control of the electron energy is accomplished by new electronics and a new filament arrangement operated at lower temperature, while a clear distinction between ECD, hot ECD and EID regimes is observed for ubiquitin charge state 8+ ions.
Complete sequence coverage is observed in fast MS2 ExD experiments of ubiquitin and ~90% sequence coverage is obtained for myoglobin, with all six primary fragment types observed in high abundance. MS2 ExD data are also presented for carbonic anhydrase. Additional offline top-down experiments are performed with the intact non-reduced NIST antibody sprayed under denaturing conditions.

A standard protein mix is separated by LC and analyzed by MS2 ExD using a new DDA workflow developed specifically for top-down analysis of complex samples. A new algorithm implemented for processing survey scans and driving the decision tree for MS2 and targeted MS3/MS4 experiments is discussed

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Dr Tatiana Samgina[1,2], Dr Irina Vasileva[2], Dr Albert Lebedev[1]
[1]Shenzhen MSU-BIT University, [2]Lomonosov Mosocw State University

EThcD method as a unique tool for top-down de novo sequencing of intact amphibian skin peptides

Tatiana Samgina 144 - EThcD method as a unique tool for topdown de novo sequencing of intact amphibian skin peptides, Meeting Room 105, August 19, 2024, 16:03 – 16.22

Frog peptides are typical representatives of natural bioactive peptides. They represent the only weapon of these animals against microorganisms and predators. De novo sequencing of an unknown protein/peptide without any genomic information is a difficult task even for modern mass spectrometry. Isobaric and isomeric amino acids, poor sequence coverage, inter- and intramolecular disulfide bonds, and other possible post-translational modifications force to use besides mass spectrometry certain chemical procedures requiring additional time. Moreover, their yield rarely reaches 100%, while they often lead to losses of minor ingredients of the mixture. The ideal analysis would involve introduction of a mixture of intact peptides into the mass spectrometer without any sample preparation and obtaining sequence information for all components using efficient software.

Methods

The analyses were carried out in CID, HCD, ETD, and EThcD modes with registration of positive ions with Orbitrap Fusion and Orbitrap Elite ETD instruments coupled to Easy nano-LC 1000 chromatograph (Thermo Fisher Scientific, USA). The experiments were carried out with the secretions of amphibian species Rana arvalis, Rana temporaria, and Pelophylax Esculentus complex, captured in Russia and Slovenia. Mild electrostimulation of the amphibian skin was used to induce the secretion released. The animals were released into their habitat immediately after the "milking" procedure. Methanol was used to inhibit the activity of amphibian proteases.

Novel Aspect

An efficient EThcD approach was developed for the top-down de novo sequencing of intact natural peptides without any chemical derivatization

Preliminary Data or Plenary Speaker Abstract

The developed EThcD method was initially tested for efficient (>90%) differentiation of Leu/Ile isomeric residues by detecting the ions of characteristic w-series. The experimentally optimized conditions made it possible to avoid migration of the radical center, which is important in the case of several isomeric residues in the neighboring positions. Later it became clear that it was possible to determine the full sequence of the intact peptides using exclusively EThcD spectra without any other MS/MS method or preliminary chemical derivatization. Moreover, a detailed study of EThcD fragmentation enabled finding a new series of ions formed as a result of bond breaks within the unmodified disulfide ring.

Thus, the EThcD method makes it possible de novo sequencing of natural intact frog peptides in a top-down manner. To create the most effective sequencing method it is necessary to automate the process of processing the results, since the work has been carried out so far by manual interpretation of the spectra

Prof. Dr. Christian Neusüß[1]
[1]Aalen University

Characterization of proteoforms of intact proteins by 2-dimensional CE-MS techniques

Christian Neusüß 52 - Characterization of proteoforms of intact proteins by 2-dimensional CE-MS techniques, Meeting Room 105, August 19, 2024, 16:22 - 16:41

Electromigrative techniques are powerful tools for the separation of therapeutic proteins, proteoforms and their degradation products or production-related impurities. Mass spectrometry is an ever-evolving information-rich technology with strong improvements especially on the intact protein level in recent years. The combination of both techniques contributes significantly to the detailed characterization of proteins. However, CE-MS is still restricted by the incompatibility of separation conditions of various CE methods with ESI-MS as well as the sensitivity and ease-of-use of the CE-MS interfaces. Furthermore, low injection volumes in CE-MS limit its application for biological samples. These limitations can be overcome using two-dimensional separations, coupling nanoLC or MS-incompatible CE separations to CE-MS [1,2].

Methods

The coupling of CE as well as nanoLC with CE-MS has been performed using a customized, electrically isolated mechanical valve transferring mid-nL-volumes from the first to the second separation dimension. Both CE-CE and LC-CE are coupled to QTOF, Orbitrap and timsTOF MS using the recently introduced nanoCEasy interface, demonstrating both sensitivity and ease-of-use [3]. Size based separation in the first dimension is performed using a generic gel-based CE method with SDS as protein complexing agent. NanoLC is set-up applying appropriate C4 material with large pore size. NanoLC and CZE-MS conditions were optimised independently to find ideal conditions for the combined setup.

Novel Aspect

Unparalleled CE(SDS)-CE-MS technique for characterization of biopharamaceuticals
Unique nanoLC-CE-MS setup for high sensitive proteoform analysis
First selective comprehensive nanoLC-CE-MS data

Preliminary Data or Plenary Speaker Abstract

The concept of two-dimensional separation with the incorporation of CE-MS as second dimension will be presented and discussed in the context of one-dimensional separations. The potential will be demonstrated for charge- and size-based separations in the first dimension. Generic CZE and CE(SDS) methods commonly applied for impurity analysis of harmaceutical proteins have been successfully coupled to CE-MS. It will be shown, that is possible to obtain both MS as well as MS/MS data of CE(SDS)-separated fragments of mAbs. Limitations of the method regarding handling issues will also be discussed.
Furthermore, the high potential of nanoLC-CE-MS for intact protein analysis will be demonstrated. Initially a heart-cut nanoLC-CE-MS was setup and the performance regarding improved sensitivity as well as separation of proteoforms was evaluated. Due to the increased loadability of the nanoLC, the nanoLC-CZE-MS setup exhibits a 280-fold increased concentration sensitivity compared to CZE-MS alone. Separation performance is demonstrated detecting >300 proteoforms of human alpha acid glycoprotein. Very recently we extended this approach towards selective comprehensive analysis in a novel online nanoLC-CE-MS configuration, allowing the deep-proteoform characterization of a range of proteins separated by the nanoLC using several CE-MS runs for the further separation of proteins and proteoforms. The combination of high sensitivity and orthogonal selectivity in both dimensions makes the here presented nanoLC-CZE-MS approach capable for detailed characterisation of intact proteoforms at physiologically relevant concentrations. Further developments and future applications of nanoLC-CE-MS will be discussed.

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Ion mobility curated internal fragments enhance on-line top-down proteomics experiments

Muhammad Zenaidee 369 - Ion mobility curated internal fragments enhance on-line top-down proteomics experiments, Meeting Room 105, August 19, 2024, 16:41 - 17:00

Top-down proteomics (TD-proteomics) has become the central method for the analysis of proteoforms due to the ability to analyse intact proteins whilst conserving protein isoforms, sequence variants, and PTMs. In TD-proteomics, intact proteins from a complex mixture are separated using liquid chromatography (LC), and each intact protein analysed via mass spectrometry (MS), returning proteoform information.

However, at the LC time scale, fragmentation of intact proteins are limited; peaks go unassigned within the mass spectrum. Internal fragments (fragments containing neither the C- nor N-terminus) coupled with ion mobility can enhance the confidence of internal fragment assignments in TD-MS experiments and extend the protein sequence information. Here, we utilise an ion-mobility curated TD-proteomics method to obtain proteoform information on a protein mixture.

Methods

Pierce intact protein mix (ThermoFisher Scientific) was reconstituted in 5 % acetonitrile, 0.1 % formic acid, and analysed using an M-Class Acquity UPLC system coupled to a WATERs Select Series cyclic ion mobility mass spectrometer (Waters Corporation). Sample was loaded onto an in-house C4 column (300 Å pore size, 1.7 µm particle size, 2.1 mm ID x 30 mm) column at a flowrate of 0.3 mL/min. Data was collected using an MSe method and fragmentation was induced prior to ion mobility to allow separation of fragments with similar mass-to-charge ratios.

Each chromatographic peak were summed and MS/MS spectra were generated and analysed using ClipsMS to assign internal and terminal fragments. Ambiguous internal fragments were further investigated to discern ambiguity.

Novel Aspect

Proteoform characterisation in top-down proteomics experiments can be enhanced by the inclusion of ion mobility curated internal fragment assignments.

Preliminary Data or Plenary Speaker Abstract

Here, TD-proteomics of an intact protein mixture can be extended by the inclusion of internal fragments. For example, for the proteins in the standard mix, carbonic anhydrase II, and β- human IGF-1 LR3, human thioredoxin, Streptococcus dysgalactiae Protein G, Bovine Carbonic Anhydrase II, Streptococcus Protein AG (chimeric), and Escherichia coli Exo Klenow, the inclusion of internal fragments increased sequence coverage from ~35% to 55%, which is a significant enhancement for the sequence coverage of these proteins. Interestingly, ambiguous internal fragments (internal fragments with similar mass-to-charge ratios) could be separated by ion mobility. For example, the carbonic anhydrase II internal fragment at 451.186 m/z (2+) could be assigned to two different internal fragments DGQGSEHTV and GQGSEHTVD. Ion mobility of this fragment showed two distinct ion mobiligrams at 19.20 ms and 21.20 ms. By correlating these ion arrival times to theoretical collisional cross sections of these amino acid sequences, both internal fragments can be discerned and utilised for sequence information assignment. These data indicate that ambiguous internal fragments can be discerned by ion mobility to produce confident internal fragment assignments.

As ion mobility can separate ambiguous internal fragments to enhance the confidence of internal fragment assignments. For example, after including internal fragments, for the proteins in the standard mix, carbonic anhydrase II, and β- human IGF-1 LR3, human thioredoxin, Streptococcus dysgalactiae Protein G, Bovine Carbonic Anhydrase II, Streptococcus Protein AG (chimeric), and Escherichia coli Exo Klenow, the inclusion of ion mobility curated internal fragments increased sequence coverage from ~55% to 75%. These data demonstrate that the inclusion of ion mobility to TD-proteomics experiments can enhance the confidence of internal fragment assignments to significantly extend the extent of sequence information obtained for intact proteins present in complex samples, which will be beneficial to intact proteoform characterization.

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Food Proteomics- A Luxury or a Need for Improving Food Safety and Quality?

Keynote: Dr Melanie Downs University of Nebraska-Lincoln 761 - Food Proteomics- A Luxury or a Need for Improving Food Safety and Quality?

Much like in other sectors, proteomics methods have the potential to provide a wealth of information about food systems that is otherwise inaccessible. With an ever-growing range of novel protein sources used in foods and a changing landscape of regulations impacting food allergens, methods that can characterize, authenticate, and quantify food proteins will inevitably be beneficial. The use of proteomics for food safety and quality applications, however, is currently limited to a small number of specialized laboratories. For example, mass spectrometry has been discussed as a way to improve the performance of food allergen detection methods for many years, but immunoassays remain the dominant platform for food allergen analysis. With recent growing momentum to use quantitative, risk-based approaches to manage and regulate food allergens, the urgency to have robust quantitative methods has increased, but it remains unclear if the analytical community is ready for a change in the status quo. This presentation will describe the development and evaluation of mass spectrometry methods for food allergen quantification and illustrate applications where proteomics may be necessary to make progress in providing a safe and diverse range of foods for individuals affected by food allergies. Finally, the barriers to broad implementation of proteomics methods for foods will be discussed to provide perspectives as to whether these approaches will remain out of reach for most in the food field or move into the mainstream to support the efforts of food manufacturers, laboratories, and regulatory agencies to improve food quality and safety.

M.Sc. Genc Haljiti[1,2], Sarah Brajkovic[1], Andrea Piller[1], Armin Soleymaniniya[1], Cemil Can Saylan[1], Ezgi Aydin[1], Giorgi Tsiklauri[1], Guido Giordano[1], Veronica Ramirez[1], Jiuyue Pan[1], Lukas Wuerstl[1], Mario Picciani[1], Patrick Roehrl[1], Paula Andrade Galan[1], Qussai Abbas[1], Sebastian Urzinger[1], Sophia Hein[1], Caroline Gutjahr[1], Chris Schoen[1], Claus Schwechheimer[1], Corinna Dawid[1], Dmitrij Frishman[1], Josch Pauling[1], Ralph Hueckelhoven[1], Stephanie Heinzlmeir[1], Viktoriya Avramova[1], Mathias Wilhelm[1], Brigitte Poppenberger[1], Bernhard Kuster[1], Christina Ludwig[1]
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The Proteomes that Feed the World: Unveiling Plant Proteomics and Peptidomics

Genc Haljiti 61 - The Proteomes that Feed the World: Unveiling Plant Proteomics and Peptidomics, Meeting Room 106, August 19, 2024, 15:25 - 15:44

Plants, the lifeblood of our planet, sustain nearly all species, including humans. As the world copes with a growing population and climate change, the shift towards protein-rich diets based on crops is a global necessity. Yet, despite the extensive study of crop genomes, most of their proteomes remain unknown. This research aims to illuminate the proteomes of tissues from the 100 most vital crops for human nutrition. Additionally, we delve into the world of bioactive peptides, which play a pivotal role in abiotic stress and pathogen defense. Despite their importance, the investigation of bioactive peptides has been largely overlooked in the field of proteomics due to unique analytical challenges in sample preparation and data evaluation.

Methods

For the comprehensive global mapping of plant proteomes across diverse crops and tissues, we used an optimized workflow based on TCA/acetone precipitation, SDS solubilization, phenol extraction, tryptic digestion, SP3 sample cleanup, and high pH reverse-phase peptide fractionation followed by robust microflow LC-MS/MS measurements. MaxQuant and Prosit rescoring were used to identify and quantify the proteins.
To analyze bioactive peptides, we extracted apoplastic wash fluid (AWF) from wild-type Arabidopsis thaliana (Col-0) and separated peptides from proteins using 10 kDa size exclusion chromatographic columns. We performed reduction and alkylation on the extracted peptides but omitted any tryptic digestion step. The samples were measured on a nanoflow LC-MS/MS setup, and peptide identification and quantification was done using FragPipe with MSFragger unspecific search.

Novel Aspect

Generated data from this study will aid plant research, pinpointing vital and diverse plant-related inquiries, investigations, and future studies.

Preliminary Data or Plenary Speaker Abstract

Using the proteomics workflow, we identified and quantified over 10,000 proteins in the tomato plant Solanum lycopersicum. Furthermore, we created the first comprehensive proteome of the quinoa plant, Chenopodium quinoa, detecting over 20,000 proteins in 6 hours of instrument time per tissue. Peptidome analysis resulted in over 19,000 quantified peptides in the AWF of Arabidopsis thaliana grown under standardized conditions. A range of 7-40 peptide lengths was covered, with 63 % showing a charge state > 2. A GO enrichment analysis showed that the most enriched pathway was the apoplast, and pathways such as cell-to-cell junction, extracellular matrix, or secreted were also prominent. Peptides from those pathways were distributed throughout the entire range of mass spectrometric intensities. Finally, the proteome and peptidome raw data of all studied crops, alongside identification and quantification results, will be routinely available to the public via resources such as PRIDE and ProteomicsDB.

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Prof Michelle Colgrave[1]
[1]Edith Cowan University

Proteomics application to deliver healthy and sustainable foods of the future

Michelle Colgrave 606 - Proteomics application to deliver healthy and sustainable foods of the future, Meeting Room 106, August 19, 2024, 15:44 - 16:03

Estimates predict that 70 per cent more food will be required to feed the growing global population which will reach 9.7 billion by 2050. The challenge will be how to meet this global food gap while maintaining our planet's health. In Australia, our traditional animal and plant proteins offer an opportunity to address this gap, with emerging sources of protein seen as complementary. We are witnessing changing dietary patterns, with increasing numbers of flexitarians, due to concerns over the environment, animal welfare and personal health and nutrition. In response to the growing demand for protein, technologists have been searching for cost-effective, resource-efficient and environmentally friendly protein technologies and solutions that can support traditional industries or create new industries.

Methods

To this end, we have started exploring different crops and food sources from pulses to insects to algae and even microbes. As we pivot towards these under-utilised resources, potential exists for increasing prevalence of allergy or cross-reactivity. Herein, a combination of discovery and quantitative proteome measurements were employed for evaluation of novel protein sources. Bioinformatic analysis is used to reveal potential allergens and also bioactive components.

Novel Aspect

The role of proteomics as a powerful tool to characterise both nutritional and antinutritional proteins in emerging protein sources.

Preliminary Data or Plenary Speaker Abstract

Lupin seeds possess high protein content (35-44%) and many health-promoting benefits (lowering cholesterol and blood pressure, managing glucose levels). But lupin also contains proteins that can trigger life-threatening anaphylaxis. Using proteomics, the major lupin seed storage protein families (α-, β-, δ-, and γ-conglutins) across a panel of 46 genetically diverse narrow-leafed lupin (NLL) genotypes has been evaluated. The β-conglutin proteins, which are the major allergens from lupin, were substantially reduced within several of the domesticated cultivars, wherein some degree of compensatory elevation of the bioactive γ-conglutin proteins was noted. These studies are extended to additional pulse protein sources including mung bean to reveal potential antinutritional components. Yet these same techniques can be employed to study complementary, sustainable sources of protein such as insects. Despite being consumed by people for millennia; little is known of their safety. Insects are a member of the arthropod family, like crustaceans, wherein shellfish allergies are both relatively common and potentially severe. This study explores the cross-reactivity of the immune system with insect proteins (e.g., tropomyosin and arginine kinase) as a potential health concern. Thus, proteomics serves as a technology to enable safe development of novel proteins allowing understanding of the allergenic potential of such foods.

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Dr Leigh Donnellan[1], Dr Arineh Tahmasian[2], Dr Clifford Young[1], **Prof Peter Hoffmann**[1]
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Diving Deep into the Faba Bean Proteome

Peter Hoffmann 495 - Diving Deep into the Faba Bean Proteome, Meeting Room 106, August 19, 2024, 16:03 - 16:22

It is estimated that global food systems will need to double production by 2050 to meet the requirements of a growing population. To meet this demand and mitigate the environmental impacts of meat production, alternative protein sources such as pulse crops are being investigated for their sustainability. Faba bean (Vicia faba) is an important pulse crop to Australian farmers, with approximately 678000 tonnes produced in 2020-2021. It plays an important roles in nitrogen fixation, disease break and weed control within crop rotations. Nutritionally, faba bean is desirable because of its high-quality plant protein and dietary fibre. It is therefore a crops that could satisfy the criteria for sustainable alternative protein sources, while advancing the current market-trade for Australia.

Methods

Most research on the faba bean has focussed on the removal of vicine and convicine, which are antinutritional compounds that cause favism (haemolytic anaemia) in glucose-6-phosphate dehydrogenase deficient individuals. Conversely, only a few studies have investigated attributes such as crop quality, yield, resistance to environmental stressors or protein content. Furthermore, proteomic investigation to potentially address these questions have been extensively hampered by the low number of annotated proteins in the corresponding UniProt database (125 proteins).

Novel Aspect

First comprehensive proteomics study on faba beans

Preliminary Data or Plenary Speaker Abstract

Herein, we describe the first large scale investigation of the faba bean proteome from 20 varieties grown at two different locations in South Australia. Utilising theoretical protein sequences generated from two recently sequenced faba bean genomes, we produced an extensively annotated faba bean FASTA file. Using the combination of optimised protein extraction procedures, extensive high-pH fractionation and Zeno SWATH DIA, a spectral library containing more than 6000 protein groups and 47000 peptides was generated as well as a FAIMS DDA analysis on a Orbitrap Exploris 480. This study is the first step in generating a comprehensive proteomic resource to aid researchers identify faba bean varieties with desirable nutritional traits for sustainable farming.

Dr Larissa Buedenbender[1], Dr Miguel Balado[2], Dr Marta A. Lages[2], Dr Patricia Fernandez Puentes[1], Dr Valentina Calamia[1], Dr Manuel L. Lemos[2], Dr Jaime Rodríguez[1], Dr Carlos Jiménez[1]
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chelOMICS for a holistic understanding of siderophore-mediated host-pathogen interactions in aquaculture infections

Larissa Buedenbender 512 - chelOMICS for a holistic understanding of siderophore-mediated host-pathogen interactions in aquaculture infections, Meeting Room 106, August 19, 2024, 16:22 - 16:41

The ever-growing human population places a great demand on food supplies. However, due to increasing environmental impacts, food resources, particularly of wild marine fish, are becoming scarcer. Consequently, aquaculture is necessary to feed the world's population. Outbreaks of infectious diseases, such as vibriosis, pose a persistent challenge to fish farming and result in significant economic losses. Thus, new and effective approaches are required to enhance health monitoring and rapid disease diagnostics to facilitate appropriate measures to control outbreaks. Siderophores are iron-chelating metabolites utilised by pathogenic bacteria to uptake iron from their host. These metabolites are thought to be key virulence factors enabling the infection process. Since several siderophores are species-specific, siderophores have been proposed as potential biomarkers for disease diagnostics.

Methods

Here, we present the integration of untargeted LC-MS/MS metabolomics and proteomics of spleen extracts to provide a holistic understanding of the metabolic response of the high-value flatfish, Solea senegalensis, to challenge with different doses of Vibrio anguillarum. We further studied the role of siderophores in disease progression and their use as biomarkers for vibriosis.
Proteomics data of trypsin-digested extracts were acquired on a nanoLC-timsTOF Pro mass spectrometer (Bruker Daltonics) in PASEF-DIA mode. While organic extracts for metabolomics were chelated with FeCl3, to stabilise iron-chelating siderophores, and analysed in DDA mode on an LTQ-Orbitrap Discovery mass spectrometer coupled to an Accela HPLC (Thermo Scientific). Data analysis was performed using open-source software, such as DIA-NN, MZmine and various R packages.

Novel Aspect

•Proteo-metabolomics deepens understanding of host-pathogen interactions aiding the development of diagnostic tools and treatments
•Iron-chelating siderophores are potential infection biomarkers

Preliminary Data or Plenary Speaker Abstract

Combining different OMICS strategies, such as proteomics and metabolomics, provides a more holistic understanding of the interrelationship of active biomolecules and their functions. Through integrated omics, we found that Vibrio infection most significantly impacts amino acid and lipid metabolism in Solea senegalensis. The acquired in-depth knowledge about the immune response of S. senegalensis to V. anguillarum could provide a basis for future applications to reduce susceptibility to vibriosis through the development of metabolic intervention approaches (e.g. dietary treatments) to mitigate the impact of vibriosis on aquaculture.

Furthermore, by screening for the characteristic iron isotopic pattern in the untargeted LC-MS/MS metabolomics data, we identified ferri-piscibactin in spleen samples of highly infected sole, but it could not be detected in the control and low infection groups. Piscibactin is a siderophore known to be produced by several pathogens of the Vibrionaceae family and could potentially be used as a biomarker for more accurate diagnosis of aquaculture infections.

Acknowledgements: This project has received funding from the European Union's Horizon Europe programme under the Marie Skłodowska-Curie Action grant agreement No 101066127.

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Dr Omar Mendoza Porras[1], Anca Rusu[1], Bert Koster[2], Chris Stratford[2], Dr Richard Smullen[3], Dr David Beale[4], Dr Nicholas Wade[5]
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UNDERSTANDING THERMAL STRESS IN SALMON TISSUES USING PROTEOMICS AND METABOLOMICS

Omar Mendoza-Porras 453 - Understanding Thermal Stress in Salmon Tissues Using Proteomics and Metabolomics, Meeting Room 106, August 19, 2024, 16:41 - 17:00

Increased water temperatures as one effect of climate change directly affects marine and aquaculture fish beyond their optimal temperature range with detrimental effects for physiology and homeostasis. The optimal temperature for salmon growth is in the 16-18°C range. In southern Australia, summer marine heatwaves have increased seawater temperature beyond this range, reducing feed intake in farmed salmon, as well as compromising osmoregulation, flesh decolouration, condition factor and hepatosomatic index.

Methods

Proteomics and metabolomics were used to identify thermal stress biomarkers in plasma, gill and mucus of salmon collected across a fine-scale temperature ramp that covered 6 temperatures between 15°C and 20°C. In this way, targeted metabolomics was applied to measure 220 metabolites involved in central carbon metabolism to determine the salmon plasma response to heat stress. DIA and targeted proteomics were used to identify and measure biomarkers for thermal stress in salmon gill and mucus.

Novel Aspect

Our work provides the foundation to monitor heat stress to enable the development of functional diets to mitigate thermal stress

Preliminary Data or Plenary Speaker Abstract

In gill and mucus, serpin and heat shock proteins increased in abundance as the temperature increased. Integrated bioinformatic analysis of significant proteins and metabolites revealed enrichment across important protein functional categories and pathways involved in responses to heat and stress, antioxidant, and superoxide activities as well as compromised cell processes and energy regulation. This work demonstrates that omics technologies in aquaculture can be used to monitor the abundance of thermal stress biomarkers over time as proxy for salmon health. This would allow the manufacturing of advanced diets that provide specific nutrients intended to mitigate thermal stress and attain enhanced growth, immunity and, resilience leading to increase production yield while ensuring environmental sustainability and promoting animal welfare and social license

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Lucas Abruzzi[1,2], Taelor Zarkovic[1,2], Scott Borden[1,2], Armin Saatchi[1,2], Gregory Vandergrift[1,2], Arden Hessels[1], Dr. Collin Kielty[2,6], Piotr Burek[2,6], Allie Miskulin[2,6], Lea Gozdzialski[2,6], Ava Margolese[2,6], Eric Poarch[2,6], Miriam Sherman[2,6], Derek Robinson[2,6], Oscar Sandford[2,6], Zoe Riell[2,6], Allan Custance[2,6], Abby Hutchinson[2,6], Josh Jai[2,6], Kayla Gruntman[2,6], Katy Booth[2,6], Mo Jackson[2,6], Jeremy Riishede[2,6], Rebecca Martens[2,6], Pablo Gonzalez Nieto[2,6], Taylor Teal[2,6], Sarah Littlechild[2,6], Devon Cassidy[2,6], Becca Louw[2,6], Irene Shkolnikov[2,6], Fred Cameron[5], Director, Policy and Partnerships at British Columbia Ministry of Mental Health and Addictions Kenneth Tupper[4], Professor Bruce Wallace[2,6], Professor Dennis Hore[2,6], **Professor Chris Gill**[1,2,3,6]
[1]Vancouver Island University, [2]University of Victoria, [3]University of Washington, [4]Ministry of Health, [5]Solid Outreach Society, [6]Canadian Institute for Substance Use Research

Harm Reduction Drug Checking by Quantitative Paper Spray Mass Spectrometry: Where are we Now?

Keynote: Professor Chris Gill Vancouver Island University 237 - Harm Reduction Drug Checking by Quantitative Paper Spray Mass Spectrometry: Where are we Now?, Meeting Room 109, August 19, 2024, 15:00 - 15:25

Illicit drug overdose deaths in BC Canada reached crisis levels in 2016. Currently we are experiencing an unprecedented rate of 43 deaths per 100,000 individuals with fentanyl detected in over 80% of illicit drug toxicity deaths. On-site drug checking is increasingly being included within public health's overdose prevention responses as an individual level harm reduction service as well as providing drug market data and monitoring functions. Methods such as immunoassay test strips as well as Raman and FTIR spectroscopy are most often being implemented for drug checking despite inherent sensitivity/specificity limitations. We present our successes using paper spray mass spectrometry (PS-MS) for rapid, quantitative, on-site harm reduction drug checking within the context of unprecedented rates of overdose.

Methods

A paper spray (PS) ionization triple quadrupole mass spectrometer (VeriSprayTM PS interface, FortisTM QqQ, Thermo Scientific) is used. The system is now permanently operating at a drug checking site (Substance) in Victoria, BC. Drug targets (>100) include opioids including fentanyl analogues and nitiazenes, benzodiazepines, stimulants, psychedelics, and more. One mg solid samples are diluted in methanol with labelled internal standards, spotted (10 µL) on PS-MS strips, and dried. Solvent (90/10/0.1% Acetonitrile/water/formic acid) and high voltage (+3800 V) are applied to the strips to initiate extraction and ionization. Quantitative analyses use MRMs for each analyte/IS pair. A short full scan is also implemented detecting non-targeted drugs. Data analysis and reporting is automated (one-click), generating a simplified, quantitative content summary for interpretation.

Novel Aspect

Rapid, on-site, quantitative harm reduction drug checking and illicit drug supply surveillance using paper spray mass spectrometry

Preliminary Data or Plenary Speaker Abstract

PS-MS is demonstrating its potential within drug checking interventions in British Columbia, Canada. The approach appears to avoid pitfalls common with other direct drug checking methods, providing sensitive, wide-range linear calibrations with low ng/mL range detection limits, without sample cleanup steps. In August 2019, we performed the first successful on-site PS-MS pilot-testing for harm reduction drug checking at a supervised consumption site in Vancouver (Powell Street Getaway). Since that time, over 20,000 tests have been conducted and reported to service users at Substance.

When compared to the more frequently utilized FTIR and Raman instruments, PS-MS provides trace-level detection, extremely relevant to the current overdose crisis in in which low-level actives such as potent fentanyl analogues (carfentanil etc), nitazines and xylazine are frequently linked to overdose. Substance utilizes PS-MS within a multi-method approach to drug checking that includes immunoassay strip tests and FTIR or Raman. Ultimately, it is PS-MS that provides the most credible and comprehensive results to service users, including quantitation of notable active components. In addition, PS-MS data provides public health monitoring and reporting of the illicit drug market. With illicit drug overdose linked to potent synthetics at unpredictable concentrations, such reporting can be useful for individuals as well as service providers and public health. Substance has further demonstrated the practicality of PS-MS to function beyond the lab as the PS-MS is located at a street storefront site within the local drug user organization's services. Access to mass spectrometry at point-of-care drug checking enables real-time results and relational work between technicians, harm reduction works and service users.

Overall, the PS-MS is demonstrating its value in responding to the challenges of this tragic public health crisis by being engaged in street-level harm reduction and providing highly relevant and usable results to individuals as well as public health.

Dr Maiken Ueland[1,2], Dr Amber Brown[1], Dr Daniel Pasin[1], Dr Taoping Liu[3,4], Dr Wentian Zhang[3], Assoc Prof Jodie Ward[1], Professor Steven Su[3,5], Professor Shari Forbes[6]
[1]Centre for Forensic Sciences, School of Mathematical and Physical Sciences, University of Technology Sydney, [2]Hyphenated Mass Spectrometry Laboratory, School of Mathematical and Physical Sciences, University of Technology Sydney, [3]School of Biomedical Engineering, University of Technology Sydney, Sydney, NSW 2007, Australia, [4]Academy of Advanced Interdisciplinary Research, Xidian University, [5]College of Artificial Intelligence and Big Data for Medical Sciences, Shandong First Medical University, [6]Department of Chemistry and Biochemistry University of Windsor

When disaster strikes, locating victims using electronic nose technology

Maiken Ueland 122 - When disaster strikes, locating victims using electronic nose technology, Meeting Room 109, August 19, 2024, 15:25 - 15:44

The occurrence of mass disasters are increasing due to changing climate conditions bringing on more destructive weather events, additionally man-made disasters are becoming more common. Locating victims during these events remains a major challenge. Currently, scent detection canines are the preferred tool, however, they are susceptible to false responses, fatigue and are costly to train and deploy. There is therefore a need for an improved approach. There exist analytical instruments to analyse VOCs however these rely on a two-step process whereby samples are collected in field and analysed in the laboratory, which is not suitable for disasters where immediate answers are required. Here we show an alternative technologically based detection tool, an electronic nose.

Methods

Simulated mass disaster scenarios using donated human cadavers at the Australian Facility for Taphonomic Experimental Research (AFTER) were created where victims were completely obstructed from view. An in-house electronic nose was tested for its ability to detect victims over time in the disaster scenario at differing locations. Comparison data was obtained using thermal desorption tubes collected in the field and analysed using comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GCxGC-TOFMS).

Novel Aspect

The work presents an e-nose that is cheap and easy to deploy by non-expert users at mass disaster scenes.

Preliminary Data or Plenary Speaker Abstract

The electronic nose was able to detect hidden human victims in a building collapse. The results were comparable to that of the GCxGC-TOFMS. In addition, no false positives were detected over building debris or over non-human organic material.

Senior Application Specialist Terry Bates, Scientist Alex Maggitti, **Svp Jeff Zonderman**, Applications Development Manager Francois Francois Espourteille
[1]Bruker, [2]DrugScan Inc, [3]Bruker, [4]Bruker

Rapid Chromatography-Free Quantitative Screening of Synthetic Cannabinoids in Urine Using a Novel DART-TripleQuad MS

Xianming Liu 387 - Rapid Chromatography-Free Quantitative Screening of Synthetic Cannabinoids in Urine Using a Novel DART-TripleQuad MS, Meeting Room 109, August 19, 2024, 15:44 - 16:03

Immunoassay-based (IA) detection for drugs of abuse is commonly used for urine-based screening due to rapid result generation. Since IAs suffer from false positives, a positive IA result must be confirmed by another analytical approach, typically a chromatography-based method. LC-MS and GC-MS are most commonly used as confirmatory assays due to their high degree of sensitivity, specificity, and accuracy. They are well established and commonly achieve sub-ng/mL detection limits, but often rely on costly carrier gases, solvents and are limited in throughput with time-consuming chromatography and sample preparation. Here we report the development of a chromatography-free method using direct analysis in real time-mass spectrometry (DART-MS) that is shown to accurately identify and quantify panels of drugs like synthetic cannabinoids.

Methods

Triplicate calibration series were prepared by spiking certified drug-free urine with standards 1-18 (0.1-2500 ng/mL) using deuterated AB-PINACA as an internal standard. Pinpoint sample preparation protocol (Pinpoint Testing) was use for all samples. 2 µL aliquots of each reconstituted sample were transferred onto a QuickStrip HTS-96 screen and dried under 40°C N2 gas. The prepared QuickStrip-HTS 96 screen was loaded onto the XY stage of an EVOQ DART-TQ+ (Bruker Daltonics) mass spectrometer for DART-MS-MS analysis. Accuracy was determined in triplicate using certified drug-free urine without detectable levels of 1-18 at 2 levels for each analyte within the linear range of each calibration series. Results were validated against LC-MS of 20 positive urine samples confirmed for one or more analytes.

Novel Aspect

Chromatography-free workflow for rapid screening and DART-MS/MS quantitation of synthetic cannabinoids that minimizes false-positives and solvent waste.

Preliminary Data or Plenary Speaker Abstract

The integrated DART and EVOQ DART-TQ+ mass spectrometer parameters were optimized for high sensitivity, precision, and fast analysis time. With DART gas temperature and grid voltage optimized at 350°C and 100 V, respectively, unique MS/MS transitions, collision energies, and MS scan times were successfully identified for 1-18. DART-MS analysis of the synthetic cannabinoid panel resulted in a good linear correlation of $R2 > 0.95$ and an accuracy between 87 and 107% for all 18 analytes across the defined calibration ranges. The lower level of quantitation (LLOQ) of between 0.1 to 5 ng/mL and cross-validation of the samples showed good correlation with LC-MS data, indicating that this rapid chromatography-free workflow is sufficient in detecting all 18 analytes at or below the common cutoff values without the high rate of false positives associated with IA based screening approaches.
The results presented herein demonstrate the suitability of the chromatography-free DART-MS workflow as a rapid, quantitative, and selective alternative to conventional IA-based urine screening by offering a quantitative method with the benefits of minimizing false positives typically associated with IA based screening, avoiding costly and unnecessary chromatography-based confirmatory testing.

Louise O'Grady[1], Prof Xavier Conlan[1], Dr Lawrence Webb[1]
[1]Deakin University

Identifying Psychedelics in Australian Acacia Species: Wattle We Do?

Louise O'Grady 25 - Identifying Psychedelics in Australian Acacia Species: Wattle We Do?, Meeting Room 109, August 19, 2024, 16:03 - 16:22

Recent amendments to the classification of some psychedelic substances within Australian legislation highlights the challenge of the changing licit use of hallucinogens due to their emerging medical applications, including the treatment of depression, anxiety, addiction, and PTSD. N,N-dimethyltryptamine (DMT) is a psychedelic compound, similar in function to ketamine or LSD, that is forensically relevant due to its increasing illicit use and clandestine manufacture. The psychedelic, its precursors, and similar compounds are naturally produced within a wide variety of plant species, including Australian native Acacia species. This research focuses on the development of a robust analytical protocol for the characterisation, and quantitation of DMT within forensically relevant plant material, utilising a rapid liquid chromatographic separation and mass spectral detection.

Methods

A variety of Acacia species were sourced from nurseries around Australia, consisting of over 300 plants representing approximately 150 native species, primarily from Victoria and New South Wales. The sample preparation included the lyophilisation and ball milling of plant material, followed by a methanol and acetic acid extraction. Reverse phase liquid chromatographic separation and mass spectral characterisation was conducted using a Shimadzu Quadrupole Time-of-Flight Liquid Chromatography Mass Spectrometer. A robust and reproducible method was obtained through the alteration of a range of parameters including comparison of columns and optimisation of solvent gradients. Untargeted compound detection was achieved through MS/MS acquisition of 31 events, investigating precursor ions ranging from m/z 150-1000.

Novel Aspect

This research provides a novel, robust method for DMT identification, practical for use by forensic organisations in potential seizure samples.

Preliminary Data or Plenary Speaker Abstract

The research conducted developed a robust method for the identification of DMT within Acacia species. An effective and efficient method for chromatographic separation and mass spectral characterisation of the constituents within each sample was achieved through continuous optimisation of the system parameters. The findings of one study determined 51 (49.04%) of the 104 sampled species contained at least trace amounts of the hallucinogenic, with 41 of these DMT-containing species previously unknown to contain the psychedelic. Principal Component Analysis (PCA) was subsequently utilised to determine the feasibility of differentiating between plant species based on DMT production. Through conducting PCA, three distinct clusters were generated between the 104 species analysed. However, the results displayed that the clustering was not due to the presence or quantity of DMT within the samples, or factors such as the species or subgenera, location of growth, or the location from which the plant was sourced. The PCA generated from both positive and negative ion mode analyses highlighted a range of compounds found to drive the clustering between samples and groups of species. Through the development of a robust protocol for plant analysis, this can inform botanical research through providing an avenue for compounds identification, and forensic investigators through the further development of the understanding of DMT and the factors that may drive illicit DMT extraction.

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Dr Simon Ovenden[1], Dr Lyndal McDowall[1], Dr Nathan McGill[1,2], Dr Marija Petricevic[1], Dr Renee Webster[1,3], Mrs Jilliarne Williams[1], Dr Shannon Zanatta[1]
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The identification of VX chemical attribution signatures from four different synthetic methods

Simon Ovenden 392 - The identification of VX chemical attribution signatures from four different synthetic methods, Meeting Room 109, August 19, 2024, 16:22 - 16:41

Attribution of highly toxic chemicals designated as Schedule 1 under the Chemical Warfare Convention (CWC) is of interest to multiple national and international forums. In this context, attribution pertains to the chemical analysis of samples containing Schedule 1 compounds that provides forensic insights into their production. The results from this analysis could provide key information on the chemical used and be an important aspect of any subsequent investigations that are undertaken.
VX is a highly toxic organophosphorous nerve agent that is classified as a Schedule 1 chemical under the CWC. The identification of chemical attribution signatures (CAS) that could aid in the identification of precursor chemicals, synthetic route, production facility, process or material, would be of benefit to any investigations.

Methods

Four methods of VX synthesis were investigated. These methods varied according to precursor starting materials and reaction conditions. Method VX1 diisopropylethanolamine and O-ethyl methylphosphonochloridothionate; Method VX2 diisopropylaminoethylchloride hydrochloride and sodium O-ethyl methylphosphonothioate; Method VX3 diisopropylaminoethylthiol and ethyl methylphosphonochloridate; Method VX4 diisopropylaminoethylchloride and sodium O-ethyl methylphosphonothioate. Replicate reactions were analysed as crude reaction mixtures with minimal work up. A total of twenty-four synthetic samples were generated for analysis. Additionally, purified VX from the larger scale synthesis were also prepared for analysis.
Each of the samples was made up to 100 ppm and subjected to both LC-HRMS and GC-MS(EI). The LC-HRMS data was subjected to multivariate statistical analysis to identify chemical features that could be considered as candidates for method of production.

Novel Aspect

Identification of candidate CAS to aid in the determination of synthetic method of production of VX using LC-HRMS and GC-MS(EI).

Preliminary Data or Plenary Speaker Abstract

Following the conversion of the LC-HRMS data to mzML format, it was imported into MZmine, where it was crop filtered, retention time and mass aligned, and gap filled, to create a 104 x 420 data matrix. This data matrix was then imported into the multivariate statistical program Unscrambler. Here, the data was mean centred and Pareto scaled and subjected to both PCA and PLS-DA modelling. Important chemical features were selected using a combination of regression coefficients and selectivity profiles, resulting in thirty-nine possible CAS candidates. A combination of accurate mass measurements to propose molecular formulae, and electron ionisation fragmentation matching using associated databases, allowed for the structures of thirty-two CAS to be elucidated. The remaining seven CAS remain structurally unknown.
Identified CAS belong to a diverse range of structure classes, including phosphonic acids and phosphonates, alkylsulfides, phosphonothioates and pyrophosphonates. Eighteen of the CAS have previously reported as impurities or degradation products. However, as the synthetic methods were not described, no inference can be made regarding the suitability of these as robust CAS candidates.

The remaining fourteen identified CAS have not previously been reported from the chemical analysis of VX samples.
The majority of identified CAS were identified as being discriminators for Method VX1 (seventeen) and Method VX3 (thirteen). Four CAS were found to be discriminators for Method VX4, and one for Method VX2. Some of the identified CAS are potentially strong candidates for method discrimination. As an example, the major CAS identified for Method VX1 was O-ethyl O-[2-(diisopropylamino)ethyl] methylphosphonothiolate. This compound was only identified in Method VX1 and is the thiono isomer of VX. Method VX1 is the only method in this study where it would be expect that the thiono isomer would form. Further examples of discriminator compounds will be elaborated on.

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High-Resolution and Isotope Ratio Mass Spectrometry based profiling of Ricinus communis - A forensic approach

Lisa Scharrenbroch 95 - High-Resolution and Isotope Ratio Mass Spectrometry based profiling of Ricinus communis - A forensic approach, Meeting Room 109, August 19, 2024, 16:41 - 17:00

Recent incidents in France, Germany, and worldwide highlight the global threat posed by biotoxins. Particularly the plant toxin ricin, found in the seeds of the castor oil plant Ricinus communis (R.c.), is of special forensic interest due to its worldwide availability, high toxicity, and lack of medical countermeasures. Analytical methods for the detection of ricin have already been established, but supplementary molecular forensic analysis is crucial to link different pieces of evidence to a common source or toxin extraction process, and is highly challenging for complex biological samples. To raise the evidential value of forensic investigations in this field, we developed orthogonal methods and chemometric models and evaluated them according to their potential to retrospectively categorize toxin-containing pieces of evidence.

Methods

Biomolecular compositions of crude R.c. seeds and purified ricin extracts were characterized in a proof-of-principle study. We performed untargeted LC-HR-FTMS and subsequent multi-stage fragmentation analysis with CID and HCD on an Orbitrap Fusion instrument. Combining metabolomic and lipidomic approaches, these experiments allowed for pattern recognition of diverse biomarker profiles. Additional forensic signatures were established by stable carbon and nitrogen isotope ratio mass spectrometry (IRMS). A Delta V Plus isotope mass spectrometer was used for total isotope measurements (EA-IRMS), and targeted analysis of the biomarker ricinine (GC-IRMS). With the help of the acquired data from the comprehensive analytical approach and subsequent chemometric analysis, new profiling strategies were developed allowing us to potentially establish correlations between crime scenes, preparation sites and suspects.

Novel Aspect

Identification of characteristic patters by untargeted and targeted orthogonal MS-methods, molecular forensics, and chemometric models provides valuable forensic information.

Preliminary Data or Plenary Speaker Abstract

Untargeted LC-HR-FTMS analysis allowed the detection and identification of structurally diverse R.c. biomarkers at different concentration levels with high mass accuracy despite the sample complexity. Use of MSn-experiments with complementary CID and HCD fragmentation confirmed distinct structural elements of biomarkers, which is especially valuable for verification of hydroxylated lipids abundantly present in R.c. extracts. Various characteristic metabolite and lipid species, including amino acids, alkaloids, glycerolipids, glycerophospholipids, and sterols were identified and included in a database. Semi-quantitative analysis of identified biomarkers showed distinct patterns dependent on solvent identity and volume used for toxin extraction, allowing a differentiation between crude and purified ricin extracts of different cultivars.
Pattern recognition by HRMS gave valuable information about toxin extraction method but showed limitations when distinguishing between purified materials prepared by the same protocol. As stable isotope ratios are intrinsic attributes, IRMS analysis of crude R.c. seeds and purified extracts was used as an additional forensic signature. More than 98% of purified extracts prepared by the same method but from seeds obtained from different locations could be distinguished in pairwise comparison based on total carbon and nitrogen isotope ratios determined by EA-IRMS. Regression of δ13C and δ15N values of purified extracts against corresponding crude seeds demonstrated strong correlations independent of purification solvent and volume. Additional targeted GC-IRMS analysis of the

biomarker ricinine implied that δ15N values of ricinine are not influenced by toxin extraction, allowing a direct correlation of purified extracts to source seeds. Ultimately, the combination of data obtained by untargeted LC-HR-FTMS and EA-IRMS experiments as well as targeted GC-IRMS measurements and subsequent chemometric analysis suggests that molecular forensics can help link ricin preparations to specific extraction protocols and source seeds by identifying unique, characteristic patterns. Such additional forensic signatures can be implemented in profiling strategies and are of high value for forensic intelligence.

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Professor Shabaz Mohammed[1,2]
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Top down and bottom up proteomics on an Orbitrap Exploris 480 - Omnitrap instrument equipped with ExD, UVPD and IRMPD

Keynote: Professor Shabaz Mohammad University of Oxford 313 - Top down and bottom up proteomics on an Orbitrap Exploris 480 - Omnitrap instrument equipped with ExD, UVPD and IRMPD, Meeting Room 110, August 19, 2024, 15:00 - 15:25

Preliminary Data or Plenary Speaker Abstract

Mass-spectrometric analysis has achieved remarkable results in the analysis of primary structures of biomolecules. In the last few years, MS designs have started to move beyond performing simply efficient and speedy CID, expanding their abilities to resolve analytes (e.g., ion mobility) and to provide access to alternative activation techniques. One such approach involves the development of a multi-segmented ion trap that allows the possibility of several discrete ion trapping environments. In this presentation, we will describe the design, development and characterization of an Orbitrap Exploris™ 480 MS that has been equipped with an Omnitrap platform. We characterized a number of proteins including ubiquitin, myoglobin and carbonic anhydrase. We assessed efficiency in terms of speed, sensitivity and sequence coverage obtained for each individual activation technique and found that the results were in line with what was previously observed using other instruments with the additional benefit of all modes being accessible on a single instrument. We also explored how IRMPD as a supplemental activation affects fragmentation yields of ECD, EID and UVPD. Additionally, we explored the effect of IRMPD activation timing with the laser being triggered before, during and after the ExD and UVPD events. For some proteins, we managed to obtain comprehensive sequence coverage in ExD and AI-ExD experiments. In addition, we will describe the results of several large-scale proteomics experiments (2DLC and 5 enzymes), where we assessed the performance for each activation approach. Furthermore, we will show that the multiple activation techniques available on this system create a comprehensive analysis suite for PTMs such as glycosylation both N- and O-type. We show how the extensive abilities of this versatile platform can augment both top down and bottom-up proteomics.

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Dr Chaoshuang Xia[1], Professor Juan Wei[2], Professor Cheng Lin[1], **Professor Catherine Costello**[1]
[1]Boston University Chobanian & Avedisian School Of Medicine, [2]Shanghai Jiao Tong University

ExD fragmentation and multistage MSn facilitate high-confidence characterization of intact glycopeptides and glycolipids

Catherine Costello 671 - ExD fragmentation and multistage MSn facilitate high-confidence characterization of intact glycopeptides and glycolipids, Meeting Room 110, August 19, 2024, 15:25 - 15:44

Site-specific characterization of glycoconjugates is critical to elucidation of their functional relevance, e.g, in the context of antigenicity, pathogenesis and disease progression. Therefore, the ability to accurately characterize the structures of glycoproteins and other glycoconjugates is of great importance for unravelling the diverse functions of glycosylation. However, the heterogeneity of glycosylation at each occupied site, branching patterns, and the lability of glycosidic linkages still present challenges for accurate and complete structural analysis of the many types of glycoconjugates. We report here on a well-defined electron-activated dissociation (ExD) approach for improving the analysis of N- and O-linked glycoconjugates and multistage dissociation protocols for maximizing both information content and confidence in the assignments.

Methods

Proteins were solubilized, reduced, and alkylated prior to protease digestion. Digests were analyzed using LC (C18-Acuity-nanoUPLC [Waters])-MS/MS. Both native and permethylated glycosphingolipids were analyzed. ExD MS/MS experiments were performed on a Q Exactive-HF instrument [Thermo Scientific] modified with an Omnitrap platform [FasmaTech]. The precursors were irradiated with electrons at steps between 3 eV - 50 eV in the Omnitrap for 30 to 200 ms. Collision-induced dissociation (CID), higher energy collision-induced dissociation (HCD), electron-transfer dissociation (ETD), and electron-transfer/higher-energy collisional dissociation (EThcD) MS/MS and MSn experiments were conducted on a Fusion Lumos Tribrid Orbitrap mass spectrometer, using various combinations of dissociation modes. The ETD/EThcD reaction time was 100 ms. All spectra were acquired with 10 microscans. MS/MS spectra were interpreted manually.

Novel Aspect

EED fragmentation and multistage dissociations offer highly confident, site-specific identification of N- and O-linked glycopeptides and other glycoconjugates.

Preliminary Data or Plenary Speaker Abstract

A tryptic-glycopeptide 58SRNLTK63 + Hex5HexNAc2 (from RNase B) containing one potential N-glycosite and two potential O-glycosites and an O-glycopeptide 158AVESTVATLE168 + Hex1HexNAc1NeuAc2 (obtained from κ-CN) containing three potential O-glycosites, were employed as model glycopeptides. In the stepped-collision energy HCD spectrum of the [M + 2H]2+ peak of the glycopeptide with potential for both N- and O-linked glycosylation, the high abundance fragments could be attributed to glycosidic cleavages (B-, Y- ions) and peptide backbone cleavages (b-, y- ions), providing information on glycan composition and the peptide sequence, but did not allow assignment of the glycan site occupancy. HCD spectra of the κ-CN [O-linked glycopeptide also lacked critical information. In contrast, electron-capture dissociation (ECD), a non-ergodic process employing very low-energy electrons for activation of the precursor ion, has been reported to cleave the backbone N-Cα bonds in peptides, forming c- and z-ions. ECD (3 eV) fragmentation of the [M + 2H]2+ peaks of the RNase glycopeptide generated a series of B- and Y-ions that revealed the glycan composition and the intact glycopeptide site occupancy as fully N-linked. However, ECD could not specify the O-linked glycosylation sites on κ-CN. Similar results were observed in the ETD experiments. Hot-ECD (12 eV) and electron excitation dissociation (EED at 17 eV) fragmentation techniques allowed the unambiguous determination of the glycosylation site locations and both the N- and O-linked glycan structures. Much higher electron energy (35 eV) the electron ionization

dissociation (EID) spectra obtained for N- and O-linked glycopeptides were dominated by low-m/z fragments. In contrast, glycolipid spectra recorded at >30 eV were information-rich, regarding the glycan, LCB and FA and provided alkyl chain fragmentation that enabled double-bond site localization. Conditions optimized with the standards were then used for analysis of project-related samples.

Dr. Rachel Loo[1], Jessie Le[1], Boyu Zhao[1], Joseph Loo[1]
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The Big Break Up—Understanding How a Subunit or Polypeptide Abandons its Noncovalent Complex in Native Top-Down MS

Rachel Ogorzalek Loo 246 - The Big Break Up—Understanding How a Subunit or Polypeptide Abandons its Noncovalent Complex in Native Top-Down MS, Meeting Room 110, August 19, 2024, 15:44 - 16:03

Small molecule MS/MS is well-understood from dissociation time scale and transition state theory. Applying slow, low energy activation in multiple collisions favors rearrangement-driven pathways, while depositing energy quickly, in one or a few higher energy collisions favors direct dissociations. For native top-down MS, protein multimeric complexes subjected to CID on QTOFs typically decompose into a charge-enriched subunit and low charge residual complex, whereas those subjected to SID release noncovalently-bound oligomers with similar charge. The former (asymmetric) mechanism is attributed to unfolding or salt-bridge rearrangements, while the latter (symmetric) is “direct” (no unfolding or few salt-bridge rearrangements). We extend our mechanistic understanding of native top-down CID to novel instances where backbones cleave or where statistical, multiple collisions eject multimers.

Methods

Membrane proteins aquaporin Z (AqpZ) and ammonium transporter (AmtB) were expressed in E.coli, purified and buffer-exchanged into 200mM ammonium acetate and 40mM n-Octyl-β-D-Glucopyranoside (OG) prior to analysis. Soluble proteins including alcohol dehydrogenase, aldolase, and GroEL were also examined. Native top-down MS by HCD, ECD or activated-ion ECD employed a ThermoFisher Q Exactive UHMR Orbitrap equipped with an ECD cell (eMSion). Proteins were delivered by a nanoESI source and Pt-coated borosilicate pulled glass capillaries. Collision-based fragmentation was also induced via activation in-source. Alternatively, complexes were analyzed on a 15-Tesla Bruker SolariX Fourier transform-ion cyclotron resonance mass spectrometer (FTICR) with activation in-source and/or at the downstream hexapole or on a Synapt G2si QTOF activated in-source or in the transfer region.

Novel Aspect

Energetics of CID, combined with structural aspects, govern the type of products ions observed in native top-down MS.

Preliminary Data or Plenary Speaker Abstract

SID’s high energy, single collision activation of noncovalent complexes accesses decomposition pathways that differ from those of QTOF CID, which typically releases charge-enriched monomers. Here, we explore additional low energy pathways accessed by Orbitrap, FTICR, and QTOF CID for strongly bound complexes (often with salt-bridge interactions) and those that decompose readily. Native top-down (nTD) orbitrap spectra of yeast alcohol dehydrogenase (ADH tetramers, 147 kDa) differ from complex-down (CD) spectra. The former’s HCD-activated tetramers release primarily N-terminal fragments, whereas the latter’s HCD-activated monomers yield both N-/C-terminal products, establishing that nTD backbone cleavages arise directly from tetramers. QTOF-CID of ADH tetramers yields both monomers and covalently-cleaved products, but increased energy favors backbone-cleavages. Interestingly, monomers’ average charge increases with activation energy, again establishing that the backbone-cleaved products don’t arise from subsequent monomer dissociations and suggesting that, at high activation energies, only dissociations ejecting the most highly charged monomers proceed quickly enough to compete with backbone cleavage. Furthermore, the average charge of backbone segments decreases with increasing activation energy in a pattern suggesting higher energies increase covalent cleavage from lower-charge density subunits/regions.

HCD-activated aldolase tetramers (158 kDa) primarily decompose to y74 and 4M-y74 products, with the complementary ions indicating direct production from tetramers. Higher activation energies decrease y74's average charge, while increasing that of 4M-y74, implying again that higher energies increase cleavage from lower-charge density regions.

Combined with membrane protein data, these behaviors imply low energy asymmetric dissociations proceed by rearranging salt bridges until a favorable combination can free a subunit. With sufficient energy to cleave backbones, asymmetric dissociations compete with covalent cleavages. Lower charge state precursors, with more opposite charges (potentially more salt bridges), are most likely to break covalent bonds. Such considerations add to our understanding of how dissociation of protein complexes (nTD) can be linked to their original structures.

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Fuzhong Ni[1], Mr. Bingyin Xu[1], Dr. Fuxing Xu[1], Miss Ziyang Song[1], **Prof. Li Ding**[1]
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Implementation of Electron Capture Dissociation in an RF Linear Ion Trap without Assistance of Magnetic Field

Li Ding 169 - Implementation of Electron Capture Dissociation in an RF Linear Ion Trap without Assistance of Magnetic Field, Meeting Room 110, August 19, 2024, 16:03 - 16:22

The electron capture dissociation (ECD) can be implemented in an ICR cell as well as in some simpler ion storage or transportation devices where ions are confined by radio frequency (RF) electric field. Often, a magnetic field is used to guide the low-energy electrons to travel along a central axis of the RF field without being heated up, before they react with the multiply charged ions. One exception without using magnetic field is the Omnitrap, which employs the digital waveform, where the electron energy is retarded to zero when they reach the ion trap center. ECD in the conventional sinusoidal RF was regarded as inefficient since the low energy electrons would be easily interrupted by the varying electric field.

Methods

A 2D RF ion trap has a pair X electrodes and a pair of y electrodes, with a slot to let electron to injected into the trap. An electron source includes the indirectly heated cathode, accelerating and focusing lenses, which help to generate a flat electron beam. SIMION software was used to study the electron beam profile, the energy of electron when it reaches the central axis of the 2 D trap, for different initial cathode potential, angular distribution and RF phase of electron emission.

Novel Aspect

High efficient ECD in conventional RF trap without using magnetic field,using dynamic cathode bias.

Preliminary Data or Plenary Speaker Abstract

ECD of peptide ions normally need electron energy below 3 eV, sometimes even below 1 eV to avoid unnecessary side chain fragment. Electron injection through the slot in y rod must take place during the positive phase of the sinusoidal wave for the y rod. The electron beam is compressed in x direction and retarded in y direction, so the remaining kinetic energy will be gradually reduced to < 3 eV. If the potential of the cathode is carefully selected, the energy of electron can be very closed to zero when it reach the centre of the trap. However, the variation of sinusoidal wave voltage dynamically changes the energy of electron, and electron may not be possible to reach the centre reaction region in some phases. It has been found that a phase window of $\pi/4$ exists for electron reach the reaction region with energy below 3 eV when a fixed electric potential of cathode is used. By implementing a periodic cathode bias voltage with a specially designed waveform, ECD phase window may be extended to almost $\pi/2$ with electron energy below 1 eV at reaction region.

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Dr Oliver Hale[1], Prof Helen Cooper[1]
[1]University Of Birmingham

Declustering protein complexes by infrared photoactivation for in situ native mass spectrometry.

Oliver Hale 351 - Declustering protein complexes by infrared photoactivation for in situ native mass spectrometry, Meeting Room 110, August 19, 2024, 16:22 - 16:41

Native mass spectrometry has typically relied on sample preparation methods like buffer exchange to reduce non-specific adducts. These methods are incompatible with in situ native mass spectrometry, where protein complexes are analyzed directly from a biological environment e.g. tissue. For example, when using nanospray-desorption electrospray ionization (nano-DESI), gas-phase declustering of protein from adducts can be performed with collisional activation. Limitations of collisional activation led us to investigate declustering by infrared laser (IR) photoactivation by modification of a linear ion trap mass spectrometer with a CO2 laser. Declustered protein complexes retained non-covalent interactions with their endogenous ligands. We propose IR declustering as an advancement for in situ native MS on native MS-focused mass spectrometers.

Methods

A home-built nano-DESI ion source was attached to an LTQ Velos Pro modified with a CO2 laser for irradiation through the rear flange. The solvent system was aqueous ammonium acetate (200 mM) + 0.125% (by volume) of the detergent C8E4 and set to a flow rate of 1.7 µL/min. Trap pressure was approx. 1.7e-5 Torr with helium provided as the damping gas. Tissue sections were continuously sampled by scanning under the nano-DESI probe. Ions were irradiated by 10.6 µm photons along the beam path through the high-pressure cell and transfer optics. The automatic gain control target was set to 5e4 with injection time = 750 ms. The ion trap mode was set to "High mass".

Novel Aspect

IR declustering of protein complexes analysed by nano-DESI under native conditions on a linear ion trap.

Preliminary Data or Plenary Speaker Abstract

Nano-DESI analysis was performed on hSOD1G93A mouse brain and sheep eye lens tissue sections. For the mouse brain, proteins were undetectable when declustering was performed with collisional activation. With IR declustering, protein complexes up to approx. 32 kDa molecular weight were detectable and the background signal was substantially reduced. Protein complexes were detected intact with endogenous ligands, including SOD1 bound to metal ions and Arf1 bound to its endogenous ligand GDP.
The same analysis with eye lens tissue enabled analysis of protein complexes up to ~47 kDa in molecular weight including heterodimeric complexes of beta-crystallins. In all analyses, declustering of the proteins by IR declustering proved essential to achieve the highest protein signal and reduce background signal. We propose IR declustering as an enabling technology for in situ native protein ion analysis.

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Ion Fragmentation for Detailed Lipid Structural Analysis using Atomic Hydrogen/Oxygen Irradiation (HAD/OAD)

Hidenori Takahashi 434 - Ion Fragmentation for Detailed Lipid Structural Analysis using Atomic Hydrogen/Oxygen Irradiation (HAD/OAD), Meeting Room 110, August 19, 2024, 16:41 - 17:00

In recent years, a variety of innovative LC-MS/MS techniques have emerged for the comprehensive structural analysis of lipids with complex structures. These techniques can be divided into two main categories: those that employ novel fragmentation methods such as EIEIO (Electron Impact Excitation of Ions from Organics) and UVPD (Ultraviolet Photodissociation), and those that involve derivatization techniques like MELDI (mCPBA Epoxidation for Lipid Double-bond Identification) and Paternò–Büchi reactions. In our research, we have developed a new radical-induced ion fragmentation method that utilizes atomic hydrogen (hydrogen abstraction dissociation, HAD) and atomic oxygen (oxygen attachment dissociation, OAD) [1-3].

Methods

Atomic hydrogen and atomic oxygen are generated via microwave discharge of water vapor. The generated radicals were introduced into the quadrupole ion trap to initiate HAD/OAD of the target precursor ions. When HAD/OAD is applied to lipid analysis, irradiation of the precursor ions with hydrogen atoms (HAD) causes sequential cleavage of C-C bonds in fatty acids, while irradiation with oxygen atoms (OAD) selectively cleaves C=C bonds. The unique advantage of this technique is its ability to switch between HAD and OAD within a single instrument, enabling the acquisition of structure-dependent information for lipid analysis. Unlike EIEIO, which utilizes electrons, this technique utilizes charge-neutral radical atoms and can be applied in both negative and positive ion modes.

Novel Aspect

The use of charge-neutral hydrogen/oxygen atoms in radical-induced dissociation enables structural analysis independent of precursor ion valence, facilitating comprehensive characterization.

Preliminary Data or Plenary Speaker Abstract

HAD offers crucial insights into modification sites, such as hydroxyl groups in fatty acids, while OAD is instrumental in elucidating the structure of carbon-carbon double bonds. By utilizing the synergistic potential of HAD and OAD, we have successfully elucidated the structures of unique metabolites known as 'goadvionin,' which are hybrid compounds of fatty acids and peptides [Onaka et al., Nat. Chem. 12, 869–877, 2020]. These structures were unresolvable using conventional high-resolution mass spectrometry or NMR techniques. Additionally, HAD has played a key role in identifying novel biomarkers for Niemann-Pick disease type-C, which were previously undetectable using traditional mass spectrometry methods [Maekawa et al., Int J Mol Sci. 2019 Oct; 20(20): 5018].
Furthermore, OAD has been employed in untargeted lipidomics studies, as demonstrated by Arita and Tsugawa et al., and a comprehensive analysis platform, including software (MS-DIAL), has been developed [Communications Chemistry, 5-162, 2022]. Through the examination of samples derived from humans and mice, a total of 648 unique lipids across 24 lipid subclasses were characterized based on their resolved carbon-carbon double bond positions.
In this presentation, we will provide a detailed overview of the mechanism behind this technique and present examples of carbon-carbon double bond position-resolved lipid structural analysis using a conventional HPLC system, direct ionization method (PESI), supercritical fluid chromatography (SFC), and MS imaging (MSI) in combination with atomic radical irradiation.
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Plenary - Mass Spectrometry in Research of Chemical Reactions, Plenary 3, August 20, 2024, 08:30 - 09:30

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Prof Jana Roithová[1]
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Mass Spectrometry in Research of Chemical Reactions

Understanding reaction mechanisms is the key to developing new chemical reactions. Electrospray ionization mass spectrometry has a unique dynamic range that allows the study of reaction mixture compositions, including low-abundant reactive intermediates. In the lecture, I will show several approaches to studying reaction kinetics via mass spectrometry coupling with flow-chemistry or batch-chemistry setups.[1-3]

Methods

Electrospray ionization mass spectrometry; ion-mobility separation; helium tagging photodissociation spectroscopy; flow chemistry; delayed reactant labeling; cyclic voltammetry.

Novel Aspect

New methods in research of reaction mechanisms based on mass spectrometry.

Preliminary Data or Plenary Speaker Abstract

Understanding reaction mechanisms is the key to developing new chemical reactions. Electrospray ionization mass spectrometry has a unique dynamic range that allows the study of reaction mixture compositions, including low-abundant reactive intermediates. In the lecture, I will show several approaches to studying reaction kinetics via mass spectrometry coupling with flow-chemistry or batch-chemistry setups.[1-3]

Monitoring the kinetics associated with the reactive intermediates opens a way to understand the effects of catalysts and other reaction mixture components on forming products and by-products. One of the rising fields in catalysis is electrocatalysis. However, processes at the electrodes are extremely difficult to study. However, electron transfer processes usually generate charged species; therefore, mass spectrometry can be an ideal tool to study these processes, provided we have an efficient way of transferring the generated species to a mass spectrometer. I will present our solution, allowing us to study the electrochemically generated intermediates and evaluate their kinetics at an electrode.[5,6] We also couple the setup with cryogenic ion spectroscopy and study the structure of the reactive intermediates by vibrational and electronic spectroscopy.[4]

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Professor Thomas Kislinger[1]
[1]Princess Margaret Cancer Centre

Prostate Cancer Reshapes the Secreted and Extracellular Vesicle Urinary Proteomes

Keynote: Professor Thomas Kislinger University of Toronto 613 - Prostate Cancer Reshapes the Secreted and Extracellular Vesicle Urinary Proteomes, Plenary 3, August 20, 2024, 10:00 - 10:25

For the 135,000 North American men diagnosed with low- or intermediate-risk prostate cancer (PC) each year, clinical outcomes are heterogeneous: 50-80% will be disease-free 10 years following curative-intent therapy while 10-20% will experience recurrence within 18 months, portending lethality. Conversely, 25% diagnosed with low-risk PC elect to enter Active Surveillance (AS), where their disease is monitored by repeat prostate-specific antigen (PSA) tests and needle biopsy to rule out the presence of aggressive PC. Current clinical prognostic factors (Gleason Score, PSA, and T category) do not accurately predict disease aggression and clinical outcome for individual men resulting in under-treatment of occult aggressive disease and over-treatment of indolent disease. Molecular urine biomarkers that assist the clinical decision process are urgently needed.

Methods

We have performed proteomics on over 600 prostate related fluid samples from 190 unique patients, spanning the entire risk spectrum of prostate cancer (benign prostate hyperplasia (BPH) and Gleason Scores 6-10). Our cohort is richly annotated with an average follow-up time of over seven years. We performed differential ultracentrifugation to isolate two populations of extracellular vesicles (P20 and P150) (Correll VL, et al. J Extracell Vesicles. 2022), as well as the soluble urine fraction using a PVDF membrane-based capture approach (Berger et al. Mol Cell Proteomics, 2015). All samples were analyzed by DDA-based LC-MS using a QExactive HF and search with MaxQuant. All obtained data was parsed into a relational database management system for streamlined analysis.

Novel Aspect

We report the most comprehensive interrogation of prostate related fluids proteomes across the entire risk spectrum of prostate cancer.

Preliminary Data or Plenary Speaker Abstract

Urine is a complex biofluid that reflects both overall physiologic state and the state of the genitourinary tissues through which it passes. It contains both secreted proteins and proteins encapsulated in tissue-derived extracellular vesicles (EVs). To understand the population variability and clinical utility of urine, we quantified the secreted and EV proteomes from men with prostate cancer. In total, we detected over 6,000 proteins with considerable overlap observed among the three analyzed fractions. EVs of different sizes and densities contained proteins from different subcellular origins, suggesting distinct biogenesis. Urinary EVs, in particular the P20 population, are faithful surrogates of tissue proteomes, but secreted proteins in urine or cell line-derived EVs are not. Using samples obtained from patients on active surveillance we observed a fraction of the urinary proteome that was longitudinally stable. Since our unique dataset represents one of the largest urine proteomics studies, we were able to leverage longitudinally stable proteins to derive new biomarkers that can accurately and non-invasively distinguish malignant from benign prostatic lesions, and risk-stratify prostate tumors. Future validation using robust quantitation by targeted proteomics assays with stable isotope labeled standards in large, racially diverse cohorts will be required. We have used our data to generate an interactive portal to facilitate investigation into the urinary secreted and EV proteomes. In summary, our data quantified the complexity of the urinary proteome and revealed

the synergistic value of secreted and EV proteomes for translational and biomarker studies (Khoo A, et al. bioRxiv. 2023).

Dr Nicola Gray[1,2], A/Prof Joshua D Chandler[3], Dr Yuliya Karpievitch[4], Ho Yin Ho[4], Prof Elaine Holmes[1,5], Prof Sarath Ranganathan[6,7,8], A/Prof Rabindra Tirouvanziam[3], Dr Shivanthan Shanthikumar[6,7,8], Prof Charles R Esther Jr[9]
[1]Centre for Computational and Systems Medicine, Health Futures Institute, Murdoch University, [2]Australian National Phenome Centre, Health Futures Institute, Murdoch University, [3]Department of Pediatrics, Emory University and Center for CF and Aiways Disease Research, Children's Healthcare of Atlanta, [4]Telethon Kids Institute, [5]Institute of Global Health Innovation, Faculty of Medicine, Imperial College London, [6]Royal Children's Hospital Melbourne , [7]Murdoch Children's Research Institute , [8]Melbourne Medical School, University of Melbourne, [9]University of North Carolina at Chapel Hill

Signatures of future bronchiectasis risk in children with cystic fibrosis via lipid profiling of bronchoalveolar lavage fluid

Nicola Gray 496 - Signatures of future bronchiectasis risk in children with cystic fibrosis via lipid profiling of bronchoalveolar lavage fluid, Plenary 3, August 20, 2024, 10:25 - 10:44

Many children with cystic fibrosis (CF) experience early airways disease that ultimately leads to bronchiectasis (BE). However, a subset of children with CF do not develop BE, a phenomenon that is not yet fully understood. Early life biomarkers that predict later structural lung disease (or lack of such) in children with CF have the potential to facilitate personalised care, offer insights into disease pathophysiology, and suggest novel therapeutic targets. To explore novel biomarkers associated with future BE, we examined lipid and bile acid signatures (in relationship to other biomarkers) in longitudinal bronchoalveolar lavage (BAL) samples from children with CF classified by the development of BE or not.

Methods

Biobanked bronchoalveolar lavage (BAL) samples were identified from two cohorts (n=14 each) of children who had annual bronchoscopy and chest CT as part of the AREST-CF program. The first cohort comprised children who had no BE on chest CT at age 9 (no BE cohort), representing the mildest quartile of disease severity; the second cohort was age, sex, and genotype matched children who had BE at age 9 (BE cohort). BAL samples from ages 0-5 yrs within each cohort were analysed by targeted liquid chromatography-mass spectrometry (LC-MS)-based lipid and bile acid panels, alongside an inflammatory cytokine panel including neutrophil elastase (NE) and IL-8 as established markers of BE. Multivariate statistical analysis and Pearson correlations were used to evaluate group differences.

Novel Aspect

Lipid and bile acid signatures in early-life bronchoalveolar lavage from children with cystic fibrosis associate with future structural lung disease.

Preliminary Data or Plenary Speaker Abstract

Lipids and bile acids were measured in 157 BAL samples from 28 subjects. Following data pre-processing and analytical quality control filtering, 517 lipids spanning 18 sub-classes and 9 bile acids were included for further statistical analysis. Significant differences were noted in several lipids species between the BE and no BE cohorts across the age groups, with discriminating lipids including multiple lactosylceramides (LCERs), hexosylceramides (HCERs) and ceramides (CERs), as well as a number of phosphatidylcholines (PCs) and phosphatidylethanolamines (PEs). PC(18:0/18:3), PE(18:1/18:2), LCER(24:1) and HCER(24:1) were elevated in the BE cohort. To assess the influence of age, we performed a subgroup analysis in which biomarker concentrations were averaged within BAL collected between ages 0-2 yrs as early preschool, with averages from BAL collected between ages 3-5 yrs as late preschool. Robust lipid biomarkers, including LCER(24:0), HCER(24:1), CER(24:1), LPE(18:0), PC(18:1/18:1) and PE(18:0/18:1) demonstrated better performance at predicting future BE in the late preschool age group. Discriminating lipids outperformed, but were significantly correlated with, IL-8 and NE as established BE markers.

Glycocholic acid (GCA) and glycochenodeoxycholic acid (GCDCA) were the bile acids present at the highest concentrations. In early preschool, total bile acids, GCA, and TCA (taurocholic acid) were elevated in the BE cohort relative to the no BE cohort, and were strongly correlated with NE and IL-8. In late preschool, bile acids were similar in BE and no BE cohorts and had reduced correlations to NE, whilst total bile acids, GCA and GCGCA remained highly correlated with IL-8 in late preschool.

In summary, these data support the hypothesis that sphingolipids play an important regulatory role in CF with respect to inflammation. Children with CF showing the mildest structural lung disease at age 9 had reduced BAL lipids and bile acids in early life, representing mechanistic insights and risk factors for later BE development.

Dr Oana Marian[1,2], Dr Danqing Min[1,3,4], Dr Callum Baker[1,3], Mr James Gerofi[1,3], Dr Xiaoyu Wang[1,3], Associate Professor Nathan Johnson[5,6], Prof Anthony Don[2], Professor Stephen M Twigg[1,3,4]
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A Low Volume Exercise Intervention Remodels the Type-2 Diabetic Plasma Lipidome, Reducing Circulating Toxic Deoxyceramides.

Oana Marian 399 - A Low Volume Exercise Intervention Remodels the Type-2 Diabetic Plasma Lipidome, Reducing Circulating Toxic Deoxyceramides, Plenary 3, August 20, 2024, 10:44 - 11:03

High intensity interval training (HIIT) has recently gained interest as a potential intervention for improving outcomes in people with type-2 diabetes mellitus (T2DM) and prediabetes (PD). PD and T2DM are characterised by increased blood glucose levels and are frequently associated with obesity. Previous studies by our laboratory conducted on the randomised control trial PACE-G have shown that a low-volume combined supervised HIIT and progressive resistance training (exercise) intervention over 12 weeks improved liver fat. We sought to profile the plasma lipidome of PACE-G participants to determine: (1) how the baseline plasma lipidome differs between overweight or obese participants with either normal glucose (NG), PD or T2DM, and (2) the effect of exercise intervention on circulating lipids.

Methods

PACE-G study participants with a BMI >25 kg/m2 encompassing newly diagnosed T2DM, PD and NG were randomly assigned to either a stretching or an exercise protocol. Fasted blood plasma was collected at baseline and after a 12-week intervention period. Untargeted lipidomics was performed using LC-MS/MS on a Thermo Q-Exactive HF-X and Vanquish HPLC. Lipid annotation and chromatogram alignment was performed with LipidSearch v4.2 software, and lipid levels were quantified relative to class-specific internal standards, which were added prior to lipid extraction. Experimental data at baseline were compared by one-way ANOVA adjusted for age, sex, and BMI, and corrected for the false discovery rate using the Benjamini-Hochberg method. Baseline and follow-up data were compared within groups by paired t-test.

Novel Aspect

This study specifically identifies elevated circulating toxic deoxyceramides in T2DM, with novel reduction in their levels by an exercise intervention.

Preliminary Data or Plenary Speaker Abstract

Plasma from 160 participants with mean age of 58.2 ± 12.2 years and mean BMI of 31.9 ± 5.3 kg/m2 were analysed. A total of 635 lipids across 22 functional lipid classes were quantified. At baseline, the abundance of 96 lipids were significantly altered between the NG and T2DM groups. Relative to NG cases, the T2DM group was characterised by significantly lower levels of sphingomyelin (SM), lysophosphatidylcholine (LPC) and phosphatidylserine (PS) species and significantly higher levels of diacylglycerol (DAG) and deoxyceramide (mCer) species. Deoxyceramides are a subclass of ceramides formed through a non-canonical biosynthetic pathway and are thought to be poorly degraded and to exert cytotoxic effects. Eighty-two lipids were significantly different between PD and T2DM groups, and similar observations of significantly higher levels of mCer species and lower levels of SM, LPC and PS species were observed in T2DM cases compared with PD cases, indicating that the abundance of these lipids is altered as glucose tolerance declines. Only seven lipids differed in abundance between PD and NG cases, where PD cases had significantly lower levels of several LPC and SM species, which may indicate that LPC and SM are early circulating lipid markers of impaired glucose homeostasis.

Following 12 weeks of exercise intervention, mCer levels were significantly reduced in participants randomised to the exercise intervention in the T2DM group but not the PD or NG groups.
Using comprehensive lipidomic analysis, we demonstrate that deoxyceramides and diacylglycerols are increased with impaired glucose tolerance, after controlling for BMI, and show for the first time that HIIT can reduce levels of toxic deoxyceramides. This study therefore indicates that impaired glucose homeostasis is progressively linked to impaired lipid metabolism and it has identified a beneficial effect of a low volume exercise intervention on remodeling the plasma lipidome.

Ms Anna Emilia Hoffmann[1,2], Dr Timothy A. Couttas[1], Dr Cathrin Rohleder[1,2,3], Dr Boris Guennewig[4], Prof F. Markus Leweke[1,2,3]
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Interplay between sphingolipid metabolism and the endocannabinoid system in schizophrenia: Insights into biomarker candidacy

Anna Emilia Hoffman 71 - Interplay between sphingolipid metabolism and the endocannabinoid system in schizophrenia: Insights into biomarker candidacy, Plenary 3, August 20, 2024, 11:03 - 11:22

To date, diagnosis of schizophrenia (SCZ) is exclusively based on clinical presentations, which frequently overlap with other psychiatric disorders, impeding diagnostic accuracy and timing. Alterations to sphingolipid (SLs) metabolism, a diverse class of signalling lipids enriched in the brain, are recognised for their regulatory impact on neuronal growth, myelin maintenance and synaptic transmission. The endocannabinoid system (ECS) is known to be dysregulated in SCZ and further affects SL metabolism by enhancing SL stability and the promotion of SL synthesis de novo. This study represents the first broad lipidomic investigation of SL metabolism and the ECS, exploring the shared aspects of these systems in schizophrenia (SCZ), given both have been implicated in disease pathogenesis.

Methods

For our lipidomic investigations, we employed advanced liquid chromatography-tandem mass spectrometry (LC-MS/MS) coupled with our published retention time prediction tool ReTimeML for quantification of 48 SLs comprising different ceramides (Cer) and sphingomyelins (SM), alongside the ECS agonists anandamide (AEA) and 2-arachidonylglycerol (2-AG). Our cohort encompasses 282 participants, including pre-psychotic (prodromal, SCZpro, n=23), antipsychotic-naïve (SCZuntreat, n=90), or antipsychotic-treated SCZ (SCZtreat, n=96), compared to age-matched healthy volunteers (HVs, n=73), of which matched serum and cerebrospinal fluid (CSF) were analysed.

Novel Aspect

This presents the first investigation of SLs and ECS-agonists in SCZ, warranting further investigation into their potential as lipidomic markers.

Preliminary Data or Plenary Speaker Abstract

Results revealed an upregulation of AEA in the CSF of SCZ-diagnosed participants ($p<0.05$), which aligned with the current literature. Furthermore, we observed an increase of specific SMs in SCZtreat, that could be categorised into structural variants based on the degree of saturation of their sphingoid-backbone (d18:1, $p<0.0001$; d18:2, $p<0.05$), as well as an overall loss of Cer lipids in participants diagnosed with SCZ ($p<0.0001$). Orthogonal partial least squares discriminant analysis identified a set of differential lipids of high variable importance, occuring in each of the SCZ cohorts (SCZpro, n=18; SCZuntreat, n= 16 and SCZtreat, n= 20), when compared to HVs (VIP >1). This included changes to SLs that were specific to a SCZ group ($p<0.05$) and/or conserved across the two biofluids ($r>0.8$, $p<0.0001$).
Finally, we evaluated the correlation of Cer and AEA, based on known evidence of interaction supporting reciprocal stabilisation preventing degradation of both lipids. We observed a positive Cer-AEA association for HVs in the CSF ($r=0.480$, $p= 0.005$) and sera ($r=0.305$, $p= 0.019$), which was not observed in our SCZ groups. Notably, this correlation was lost at the prodromal stages of disease, exclusively in the CSF, before loss of correlation in the serum of patients diagnosed with first or multiple psychotic-episode SCZ. These results may signify an early pathogenic event, given the cerebral changes (CSF) were yet to reach the peripheral system (serum) at prodromal stages of disease onset.

Dr Thomas Meikle[1,2], Dr Kevin Huynh[1,2], Dr. Corey Giles[1,2], Dr Jingqin Wu[1], Dr Tingting Wang[1], Dr Aleksandar Dakic[1], Dr Andrew Gooley[3], Dr Konstantinos Kouremenos[3], Dr Belinda Whittle[3], Dr Jean Yang[4], Dr Stuart Grieve[5], Dr Stephen Vernon[6], Dr Michael Gray[5], Professor Gemma Figtree[6], Professor Peter Meikle[1,2]

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Development and clinical translation of high-throughput lipidomic profiling for the assessment of individual cardiometabolic risk

Thomas Meikle 455 - Development and clinical translation of high-throughput lipidomic profiling for the assessment of individual cardiometabolic risk, Plenary 3, August 20, 2024, 11:22 - 11:41

This presentation outlines the development of a high-throughput Clinical Lipidomics Platform – a clinically focused translation of our research-based lipid profiling technology. The Clinical Lipidomics Platform (CLP) was designed to provide an accessible approach to personalized risk assessment for cardiometabolic disease. The platform is centered around an LC-MS/MS based lipid assay, measuring 251 unique lipid species in 5 minutes. A key innovation is the online calculation of lipidomic risk scores, where individual lipid profiles obtained through the assay are processed using statistical algorithms to generate individualized health risk assessments. This approach has the potential to broaden clinical and research applications of lipidomic profiling, bridging the gap to precision medicine.

Methods

Single-phase lipid extractions are performed on 10 µL aliquots of plasma, followed by separation via a 5 minute RP-HPLC gradient, and analyzed for lipid content using an Agilent 6495C triple quadrupole instrument operating in dMRM mode. Monitored lipid species were selected to enable automated integration and data processing. Lipid concentrations are quantified using a series of isotopically labelled internal standards and normalized to an external reference sample (NIST SRM1950). Utilizing regression modelling in conjunction with large lipidomic datasets, the resulting lipid profile is used to generate a cardiovascular Lipidomic Risk Score (LRS) for each individual, providing improved risk prediction over conventional cardiovascular risk scores.

Novel Aspect

Development of cardiometabolic risk scores using lipid profiles of 251 individual species obtained via a high-throughput LC-MS/MS assay.

Preliminary Data or Plenary Speaker Abstract

We describe the clinical translation of our research-based lipidomic profiling platform, including the process of lipid selection, modifications to RP-HPLC methods, overall platform design and workflow, scalability and throughput requirements, and process automation. Further, we have outlined the use of external reference samples to correct for within- and between-batch variation.
The Clinical Platform was validated via the analysis of a large sample-set – the BioHEART-CT cohort. This set of 1,121 plasma samples was analyzed using both our high-throughput clinical assay, measuring 251 lipid species, as well as our more detailed research protocol, measuring 802 lipid species. We have compared assay performance between the two methods, and calculated a cardiovascular LRS based on each profile, designed to predict the risk of a cardiovascular event within the next ten years. Lastly, we have explored the correlation between the LRS calculated using 251 vs. 802 lipid species and evaluated risk prediction and reclassification within the BioHEART cohort using the calculated LRS.

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Dr Elizabeth Want[1], Dr Istvan Nagy, Dr Joshua Cuddihy
[1]Imperial College London

Exploring novel metabolic changes in tissue injury: possible new treatment routes

Elizabeth Want 531 - Exploring novel metabolic changes in tissue injury: possible new treatment routes, Plenary 3, August 20, 2024, 11:41 - 12:00

Tissue injury, including burns, are a major trauma affecting millions worldwide every year. These injuries can cause death and morbidity, resulting in huge healthcare costs and impacting both patients and their families. After injury, intracellular molecules are released, triggering inflammatory reactions to restore tissue function and resulting in persistent pain. This pain can persist for months or years after the injury itself, affecting quality of life. However, these pain-inducing molecules are largely unidentified, hampering our understanding of tissue injuries and the ability to treat them effectively. Using a mass spectrometry-based omics workflow, we aim to improve understanding of molecular changes during tissue injury, to advance treatment of tissue injuries, reduce pain and improve patient outcomes.

Methods

We developed a scalding burn injury model and metabolomics workflow for analysing dermal microdialysate and skin from burn-injured and control subjects. This model facilitates sample collection immediately after injury and enables molecular changes to be followed over time, while clinical samples provide insight into longer-term metabolic changes. We combined ultra-performance liquid chromatography (UPLC) and desorption electrospray ionisation (DESI) imaging mass spectrometry to elucidate metabolite and lipid changes after burn injury. Samples were analysed using a UPLC Acquity system coupled to a Synapt G2-S mass spectrometer. DESI-MS imaging was performed on skin sections from patients using a Waters Xevo G2 QTOF. Data were pre-processed and analysed using both univariate and multivariate approaches to identify significant molecular alterations due to burn injury.

Novel Aspect

These novel findings could potentially improve patient care and outcomes by reducing pain and inflammation and providing better treatments.

Preliminary Data or Plenary Speaker Abstract

For metabolite and lipid profiling, microdialysate and skin samples from burned and non-burned subjects were extracted and subjected to reversed-phase chromatography employing in-house methods. DESI-MS imaging was performed on sections of burned and non-burned skin from patients (n=72) in positive and negative ion mode. Significant metabolic changes were observed in response to burn injury, including elevated uric acid and niacinamide, known to be involved in tissue repair and wound healing. A novel and significant finding was that lysophosphatidylcholine (LPC) species were significantly altered in both microdialysate and skin after burn injury. Of specific interest were 14:0, 16:0 and 18:0-LPCs, all elevated following burn injury, due to oxidative stress. These pro-inflammatory lipids can be hydrolysed to lysophosphatidic acid, which causes pain through demyelination and activation of pain-related molecules on primary sensory neurons. DESI-MS showed significant alterations in skin lipid content 5-9 days after scalding burn injury, also revealing the increase in 18:0-LPC to be in the upper and middle third of the dermis. RNAseq data indicated several LPC metabolic enzymes to be differentially expressed after burn injury. Subsequent in vitro and in vivo studies revealed that administration of 18:0-LPC induced immediate pain and development of hypersensitivities to mechanical and heat stimuli, through the modification of ion channels.

In conclusion, burn injury causes significant and consistent alterations in small molecules and lipids, in both microdialysate and skin samples. Importantly, 18:0-LPC was observed to contribute to the development and persistence of pain. These lipids have also been found to increase in other tissue injuries (e.g. ischemia, surgical injuries), and contribute to the development of inflammation, insulin resistance, and atheroschlerotic plaques. Therefore, this is likely to be an important mechanism for the development of hypersensitivities in tissue injuries.

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Professor Lisa Jones[1]
[1]University of California San Diego

In-Cell Protein Footprinting Coupled with Mass Spectrometry for Structural Biology Across the Proteome

Keynote: Professor Lisa Jones University of California San Diego 752 - In-Cell Protein Footprinting Coupled with Mass Spectrometry for Structural Biology Across the Proteome, Meeting Room 105, August 20, 2024, 10:00 - 10:25

In recent years, protein footprinting coupled with mass spectrometry has been used to identify protein-protein interaction sites and regions of conformational change through modification of solvent accessible sites in proteins. The footprinting method, fast photochemical oxidation of proteins (FPOP), utilizes hydroxyl radicals to modify these solvent accessible sites. To date, FPOP has been used in vitro on relatively pure protein systems. We have further extended the FPOP method for in vivo analysis of proteins. The method has been successfully used to modify proteins in cells, C. elegans, and spheroids. In-cell FPOP (IC-FPOP) can provide structural information on over 2000 proteins in a single experiment.

Methods

K562 leukemia cells were cultured in a 6-well plate. Cells were either treated with the drug methotrexate or the vehicle DMSO. Cells were mixed with hydrogen peroxide (H2O2) and irradiated with a laser. No laser controls were also analyzed. After FPOP labeling, cells were lysed, proteins extracted and digested by trypsin. Peptides were analyzed by a Thermo Scientific Q-Exactive Orbitrap. Database searching was performed using Sequest in Proteome Discoverer.

Novel Aspect

This is the first time IC-FPOP was used to study off target drug effects.

Preliminary Data or Plenary Speaker Abstract

A major application of the in vivo method is for proteome-wide structural biology. In one such application, we used in-cell FPOP (IC-FPOP) to identify on and off targets of the anti-cancer drug methotrexate in leukemia cells. In total, 1067 proteins were modified in both the drug treated and vehicle treated states. Differences in oxidative labeling between the two states are present in 524 proteins. Many of these proteins are present in the folate acid pathway, the pathway where the target of methotrexate dihydrofolate reductase (DHFR) is located. This demonstrates the ability of IC-FPOP to study drug off target effects. The localization of IC-FPOP differences to the peptide level on the protein S-adenosyl-L-homocysteine hydrolase (AHCY) found biologically relevant differences. This work demonstrates that IC-FPOP can distinguish downstream structural changes that occur due to drug treatment.

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Adalet Memetimin[1,2,3], Ching Tarn[4], Peng-Zhi Mao[4], Zhenlin Chen[4], Yong Cao[1,2], Hao Chi[4], Si-Min He[4], Dr. Meng-qiu Dong[1,2]
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Improved identification of cross-linked peptide pairs by focused accurate inclusion mass screening (fAlMS)

Adalet Memetimin 582 - Improved identification of cross-linked peptide pairs by focused accurate inclusion mass screening (fAlMS), Meeting Room 106, August 20, 2024, 10:25 - 10:44

Cross-linking mass spectrometry (abbreviated as CXMS or XL-MS) is a powerful technique for mapping the interface between interacting proteins. It has enjoyed widespread use in structural analysis of protein complexes, and even inspired attempts to map protein-protein interactomes. CXMS/XL-MS relies on the successful identification of cross-linked peptide pairs, but their abundance is typically much lower than the linear, non-crosslinked peptides present in the same sample. As such, only a small fraction of MS2 spectra acquired in the data-dependent acquisition (DDA) mode is informative for the purpose of a cross-linking experiment. To address this problem, we carefully optimized AIMS (Accurate inclusion mass screening), a previously described targeted MS method, and named the modified version focused AIMS or fAIMS.

Methods

DSSO cross-linked E. coli whole-cell lysates were digested with trypsin and analyzed by LC-MS/MS on a Fusion Lumos mass spectrometer fitted with an EASY-nLC 3000 HPLC. Peptides were separated using the following reverse phase gradient: 2-6% buffer B over 4 min, 6-40% B over 156 min, 40-100% B over 10 min, holding at 100% B for 5 min, followed by a decrease to 10% B in 5 min. MS data were acquired in the DDA or fAIMS mode. MS parameters including AGC Target, resolution, isolation window, ion injection time, intensity threshold, and dynamic exclusion were optimized for fAIMS. The MS data were searched using pLink2.4.2 to identify cross-link peptides pairs.

Novel Aspect

fAIMS produces higher-quality MS2 spectra, increases the number of cross-link identifications, and reduces ambiguity in cross-link site localization.

Preliminary Data or Plenary Speaker Abstract

Compared to DDA, fAIMS significantly improved the quality of MS2 spectra, as indicated by enhanced fragment ion coverage, E-value, and Max tag length. For cross-links identified from the initial DDA run, only 43% of the precursor ions had MS2 spectra of high fragment-ion coverage, and fAIMS increased this percentage to 86%. For precursor ions whose DDA cross-link-spectrum match did not pass the 0.05 FDR cutoff, 75% of them gained identity, cross-link or not, from their fAIMS spectra. In all, fAIMS increased the number of cross-link identifications with high fragment-ion coverage by 62%. Also, fAIMS greatly proved precision in cross-link-site localization and helped maintain sensitivity of identification when faced with an expanded search space.

Professor Tak Wah Dominic Chan[1], Ms. Tin Yi Lui[1]
[1]Department of Chemistry, The Chinese University of Hong Kong

Millimeter Water-in-Oil Droplet as an Alternative Back Exchange Prevention Strategy for Hydrogen/Deuterium Exchange Mass Spectrometry of Peptide/Protein

T. W. Dominic Chan 203 - Millimeter Water-in-Oil Droplet as an Alternative Back Exchange Prevention Strategy for Hydrogen/Deuterium Exchange Mass Spectrometry of Peptide/Protein, Meeting Room 105, August 20, 2024, 10:44 - 11:03

Since the reliability of HDX-MS analysis considerably depends on the retention of deuterium labels in the post-labelling workflow, D/H back exchange prevention strategies, including decreasing pH, temperature, and exposure time to protic sources of the deuterated samples, are widely adopted. Herein, an alternative and effective back exchange prevention strategy based on the encapsulation of a millimeter droplet containing the deuterated peptide solution in a water-immiscible organic solvent ("water-in-oil droplet") is proposed. This method evolves from our accidental observation of the decelerated back exchange kinetics in millimeter droplet as compared to bulk solution. Based on this observation, we implemented the post-labelling workflows, specifically the enzymatic digestion step, of HDX-MS in water-in-oil droplet and successfully suppressed the undesirable back exchange.

Methods

Myoglobin (Mb) was chosen as the model entity in this study. H/D exchange of Mb was initiated by incubating Mb in 96% D2O. After a predefined H/D exchange duration, deuterated Mb was digested with pepsin in either bulk solution or millimeter water-in-oil droplet. For bulk digestion, the Mb/ pepsin mixture was kept in an Eppendorf tube for 2 mins in the presence of an ice-water bath. For in-droplet digestion, 0.5 µL of the Mb/ pepsin mixture was submerged in a cyclohexane reservoir for 2 mins. The Mb digest resulted from the two digestion approaches were analyzed respectively by an FTICR mass spectrometer equipped with a homebuilt liquid microjunction-surface sampling probe (LMJ-SSP) platform. Experiments were repeated using different deuteration time points.

Novel Aspect

For the first time, retarded HDX in millimeter droplet is demonstrated and applied as an unconventional back exchange suppression strategy.

Preliminary Data or Plenary Speaker Abstract

The deuterium level resulting from the two digestion approaches are compared, with a higher deuterium level indicating a lower back exchange extent occurred during the 2-min digestion. In-droplet digestion resulted in a higher deuterium level for all fragments, especially at the early deuteration time points. Taking the segment 70-106 as an illustration, the deuterium levels obtained from in-droplet digestion are 3.5 Da, 3.7 Da, 2.3 Da, 1.8 Da and 0.0 Da higher than that recorded using bulk digestion for 30s, 2min, 10min, 50min and 3h labelling time points respectively. Since deuterium labels uptake at the earlier time points normally have a faster back exchange kinetics, recording a higher deuterium level for earlier time points implied that the in-droplet digestion approach is more capable in providing instant protection. Once the deuterated sample solution is added to the cyclohexane reservoir, the deceleration of back exchange is in action and hence the fast-exchanging deuterium labels could be promptly preserved. In contrast, although the bulk digestion is conducted in the presence of an ice-water bath, heat conduction takes time and therefore delays the deceleration effect. At later labelling time points, the H/D exchange is approaching equilibrium and more intermediate-exchanging backbone amide hydrogens are constituting the plateau deuterium level. The similar deuterium level for the two approaches at the later time point indicated their comparable effectiveness in preserving the intermediate exchanging backbone amide hydrogens.

Our results proved that the water-in-oil droplet strategy exhibits a deuterium retention ability superior to the temperature reduction method, particularly for the fast-exchanging deuterium labels. The back exchange prevention effectiveness of water-in-oil droplet could be further enhanced if used in combination with temperature reduction. Preliminary results showed that conducting the digestion in a cooled water-in-oil droplet recovered a larger number of deuterium labels than using either method alone.

Prof. Joshua Sharp[1], Lyle Tobin[1], Haolin Liu[2], Dr. Sandeep K. Misra[1], Professor Lisa M. Jones[2]
[1]University Of Mississippi, [2]University of California San Diego

Radical Protein Footprinting in Stabilized Whole Blood

Joshua Sharp 28 - Radical Protein Footprinting in Stabilized Whole Blood, Meeting Room 105, August 20, 2024, 11:03 - 11:22

Radical protein footprinting is a powerful and flexible structural biology technique that has been used in living cells and even live nematodes. However, radical protein footprinting in mammalian tissues has not been achieved. Here, we describe the first example of radical protein footprinting in mammalian blood using sulfate radicals. We show the first oxidation of proteins by sodium persulfate using a FOX protein footprinting system, and the suitability of adenine dosimetry for measuring sulfate radical dose. We develop a rapid inline mixing system and improved sulfate radical quenching method to reduce background oxidation previously observed in sulfate radical footprinting both in vitro and in vivo. Finally, we show implementation of sulfate radical protein footprinting of stabilized whole mouse blood.

Methods

A programmable syringe pump with a 500 µL syringe for sample and a 50 µL syringe for 1M sodium persulfate was used for inline mixing. Syringes mixed sample and persulfate to a final concentration of 91 mM sodium persulfate. The microtee effluent line flowed sample through the FOX flash and dosimetry modules. Samples were flashed at 2Hz at flash voltages ranging from 0V to 900V. Samples were collected in a quench solution at a final concentration of 100 mM DMTU, 100 mM imidazole, 35 mM methionine. Blood samples were lysed, trypsinized and analyzed by LC-MS/MS for peptide-level RPF. RPF data were searched against UniProt Mus musculus proteome using FragPipe FPOP workflow. Peptide level oxidation was normalized and calculated using PowerPivot.

Novel Aspect

First radical protein footprinting in mammalian tissue; first sulfate protein footprinting using a commercial protein footprinting system

Preliminary Data or Plenary Speaker Abstract

One of the major hurdles in implementation of FPOP in blood is the high native catalase activity. We were unable to achieve high levels of catalase inhibition without altering the gross properties of the blood sample, preventing use of peroxide-based HRPF. A foam assay using 100 mM sodium persulfate showed no bubbles, suggesting persulfate is more stable in blood and may be a suitable radical precursor.
Previous work with sodium persulfate showed high levels of background oxidation, suggesting the standard HRPF quench protocol was insufficient for persulfate-based quenching. We developed a custom flow system for inline mixing at a 10:1 ratio of sample to persulfate to minimize exposure to persulfate and sample dilution. Imaging of dye mixing showed that we achieved homogenous mixing at a distance of 4 cm after the mixing tee; Fox photolysis occurs at 6 cm. We tested a variety of quenchers and found that imidazole at 100 mM in the standard HRPF quench prevented background oxidation.
We performed the first persulfate-based oxidation of a protein using a Fox Protein Footprinting system. Inline adenine dosimetry found oxidation response from 100 mM persulfate was substantially greater than that for 50 mM hydrogen peroxide at 900V. LC-MS/MS showed strong oxidation of test proteins in vitro.
We performed the first radical protein footprinting in mammalian whole blood. EDTA-stabilized blood fresh drawn from exsanguination of a nude mouse was used in our flow system, and mixed to a concentration of 91 mM sodium persulfate. Results showed oxidation of common blood proteins at a much higher level than in the 0V control, indicating successful radical footprinting. Work is currently

being done to measure the radical dose response in whole blood to different voltages, and to correlate protein oxidation with in vitro tertiary structure.

Mr. Kuang-Ting Kuo[1,2], Dr. Bilel Bdiri[3], Dr. Yuanjun He[3], Dr. Marcel Koenig[3], Dr. Theodore Kamenecka[3], Dr. Patrick Griffin[1,2,3]
[1]Department Of Molecular Medicine, The Scripps Research Institute, [2]Skaggs Graduate School of Chemical and Biological Sciences, The Scripps Research Institute, [3]Department of Molecular Medicine, The Herbert Wertheim UF Scripps Institute for Biomedical Innovation & Technology

Integration of Structural Proteomics and Computational Simulation for Elucidating Structural-Activity Relationships of Novel PPARγ Inverse Agonists in Metabolic Disease

Kuang-Ting Kuo 275 - Integration of Structural Proteomics and Computational Simulation for Elucidating Structural-Activity Relationships of Novel PPARγ Inverse Agonists in Metabolic Disease, Meeting Room 105, August 20, 2024, 11:22 - 11:41

The peroxisome proliferator-activated receptor gamma (PPARγ) is a target for treating metabolic disorders. Our previous research showcased SR10171, a partial PPARγ inverse agonist, acting as an insulin sensitizer in obese mice without inducing osteoporosis and bone marrow adiposity. Additionally, PPARγ inverse agonists exhibited effectiveness in impeding bladder cancer proliferation, indicating a prospective therapeutic avenue for metabolic diseases. Despite numerous studies highlighting PPARγ inverse agonism, the mechanism underlying non-covalent inverse agonism remains unclear. Here, we embark on structural and functional characterization of a series of novel SR10171 analogs. We delve deeper into their structure-activity relationship (SAR) and the underlying mechanisms of action in human adipocytes and bladder cancer cell lines, leveraging computational simulations and quantitative proteomics for comprehensive SAR.

Methods

The binding affinity of novel SR10171 analogs was assessed using a LanthaScreen binding assay. Pharmacological characteristics were investigated using AlphaScreen-based assays, cell-based promoter-reporter assays, and adipogenesis assays in 3T3-L1 cells. Based on these evaluations, compounds were categorized using principal component analysis (PCA). The conformational dynamics of PPARγ-LBD:compound complexes were explored utilizing both structural proteomics methods, including differential hydrogen-deuterium exchange mass spectrometry (HDX-MS) and crosslinking mass spectrometry (XL-MS) with the MS-cleavable crosslinker DSSO. HDX data were correlated with their biochemical properties to cluster compounds. Molecular docking and dynamic simulations were employed to further elucidate potential binding mechanisms. The impact on cellular protein expression resulting from treatment with high-priority inverse agonists was analyzed using tandem mass tag (TMT)-based quantitative proteomics.

Novel Aspect

Structural activity relationship of novel PPARγ inverse agonists and their underlying mechanisms of action in the context of metabolic disorders

Preliminary Data or Plenary Speaker Abstract

In collaboration with the Kamenecka lab at UF Scripps, we conducted a screening of novel SR10171 analogs and identified a set of compounds exhibiting enhanced binding potency relative to SR10171. Most analogs demonstrated PPARγ inverse agonism in biochemical assays, including enhanced interactions between PPARγ-LBD and corepressor peptides, as well as suppression of PPARγ transcriptional activity and adipogenesis in L1 cells. Based on their pharmacological properties, PCA analysis classified the novel compounds into three groups: non-inverse agonists (non-IA) with partial agonism or antagonism, partial-IA with partial inverse agonism, and full-IA with full inverse agonism, respectively. Differential HDX-MS analysis of PPARγ-LBD in complexes with novel compounds revealed ligand-dependent dynamics primarily within regions helix 2 (H2)-H3, H5-H7, and H10-H12. Pearson correlation analysis highlighted the relationship between stabilization of the ligand binding

pocket and destabilization of H12 with PPARγ repression. Mutagenesis of residues in regions correlated with PPARγ repression altered the pharmacology of partial-IA and/or full-IA on PPARγ activity, behaving as agonists in transcriptional activity assays. Additionally, quantitative differential XL-MS identified enriched crosslinks surrounding the ligand binding pocket, suggesting improved binding pocket stability. All these observations underscore the significance of these regions in reversible PPARγ inverse agonism. Furthermore, the top predicted binding modes through molecular docking using the inactive conformation of PPARγ-LBD (PDB: 6c5q and 8b8x) revealed potential binding modes of compounds to ligand pockets along with H3. Ongoing molecular dynamic simulations aim to provide further insights into distinguishing binding modes, guiding our optimization of first-in-class PPARγ inverse agonists. Notably, partial-IA demonstrated superior potential for insulin sensitization in L1-derived adipocytes. A subset of inverse agonists also exhibited significant repression of bladder cancer proliferation via cell cycle arrest and enhanced apoptosis. Currently, we are optimizing our TMT conditions to elucidate proteomic changes for our high-priority inverse agonists in diabetes and cancer.

Terese Eisgruber[1], Dr. Marie Niedermaier, Prof. Florian Stengel
[1]University Of Konstanz, Stengel Group

INVESTIGATING MODIFICATION-SPECIFIC INTERACTIONS OF LINKER HISTONE H1 BY MASS SPECTROMETRY-BASED PROTEOMICS

Terese Eisgruber 233 - Investigating Modification-Specific Interactions of Linker Histone H1 by Mass Spectrometry-based Proteomics, Meeting Room 105, August 20, 2024, 11:41 - 12:00

Histones, structurally organizing deoxyribonucleic acid (DNA) into chromatin, are subject to a diverse set of post-translational modifications (PTMs), which facilitate epigenetic regulation and are involved in many fundamental cellular processes. The variety of PTMs, the high number of possible modification sites, and their combinatorial co-occurrence generate a complex chemical language, the histone code. While core histone modifications have been extensively studied, the similarly intensely post-translationally modified linker histone H1 has received less attention. In this study, we build on our extensive experience in combining bioorthogonal chemistry, biochemistry, and MS-based proteomics to investigate the influence of specific H1 variants on protein-protein interactions and, thus, their role in the organization of the modular proteome.

Methods

Despite their importance for controlling the function and properties of eukaryotic proteins, studying the functional cellular impact of a specific PTM remains technically challenging. One major limitation in investigating the effect of individual PTMs on the biochemical and structural properties of a protein is the generation of specifically modified proteins. The efficiency of modifiers is often limited and not position-specific, resulting in heterogeneous populations of the protein of interest. Here, we employ an amber codon suppression method to introduce unnatural amino acids and generate site-specific modified variants of the linker histone H1.2. We apply affinity purification-MS (AP-MS) and cross-linking MS (XL-MS) to study the PTM-dependent interactome and structure of H1.2.

Novel Aspect

Investigating the role of PTM-specific protein-protein interactions of the linker histone H1.2 by MS-based proteomics.

Preliminary Data or Plenary Speaker Abstract

Building on recent progress 1,2, we generated acetylated and ubiquitinated variants of H1.2 at positions K17 and K64. We use these to further study PTM-specific interaction partners of H1.2 and to investigate the role of the modification-dependent interplay for H1.2. To this end, we extend our previous efforts to additional PTMs, in particular phosphorylation, to better understand the molecular consequences of a potential crosstalk between different PTMs. Using amber codon suppression, we introduce phosphoserine and its nonhydrolyzable analog into defined loci of linker histone H1.2. We show progress towards the generation of a set of phosphorylated H1.2 variants and their application to study the cellular interactome by MS-based proteomic approaches.

1 Eva Höllmüller, Simon Geigges, Marie L. Niedermeier, Kai-Michael Kammer, Simon M. Kienle, Daniel Rösner, Martin Scheffner, Andreas Marx, and Florian Stengel. Site-specific ubiquitylation acts as a regulator of linker histone H1. Nature communications, 12(1):3497, 2021.
2 Eva Höllmüller, Katharina Greiner, Simon M. Kienle, Martin Scheffner, Andreas Marx, and Florian Stengel. Interactome of Site-Specifically Acetylated Linker Histone H1. Journal of proteome research, 20(9):4443–4451, 2021.

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Prof Paul Pigram[1], Dr Wil Gardner, Mrs Sarah Bamford, Prof David Winkler, Dr Benjamin Muir, Mr Rongjie Sun, See Yoong Wong
[1]La Trobe University

Complexity to clarity: detecting, identifying and analysing complex materials with machine learning

Keynote: Professor Paul Pigram La Trobe University 692 - Complexity to clarity: detecting, identifying and analysing complex materials with machine learning, Meeting Room 106, August 20, 2024, 10:00 - 10:25

Our ability to analyze and understand any physical, chemical, or biological material relies on accurately determining its structure, characteristics, and responses. Contemporary analytical techniques produce large volumes of data from pointwise sample analyses (one dimensional (1D) data), maps of compositional distributions (two dimensional (2D) data), and depth profiles showing composition throughout a sample volume (three dimensional (3D) data).

Correlative analyses linking data from the same sample, obtained by different analytical techniques or different operating parameters, are becoming critically important. Different analytical perspectives on the same sample enhance the richness and depth of the conclusions that can be drawn from it.

Methods

ToF-SIMS
Machine learning
Artificial neural networks

Novel Aspect

New approaches to applying machine learning to understand hyperspectral mass spectral datasets in one, two and three dimensions

Preliminary Data or Plenary Speaker Abstract

Recent advances in analytical science have resulted in an overwhelming avalanche of data – the “big data” problem. In our lab a single time-of-flight secondary ion mass spectrometry (ToF-SIMS) experiment might collect a map (512 x 512 pixels) with 2000 mass spectral peaks of significant intensity in 2 – 10 minutes. These half a billion data points all have differing degrees of significance.

In many cases, only a small number of peaks, 10 – 200, may be judged to be characteristic of a specific sample, and the rest of the data may be discarded. However, there are significant risks that such analyses are biased, and may miss important but subtle trends.

There is a very substantial knowledge gap in our ability to find and make full use of the information and knowledge contained in large scale data sets. This gap is driving rapid international progress in the application of materials informatics and machine learning to analytical surface science.

This presentation will highlight our work on applying artificial neural networks, spatial resolution enhancement and related approaches to analysis of a variety of very large hyperspectral data sets to better understand complex materials and their interactions.

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Dr Alexander Aksenov[1], Elizabeth Coler, Alexey Melnik[4], Dr. Alexander Semenov[2], Dr. Vladimir Boginski[3]

Illuminating the Dark Matter of Metabolomics Through Molecular Community Networking

Alexander Aksenov 55 - Illuminating the Dark Matter of Metabolomics Through Molecular Community Networking, Meeting Room 106, August 20, 2024, 10:25 - 10:44

Molecular networking connects structurally similar metabolites by leveraging MS/MS fragmentation pattern similarities. This approach has enabled a slew of discoveries over the past decade. However, conventional methods rely on arbitrary global spectral similarity thresholds, despite optimal connectivity being molecule class-specific. We present molecular community networking (MCN), an advanced approach that utilizes unpruned full connectivity metabolite networks parsed using network science tools to identify naturally present "molecular communities." This strategy preserves intra-community connectivity information and optimizes connectivity patterns for each metabolite class, enabling the rescue of lost relationships and the capture of otherwise "hidden" portions of the metabolome.

Methods

Full connectivity metabolite networks were constructed using LC-MS/MS or EI GC-MS data. The Louvain clustering algorithm was employed to identify naturally occurring "molecular communities" within the unpruned networks. Each community was treated as a separate network and pruned using the maximum weight spanning tree algorithm to preserve connectivity while retaining only the most meaningful information. The resulting MCNs represent partitions of the original networks into continua of molecular space, where connections within each molecular family cluster represent the most similar pairs of metabolites across the entire detected metabolome.

Novel Aspect

Machine learning-guided optimization of molecular networking preserves intra-community connectivity, enabling enhanced metabolite discovery and “dark metabolome” mapping.

Preliminary Data or Plenary Speaker Abstract

We validated MCNs using reference spectra and experimental data, demonstrating their ability to assemble molecular space into continua reflecting structural relationships. MCNs rescue lost connectivity between related molecules fractured by conventional networking, for example, link sodiated ion variants to corresponding protonated precursors. We showcase MCN's utility in discovering new bile acid structures with dipeptide conjugation produced by human microbial cultures, revealing the metabolic capacity of the human microbiota. These molecules were previously undetectable with conventional networks. MCNs exhibit high modularity, suggesting a natural tendency for molecules to group into communities resembling "small-world" structures found in online social networks. This approach empowers molecular discovery in areas such as natural products research, including the reanalysis of existing data to explore previously unconnected molecules.

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Mengbo Li[1]
[1]WEHI

Missingness-informed protein quantification and differential expression analysis

Mengbo Li 27 - Missingness-informed protein quantification and differential expression analysis, Meeting Room 106, August 20, 2024, 10:44 - 11:03

Mass spectrometry (MS) based proteomics is a powerful tool in biomedical research, but its usefulness is limited by the frequent occurrence of missing values. We argue that missing values should always be viewed as missing not at random (MNAR) in MS-based proteomics data, because the probability of detection is related to the underlying intensity.

Methods

We propose a statistical model for non-ignorable missing values in proteomics data, termed the detection probability curve (DPC). Importantly, DPC provides a probabilistic model for missing values and can be used to inform the downstream differential expression analysis. To this end, we introduce the DPC-quantification model, where missing values are taken into account when summarizing peptides into proteins. For each protein group, we use DPC to represent missing values. An additive linear model is fitted to estimate the protein-level intensity in each sample by maximizing the posterior distribution with empirical priors. Uncertainty in protein-level estimations is incorporated into differential expression testing via a customized limma analysis.

Novel Aspect

Proposed methods enable us to learn from the missing value patterns in data to inform protein quantification and downstream analyses.

Preliminary Data or Plenary Speaker Abstract

The proposed methods are tested and evaluated on real data where we show that the DPC-quantification model eliminates missing values in protein-level data and improves the statistical power for differential expression in proteome-wide experiments while maintaining correct control of the false discovery rate. All proposed methods are implemented as an open source R package which is available on Bioconductor.

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Dr Yuji Sekiguchi[1], Dr Kanae Teramoto[2], Dr Dieter Tourlousse[1], Ms Akiko Ohashi[1], Ms Mayu Hamajima1[1], Dr Daisuke Miura[1], Dr Yoshihiro Yamada[3], Dr Shinichi Iwamoto[3], Koichi Tanaka[3]
[1]Biomedical Research Institute, National Institute of Advanced Industrial Science And Technology (AIST), [2]MS Business Unit, Shimadzu Corporation, [3]MassSpectrometry Research Laboratory, Shimadzu Corporation

Genomically predicted protein mass database (GPMsDB) for rapid and broad-spectrum identification of bacterial and archaeal isolates by mass spectrometry

Yuji Sekiguchi 290- Genomically predicted protein mass database (GPMsDB) for rapid and broadspectrum identification of bacterial and archaeal isolates by mass spectrometry, Meeting Room 106, August 20, 2024, 11:03 - 11:22

The matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) method for microbial identification is already well established and used on a routine basis in clinical microbial diagnostics. However, its application for microbial identification in other microbial research fields, such as environmental and human microbiomes, is still limited. Because the MALDI-TOF MS method relies on reference spectral libraries of cultured isolates, it often fails when applied to species not represented in such libraries, including previously uncultured microorganisms. We present a new approach and toolkit for broad-spectrum microbial identification by MALDI-TOF MS. This is enabled by a large-scale database of protein masses predicted from nearly 200,000 publicly available bacterial and archaeal genomes.

Methods

We created a large-scale database of protein masses predicted from nearly >200,000 publicly available bacterial and archaeal genomes. After screening for potentially lower-quality genome sequences, a total of 193,197 genomes from 190,160 Bacteria and 3,037 Archaea were retained. Protein sequences were predicted and theoretical molecular masses were incorporated in the database. The database, referred to as genomically predicted theoretical protein mass database for mass spectrometry (GPMsDB), contains ~163 million protein mass entries, with an average of 845 entries per genome. These serve as theoretical mass peak lists for matching experimentally measured peak lists by MALDI-TOF MS for microbial identification.

Novel Aspect

Our database substantially expands MALDI-TOF MS utility for microbial testing and also the screening of microbes in any microbial fields.

Preliminary Data or Plenary Speaker Abstract

Using a diverse set of 84 bacterial and 10 archaeal strains that captured 15 different phyla within the genome-taxonomy database (GTDB), we verified the ability to classify microorganisms at the species level and below, achieving correct identification for more than 90% of measured spectra. We further demonstrate the utility of our toolkit through the identification of uncultured strains from mice faeces using the power of combining our toolkit with shotgun metagenomics; this allows the identification of new strains through customising the database with metagenome-assembled genomes. We anticipate that our toolkit will substantially accelerate the screening of the thousands of isolates currently being generated in microbiome studies and can further support MALDI-TOF MS for environmental monitoring, clinical diagnostics, and biosafety testing.

Msc Piotr Radziński[1], MSc Jakub Skrajny[1], prof. Anna Gambin[1]
[1]University Of Warsaw

Contrastive learning encoding algorithm of MS images for memory management and segmentation enhancement

Piotr Radziński 245 - Contrastive learning encoding algorithm of MS images for memory management and segmentation enhancement, Meeting Room 106, August 20, 2024, 11:22 - 11:41

Due to its high-resolution nature, mass spectrometry imaging data often requires significant memory resources, presenting challenges for efficiently applying analytical tools like segmentation algorithms. This computational burden hinders the nuanced analysis necessary for distinguishing tissue states. Our work introduces a novel encoding algorithm that enormously reduces MSI data size, alleviating memory constraints and facilitating the application of advanced segmentation techniques. By optimizing data preprocessing through contrastive learning, our approach not only streamlines analysis but also improves segmentation accuracy, offering a valuable tool for precision medicine research within the mass spectrometry community.

Methods

We applied contrastive learning encoder-decoder architecture and strategically employed a set of loss functions to optimize the compression and preparation of MSI data for future analysis of tissues' structure. Contrastive loss is pivotal in embedding generation, distinguishing between similar and dissimilar data points. Additional loss functions are instrumental in normalizing the embeddings' distribution, which is essential for consistent and reliable segmentation. The integrity of compressed data is further ensured by a loss function that assesses reconstruction accuracy, confirming that essential information is preserved. Collectively, these loss functions enhance data quality, facilitating improved segmentation by ensuring the data's structure and clarity are optimal for precise identification of tissue states.

Novel Aspect

Introducing a contrastive learning-based encoding algorithm for efficient MSI data preprocessing, enhancing analysis while reducing computational load.

Preliminary Data or Plenary Speaker Abstract

Our study introduces a preprocessing encoding algorithm tailored for mass spectrometry imaging data to enhance segmentation accuracy. This algorithm compresses the extensive datasets into easily manageable sizes while meticulously preserving critical biochemical information. A unique aspect of our method is the compression of each pixel (i.e. mass spectrum) into a concise vector, with the size being parameter-controlled; in our application, a dimensionality of just 16 was sufficient. Preliminary testing across various MS images and subsequent application of several segmentation algorithms consistently revealed improved segmentation accuracy when utilizing our encoder. The enhancements in segmentation accuracy range from 4% to 34.3%, demonstrating the algorithm's effectiveness in maintaining vital molecular details for precise analysis. These results underscore our approach's significant impact on data manageability and the precision of tissue state identification, marking a substantial advancement in the field of MSI analysis and its application in precision medicine.

Dr Toan Phung[1,2], Dr. Kerryn Berndsen[1,2], Dr. Rosamund Shastry[1,2], Dr. Tran Phan[1], Prof. Miratul Muqit[1,2], Prof. Dario Alessi[1,2], Dr. Raja Nirujogi[1,2]
[1]Medical Research Council (MRC) Protein Phosphorylation and Ubiquitylation Unit, School of Life Sciences, University of Dundee, [2]Aligning Science Across Parkinson's (ASAP) Collaborative Research Network

CHRONICLE, A Family of Tools and Softwares for Mass Spectrometry-based Proteomics Data Processing, Analysis, Visualization, and Exploration.

Toan Phung 349 - CHRONICLE, A Family of Tools and Softwares for Mass Spectrometry-based Proteomics Data Processing, Analysis, Visualization, and Exploration, Meeting Room 106, August 20, 2024, 11:41 - 12:00

With the advent of ultrasensitive mass spectrometry-based proteomics, the generation of large volumes of data has become inevitable. This poses significant challenges for institutional level infrastructure and individuals alike, who require dedicated computing hardware as well as an automated software pipeline to effectively analyze and reanalyze this data. Importantly to align with data FAIR (Findability, Accessibility, Interoperability, and Reusability (FAIR) principles. To address this issue, we developed CHRONICLE, a family of related free and open-source software and informatics tools that can help pre-emptively solve these problems of modern proteomics.

Methods

Within CHRONICLE, we provided a selection of core tools. Based on Python and JavaScript, CATAPULT is an automated raw MS data search workflow management tool that allows the user to preconfigure DIA-NN search algorithm parameters to queue them to run as soon as the storage has received output data from the mass spectrometer. To process the search output and more, we created CAULDRON, a frontend for running premade Python and R scripts for data analysis. Finally, for data sharing and visualization, we have CURTAIN and CURTAIN-PTM that can be used to create highly customizable interactive visualizations for MS-based differential analysis results. Visualization sessions can be shared as unique web links and QR codes to anyone in presentation, communication, and publication.

Novel Aspect

CHRONICLE provides a streamline workflow for maximizing efficiency in MS-based proteomics data processing, analysis, visualization and exploration.

Preliminary Data or Plenary Speaker Abstract

A typical MS experiment data flow initially from the Mass Spectrometer to the instrument controller computer. Then, the data is moved to a shared storage appliance. Following, data analysis is triggered with software like DIA-NN, Spectronaut, MSFragger, and MaxQuant. For the data analysis, each of these steps requires manual intervention from the MS expert to progress, thus each delay can become a major efficiency issue as MS output files grow.
To tackle this issue, CHRONICLE offers Catapult, a tool that provides a path for seamless flow of data without manual intervention beyond the initial planning process for each individual experiment. Catapult is designed to watch for new files in predefined locations within the filesystem and automatically process the experiment based on predefined run configurations. The processed data can then be analyzed using conventional workflows appropriate for each search output format or with CAULDRON, a statistical data analysis tool that enables differential analysis using limma and QFeatures and dimensional reduction visualization such as PCA or PHATE.
Moreover, CHRONICLE provides compatible file formats for CURTAIN and CURTAIN-PTM for data sharing and visualization. These tools enable the creation of highly customizable interactive visualizations of MS-based differential analysis results. The visualization sessions can be shared as unique web links and QR codes, making it easy for researchers to communicate their findings with others in presentations, publications, and other communication channels.

Overall, CHRONICLE provides a comprehensive solution for efficient and automated proteomics data flow, analysis, and sharing. By offering a seamless data flow process, automated data analysis, and customizable data visualization and sharing options, CHRONICLE can help lowering the barrier for proteomics data analysis and exploration for researchers in and outside of the mass spectrometry field in the landscape of big data proteomics.

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Dr. Stefan Stefan Kooij[1], Dr Aleksandra Chojnacka[1], Professor Cees van Rijn[1], Professor Daniel Bonn[1], **Professor Garry Corthals**[1]
[1]University Of Amsterdam

Electroless ionisation mass spectrometry (ELI-MS): a simple, speedy and soft ionisation method

Keynote: Professor Garry Corthals University of Amsterdam 572 - Electroless ionisation mass spectrometry (ELI-MS): a simple, speedy and soft ionisation method, Meeting Room 109, August 20, 2024, 10:00 - 10:25

Over the last 20 years, numerous techniques have appeared in the field of ambient mass spectrometry, where the focus has been on sampling and ion generation. At the same time, tremendous advances have been made with MS analysers, fragmentation, and scanning modes. With such new MS capabilities and new robust ambient ionisations sources available, an exciting time awaits us.

In this work we introduce another new device termed ELI for electroless spray ionisation. ELI is a self-ionising spray nozzle at the top of a syringe. The spray nozzle, which consists of a micro-fabricated nozzle chip, uses the interaction of the sample liquid with the nozzle interior for charging, and therefore requires no electronics to function.

Methods

The micro-fabricated spray nozzle, comprises a silicon chip with multiple 1.9µm diameter holes, in a 0.5µm membrane layer of silicon nitride (Fig. 1 b-e). The chip is mounted in a polypropylene adapter that fits on a luer lock syringe (Fig. 1 b). By applying sufficient force to the syringe, a spray is formed that, due to the electric charge acquired in the nozzle, expands and quickly evaporates over a distance of a few centimeters, to form a cloud of ionised particles.

A typical hand-held ELI experiment consists of: filling the syringe with the sample, attaching the ELI spray nozzle, and spraying in the direction of the MS-inlet. For LC-MS use the ELI-MS chip can be placed directly at the LC-column exit.

Preliminary Data or Plenary Speaker Abstract

We will demonstrate the ELI-MS ionisation process and the simplicity of setup and operation. We will provide details on the parameters that affect charging efficiency, such as fluid type, injection rate, and salt concentration. Furthermore, we will also review charging dependability on the interaction of the solvent with the nozzle interior, as previously reported (Kooij et al., 2023).

Additionally, we discuss the comparison of ELI to existing ionisation techniques such as ESI and other ambient methods such as SAWN and paperspray. Several examples will be given for various types of sample analysis. Importantly, we will highlight how the method can be used for quantitative analysis by demonstrating the linearity of the response. Additionally, we will discuss and demonstrate that by altering the surface charge of the interior of the nozzle, the polarity of the droplet charge can be reversed, an essential step in making this method broadly applicable.

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Dr Léa Ledoux[1], Yanis Zirem[1], Pr Michel Salzet[1], Professor Isabelle Fournier[1]
[1]Prism Laboratory

Bacterioscore in 2D/3D MS imaging: pioneering in vivo cancer microbiome study and its link with diagnosis and patient survival

Léa Ledoux 355 - Bacterioscore in 2D/3D MS imaging: pioneering in vivo cancer microbiome study and its link with diagnosis and patient survival, Meeting Room 109, August 20, 2024, 10:25 - 10:44

The human microbiota is made up of a large number of bacteria. However, it was recently demonstrated that there is a causal link between the presence of bacteria and the tumor behavior as disturbances in the microbiota can potentially be associated with the development of cancer. Studies have identified differences in gastric microbiota composition between gastric cancer and control patients but there is no consensus of specific microbial-taxa involved in gastric cancer pathogenesis. Now the gastric microbiota is examined in mixed cohorts without considering the different cancer subtypes. That's why, SpiderMass, an ambient mass spectrometry based-approach, was used to calculate the relative presence of bacterial strains within tissues (healthy and cancerous) to provide a comprehensive assessment of the bacterial landscape

Methods

Six esogastric (Escherichia Coli, Lactococcus lactis, Lactobacillus gasseri, Streptococcus bovis, Streptococcus anginosus and Streptococcus salivarius) and three breast cancer bacterial strains (Streptococcus infantis, Staphylococcus lugdunensis, Methylobacterium radiotolerans) were cultured and analyzed by SpiderMass, directly on agar plates. Based on their lipidomic fingerprint, two bacterioscoring ML model were trained. These models leveraged cell spectra within m/z range 600 to 1100. To predict the bacteria strain on 2D/3D SpiderMass images (healthy or cancerous tissue), the scores was used to provide probability estimates for each bacterial strain. Furthermore, ratio scores were computed to estimate the relative presence of each bacterial strain across the entire image. The ratios offered a comprehensive assessment of the bacterial landscape in the analyzed sample.

Novel Aspect

Potential of microbiota analysis as a predictive and prognosis marker for cancer patients, offering insights for personalized treatment strategies.

Preliminary Data or Plenary Speaker Abstract

We explored the possibility of developing a bacterioscore based on data generated by SpiderMass, with a view to future in vivo diagnosis and prognosis. The bacterioscoring models were trained, for which the correct classification rate of 99% were obtained after cross-validation. Moreover, from a SpiderMass image, we were able to get the predicted distribution of each bacterial strain across the tissue. First, 12 esogastric tissues were analyzed in negative ion mode (healthy or cancerous tissues). Thus, we were able to see that the predicted ratio of 3 bacterial strain (E. Coli, S. anginosus and S. salivarius) was not significantly different according to the cancerous or healthy character of the tissue. However, L. gasseri strain were predicted to have a higher abundance in healthy tissue in contrary to L. lactis and S. bovis strain that showed a higher abundance in cancerous tissue. The ratio S. bovis to L. gasseri could serve as a diagnosis marker for esogastric tissue. In fact, when the ratio is below 1, it signifies a healthy tissue or if the ratio exceeds 1 is indicative of a cancerous tissue. It was also possible to link the bacterioscore distribution to the patient overall survival. In fact, L. gasseri was predicted to have a higher abundance in tissue from patient who are still alive than in patient who died. More important, S. bovis and L. lactis strains seems to increase when the patient undergoes a recurrence of cancer. For the breast cancer bacterial strains, S. infantis was found highly expressed in tumoral regions in contrary to M. radiotolerans and S. lugdunensis that were found more present in peritumoral tissues from transgenic mice. These findings provide direct evidence of the possibility to use microbiota analysis as a predictive marker for cancer patients.

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Mass spectrometry imaging of a single HeLa cell by tapping-mode scanning probe electrospray ionization

Yoichi Otsuka 34 - Mass spectrometry imaging of a single HeLa cell by tapping-mode scanning probe electrospray ionization, Meeting Room 109, August 20, 2024, 10:44 - 11:03

The systematic analysis of various molecular constituents in a cell is crucial for both comprehending fundamental biological concepts and realizing practical applications in the life sciences. Mass spectrometry imaging (MSI) allows for the visual representation of the distribution of various components in biological tissues. We have been developing a tapping-mode scanning probe electrospray ionization (t-SPESI) technique. In t-SPESI, a capillary probe with a microscale aperture delivers a minute amount of solvent to the sample surface while oscillating vertically. This facilitates the rapid extraction-ionization under ambient conditions. We present the implementation of a new t-SPESI unit aimed at improving the spatial resolution of imaging. The visualization of lipids inside HeLa cells and the discrimination of different cell types are discussed.

Methods

For MSI of single-cell, precise positioning of both the cell and the probe's tip is crucial. To achieve this, we have designed and developed a t-SPESI unit that can be integrated into an inverted fluorescence microscope. The new system uses feedback control of the height of the t-SPESI unit to maintain the amplitude constant. To prepare the specimens, HeLa cells grown on glass substrates were labeled with fluorescent dyes, fixed with glutaraldehyde, and rinsed with ammonium acetate. Fused silica capillary probes with 2-3 um opening apertures were used. DMF/MeOH 1/1 v/v, 0.1% formic acid was used as the solvent. Xevo G2-XS QTOF mass spectrometer was used.

Novel Aspect

An extraction-ionization method using femtoliter solvents was developed for high spatial resolution mass spectrometry imaging of HeLa cells.

Preliminary Data or Plenary Speaker Abstract

The new t-SPESI system affords us the capability of observing individual cells as both bright-field and fluorescent images and verifying the liquid bridges that are formed at the probe's tip during the measurement. MSI of HeLa mCAT cells (mock cells) and HeLa cGCS cells with high expression of glycolipid synthase was performed at a pixel pitch of 2 micrometers. We tested whether the lipid distribution information acquired by single-cell MSI is useful for cell type identification. 29 lipid ions were selected by matching peaks assigned by t-SPESI with lipids identified by supercritical chromatography tandem mass spectrometry (SFC-MS/MS). Average signal intensities of 45 cells were obtained for each of the mCAT and cGCS cells. From the Volcano plots, cells were distinguished based on the intensity information of multiple lipid ions. The principal component analysis score plots showed that most of the cells could be classified into two independent groups.
To compare the differences in the distribution of lipids in the cells, we compared the fluorescence and ion images. In the ion image of PC 34:2 [M+H]+, the signal intensity was lower in the nucleus of the cells and increased in a localized region lateral to the nucleus. Such localization was also observed in other PC, PE, and PS. On the other hand, ion images of SM d18:1/22:0 [M+H]+ did not show the similar localization as PC, and the signal intensity was distributed over a wider area than PC, suggesting that SM is distributed throughout the cell membrane and that the signal intensity of SM varies with the cell.

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Professor Jae-Chul Pyun[1]
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Laser desorption/ionization (LDI) mass spectrometry based on nanomaterials for biomedical applications

Jae-Chul Pyun 123 - Laser desorption/ionization (LDI) mass spectrometry based on nanomaterials for biomedical applications, Meeting Room 109, August 20, 2024, 11:03 - 11:22

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-ToF MS) has been widely used for the analysis of biomolecules. However, the quantitative measurement of small molecules has been limited because of the fragmented organic matrix molecules in m/z range of <500 and “hot-spots” formation from inhomogeneous analyte crystals. Recently, inorganic materials begin to replace the conventional organic matrices and this method has been called laser desorption/ionization mass spectrometry (LDI-MS). So far, many inorganic matrices have been effectively used for the quantitative analysis of small molecules, which are classified into (a) carbon-based and (b) semiconductor-based nanomaterials and (c) metal nanoparticles. In this work, several matrices for LDI-MS will be presented and their biomedical applications will be demonstrated.

Methods

Several kinds of solid-matrices for the detection of small biomolecules will be presented: (1) Top-down synthesized TiO2 nanowires were synthesized as arrays using a modified hydrothermal process directly on the surface of a Ti plate; (2) TiO2 nanowires combined with porous and non-porous gold nanoislands were synthesized to enhance the ionization efficiency of analytes through the formation of shottky barrier; (3) The parylene-matrix chip was fabricated by the deposition of nano-porous parylene-N thin film on a dried organic matrix array. Using these solid-matrices the following biomedical application of LDI mass spectrometry was demonstrated for (a) quantification of immunosuppressor, (b) screening of super bacteria a nd (c) search of new sepsis biomarkers.

Novel Aspect

Solid-matrices for the quantitative small biomolecules will be presented which can avoid the fragmented organic matrix molecules and “hot-spots” formation.

Preliminary Data or Plenary Speaker Abstract

As the preliminary work of this presentation, please see the following papers:

(1) Top-down synthesized TiO2 nanowires
- Mira Kim, et. al, Peptide sequencing with MALDI-TOF mass spectrometry based on TiO2 nanowires from wet-corrosion synthesis, ACS Applied Materials and Interfaces 10 (2018) 33790-33802.
- Moon-Ju Kim, et. al, Nanostructured TiO2 materials for the analysis of crystals from gout and pseudo-gout patients using laser desorption/ionization time-of-flight (LDI-ToF) mass spectrometry, Analytical Chemistry 91 (2019) 11283-11290.
- Moon-Ju Kim, et. al, TiO2 Nanowire Photocatalyst for Dual-Ion Production in Laser Desorption/Ionization (LDI) Mass Spectrometry, Chem. Commun 56 (2020) 4420-4423.

(2) TiO2 nanowires combined with porous non-porous gold nanoislands
- Moon-Ju Kim, et. al, Laser-Induced Surface Restructuring/Melting of Nanoporous Au-Modified-TiO2 Nanowires and In-Situ Enhancement in Desorption and Ionization, Advanced Functional Nanomaterials 31 (2021) 2102475.
- Moon-Ju Kim, et. al, Photothermal Structural Dynamics of Au Nanofurnace for In-Situ Enhancement in Desorption and Ionization, Small 17 (2021) 2103745.
- Moon-Ju Kim, et. al, Laser-Shock-Driven In Situ Evolution of Atomic Defect and Piezoelectricity in Graphitic Carbon Nitride for the Ionization in Mass Spectrometry, ACS Nano 16 (2022) 18284-18297.

(3) The parylene-matrix chip

- Jong-Min Park, et. al, Rapid and sensitive antibiotic susceptibility test of E. coli using parylene-matrix chip, Biosensors and Bioelectronics 71 (2015) 306-312.
- Jong-Min Park, et. al, Medical diagnosis of sepsis by using a parylene-matrix chip, Analytical Chemistry 91 (2019) 14719-14727.
- Jong-Min Park, et. al, Quantitative and simultaneous analysis of multiple cancer biomarkers with parylene-matrix chip, Journal of the American Society of Mass Spectrometry 31 (2020) 917-926.

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Mass spectrometry profiling is a useful method for analyzing autopsy samples, taking into account their unique preservation requirements

Stanislav Pekov 525 - Mass spectrometry profiling is a useful method for analyzing autopsy samples, taking into account their unique preservation requirements, Meeting Room 109, August 20, 2024, 11:22 - 11:41

In the realm of developing tools for analytical and technical support during surgical procedures, the primary focus currently lies in creating efficient and precise techniques for detecting tumor tissues, particularly in scenarios with minimal tumor cell presence. A potential resolution to this issue involves employing molecular profiling through mass spectrometry (MS) methodologies. Classification algorithms are commonly utilized in these methods to pinpoint tissues by referencing a library. Nevertheless, these libraries frequently lack data on substandard materials due to constraints in utilizing medical samples. The objective of this research is to showcase the viability of utilizing autopsy materials to compile datasets for constructing such reference libraries.

Methods

Molecular profiles were obtained using a custom-designed ion source that relied on an online-cartridge liquid extraction and electrospray ionization technique. The subsequent analysis was conducted on a Thermo LTQ Orbitrap XL system, which allowed for the creation of a comprehensive library of data suitable for both rapid and detailed analysis. It is worth noting that the degradation process of biomaterials could potentially introduce variability into the data. In order to simulate this degradation process, an experiment was conducted on animal cadavers. Specifically, mouse tissue samples were incubated under room conditions for varying durations ranging from one to twenty-four hours. Following the designated incubation period, samples of liver, brain, and muscular tissues were collected and subjected to molecular profiling.

Novel Aspect

The feasibility of molecular profiling is demonstrated by employing electrospray ionization on a substance, taking into account its biodegradation potential.

Preliminary Data or Plenary Speaker Abstract

Based on the initial findings of the examination, it is feasible to suggest that the utilization of MS profiling in the analysis of animal cadaver tissue samples provides abundant information for tissue identification and characterization. A statistical inquiry was carried out on a database containing over 200 profiles, employing specialized software for correlation analysis. The correlation analysis of the molecular profiles within the database revealed an expected decline in correlation as the storage time of the material at room temperature increased. Interestingly, the identification of tissue type remains achievable regardless of the degree of degradation. Moreover, the data acquired from the correlation analysis not only allows for tissue type identification but also facilitates the determination of the extent of biomaterial degradation.

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A Coated Blade Spray Mass Spectrometry Workflow for Rapid Toxicology General Unknown Screening

David Borts 91 - A Coated Blade Spray Mass Spectrometry Workflow for Rapid Toxicology General Unknown Screening, Meeting Room 109, August 20, 2024, 11:41 - 12:00

Toxicology general unknown screening (GUS) workflows are critically important for human and animal health. Traditional GUS workflows typically involve targeted lists of compounds. However, targeted approaches always suffer from incompleteness and the inability to detect new or emerging compounds. Traditional workflows also make use of generic sample preparation procedures and long chromatographic run times, both of which can be very time-consuming. Therefore, there is a need for more rapid and comprehensive toxicology GUS workflows. In this work, we present a new workflow for rapid toxicology general unknown screening based around solid phase microextraction and coated blade spray mass spectrometry. This workflow correctly identified greater than 90% of test compounds with a total workflow time of approximately 2 hours.

Methods

A test set of 32 representative small molecule compounds was spiked into blank control bovine plasma at a concentration of 1 µg/mL. Raw spiked plasma was mixed with buffer and extracted directly by solid phase microextraction using coated blades from Restek Corporation. These blades were either directly analyzed by coated blade spray mass spectrometry (CBS-MS) using a prototype CBS interface from Restek Corporation or the blades were desorbed into solvent and analyzed by LC-MS. Both variations of the workflow used a Thermo Fisher Scientific Exploris 120 quadrupole-orbitrap mass spectrometer operated in Data Dependent Acquisition (DDA) mode. Product ion spectra were searched against high resolution accurate mass (HRAM) product ion spectral databases and over 90% of test compounds were correctly identified.

Novel Aspect

First report of a solid phase microextraction coated blade spray mass spectrometry workflow for rapid toxicology general unknown screening.

Preliminary Data or Plenary Speaker Abstract

A test set of 32 compounds was used to evaluate this new, rapid toxicology GUS workflow. Test compounds included human drugs, veterinary drugs, pesticides, and natural toxins. These compounds were representative of the small molecule structural diversity typically encountered in toxicology GUS. The compound test set was based upon a Food Emergency Response Network (FERN) SOP describing an LC-MS workflow for poison and toxin screening. A total of 29 out of 32 compounds (91%) were correctly identified with direct CBS-MS analysis when the DDA-generated product ion spectra were searched against the Thermo Fisher Scientific mzCloud database.

However, one challenge encountered was related to automated searching of the mzCloud database using Thermo Fisher Scientific Compound Discoverer software. Automated searching did not always accurately identify compounds present, even when high-quality product ion spectra were generated. This limitation is thought to be related to the Compound Discoverer software's bias towards chromatographic peaks versus infusion or chronogram data.

To overcome this limitation, coated blade extraction (CBE) with rapid LC-MS analysis was explored. Despite our concerns about the effects of analyte dilution with this approach, greater than 90% of compounds present were again correctly identified. CBS extraction efficiency and CBS-MS analyte consumption were investigated to better understand and explain the high success rate with the CBE + LC-MS approach.

These new CBS-MS and CBE + LC-MS toxicology GUS approaches both accurately identified over 90% of test compounds with a total workflow time of approximately 2 hours. This compound identification rate compares very favorably to current state-of-the-art LC-MS toxicology GUS workflows. And the 2 hour turnaround time is a notable improvement over current toxicology GUS workflow times, which can easily be 24 hours or more. A more rapid workflow for toxicology GUS is extremely valuable for situations in which human or animal health are at stake.

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Professor Kevin Thomas[1]
[1]University of Queensland

Advancements and Challenges in Mass Spectrometric Techniques for Comprehensive Environmental Analysis

Keynote: Professor Kevin Thomas University of Queensland 763 - Advancements and Challenges in Mass Spectrometric Techniques for Comprehensive Environmental Analysis, Meeting Room 110, August 20, 2024, 10:00 - 10:25

Chemicals are integral to nearly every facet of our lives. Our reliance on synthetic chemicals has led to the creation of over 200 million unique molecules, contributing to an ever-expanding anthropogenic chemical space. Despite significant progress in developing chemicals for a wide range of applications, our ability to identify them once released into the environment largely depends on the development of targeted and non-targeted hyphenated mass spectrometric techniques.

Methods

This keynote presentation explores two widely applied techniques in contemporary environmental analysis: Pyrolysis coupled to Gas Chromatography Mass Spectrometry (Py-GC/MS) for targeted analysis of environmental polymers, and Liquid Chromatography High Resolution Mass Spectrometry (LC-HRMS) for untargeted analysis of known unknowns (suspect screening) and unknown unknowns (non-target analysis) of contaminants. Py-GC/MS analysis of plastic mixtures in environmental samples is a relatively new technique that has been rapidly adopted and applied worldwide. As an indirect technique, where polymer concentrations are quantified from the thermal degradation products, Py-GC/MS is subject to potential interferences from other materials and chemicals that may form the same products following pyrolysis. Therefore, it is crucial to understand all the uncertainties that may influence the reliable identification and quantification of specific plastics. HRMS is currently the most comprehensive approach for screening and discovering organic compounds or Contaminants of Emerging Concern (CECs) in environmental samples. However, clear challenges persist around standardisation, the extent of the anthropogenic chemical space that can be identified, and how data are reported. While these are clearly very different stories, there are also a number of similarities in how we, as a community, apply mass spectrometric techniques to reliably and effectively characterize the chemical space around us. This presentation aims to shed light on these techniques and their applications in environmental analysis.

Novel Aspect

Exploring new frontiers in environmental analysis through innovative mass spectrometric techniques for comprehensive chemical space characterisation

Alicia Macan Schönleben[1], Fatima den Ouden[1], Dr Shanshan Yin[1,2], Stijn Bosschaerts[1], Prof Alexander van Nuijs[1], Dr Giulia Poma[1], Prof Adrian Covaci[1]
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Occurrence and Patterns of Emerging Organic Chemicals in Vegan and Vegetarian Products

Alicia Macan Schönleben 236 - Occurrence and Patterns of Emerging Organic Chemicals in Vegan and Vegetarian Products, Meeting Room 110, August 20, 2024, 10:25 - 10:44

In recent years, plant-based diets (such as veganism or vegetarianism) became increasingly popular in Western countries. Along with this popularity, the demand for plant-based products (PBPs) and thereby also the market for such products, is rapidly growing, offering a large variety of often ultra-processed alternatives. While many studies focus on the positive effects, only few focus on chemical contaminants that might accumulate in PBPs. The degree of industrial processing has been associated with different chemicals – among these organophosphorus flame retardants (PFRs) and plasticisers – suggesting higher contamination levels in more processed food. With their increasing popularity and consumption, it is crucial to investigate the chemical contamination of PBPs to accurately estimate the associated risk and to ensure food safety.

Methods

In this study, the contamination levels and patterns of PFRs and plasticisers were investigated in PBP samples (n=52) purchased in Belgium, Germany, and the United Kingdom in 2023. Additionally, their respective food contact material (FCM) was analysed for the same compounds. For the analysis of 37 selected compounds (PFRs, legacy phthalates, alternative plasticisers), the samples were extracted with acetonitrile (ACN) and prepared using a combination of solid phase extraction (SPE) and dispersive SPE, while FCM samples were extracted with ACN. Samples were analysed with liquid-chromatography coupled to tandem mass spectrometry for PFRs and plasticizers, while di(2-ethylhexyl) phthalate (DEHP) and di(2-ethylhexyl) terephthalate (DEHT) were analysed by gas-chromatography coupled to mass spectrometry. Statistical analysis was performed using SPSS v.20 and R.

Novel Aspect

This is the first study quantifying PFRs and plasticisers in PBPs and assessing their chemical food safety.

Preliminary Data or Plenary Speaker Abstract

Tris(2-ethylhexyl) phosphate (TEHP) had highest detection frequencies (DF) in both PBPs (81%) and FCMs (83%) among PFRs, while cresyl diphenyl phosphate (CDPHP, 88%) and 1,2-cyclohexane dicarboxylic acid diisononyl ester (DINCH, 97%) had the highest DF for PBP and FCM among plasticisers, respectively. The most contaminated food category for both compound classes was vegan cheese (1154 and 123 ng/g wet weight (ww) for plasticisers and PFRs, respectively), with tris(1,3-dichloroisopropyl) phosphate (TDCIPP), DEHP and DEHT contributing most to this contamination. This food category consists mostly of coconut oil as protein base, and plasticisers have been shown to accumulate in vegetable oil. Natural products (such as tofu) had the second highest contamination levels in both compound classes, due to high acetyl tributyl citrate (ATBC, mean 534 ng/g ww) and 2-ethylhexyl diphenyl phosphate (EHDPHP, mean 68.8 ng/g ww) levels. While certain compounds were abundant in FCM (e.g. DINCH) and not in the paired food, others were abundant in both paired matrices, suggesting higher migration potential for those compounds (e.g. ATBC). Particularly, two natural samples showed very high ATBC concentrations in both PBP (259 and 1336 ng/g ww) as well as respective FCM (85,960 and 227,900 ng/g plastic), suggesting the plastic packaging as a possible contamination source in these samples. Compared to previous studies

with animal products, PBP generally showed higher contamination levels for both compound classes. PBP contamination levels were most comparable to oil/fat and processed snacks/pastries from previous studies. These results are in line with previous studies associating more processed food with higher contamination levels, since PBP are often considered ultra-processed under the NOVA classification system. Finally, to estimate the chemical food safety of PBP, a dietary exposure risk assessment was performed by calculating the estimated daily intake of the compounds based on a vegan, vegetarian and omnivore diet.

Dr. Michaela Lerch[1,2], Dr. Xianyu Wang[2], Dr. Pawel Rostkowski[3], Dr. Jean Froment[3], Dr. Pernilla Bohlin-Nizzetto[3], Prof. Sarit Kaserzon[2], Dr. Derek C.G. Muir[4], Prof. Susan Bengtson Nash[1]
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Mass Spectrometric Profiling of Antarctic Sea Water for Expedited Chemical Regulation

Michaela Lerch 282 - Mass Spectrometric Profiling of Antarctic Sea Water for Expedited Chemical Regulation, Meeting Room 110, August 20, 2024, 10:44 - 11:03

Chemical pollution is - besides climate change and biodiversity loss - one of three critical issues humanity needs to resolve for a sustainable future and is correlated with early death worldwide [1,2]. Tackling this problem requires timely, comprehensive, and global regulation. However, the large number of substances produced yearly combined with the complexity of formulating effective regulations are hindering progress [3].
The following measures are proposed to speed up proceedings: Firstly, sampling in Antarctica is performed. Antarctica's remoteness allows empirical confirmation of mobility and persistence of chemicals. Secondly, mass spectrometric suspect screening is combined with targeted methods to investigate a wide range of chemicals. Thirdly, proof of persistence in the environment is considered to be sufficient for regulatory action [4].

Methods

In January 2023, seawater samples (n=52, 1 L) were collected across the circumpolar current (South Orkney islands). Samples were processed on the ship by adding stable isotopically labelled standards and loading the water onto conditioned solid phase extraction (SPE) cartridges. Subsequently, the SPE cartridges were dried and shipped to Australia. Extraction of the SPE cartridges included elution with methanol (100%), ammonia in methanol (2%), formic acid in methanol (2%), and ethyl acetate:methanol (1:1). The fractions were concentrated and analysed via liquid chromatography coupled to mass spectrometry. Two analysis strategies are applied: targeted analysis for quantitation of selected PFAS and currently used pesticides, as well as suspect screening for identification of additional substances such as polar pharmaceuticals and personal care products.

Novel Aspect

The application of mass spectrometric profiling combines targeted and non-targeted analysis of Antarctic seawater samples covering multiple substance classes.

Preliminary Data or Plenary Speaker Abstract

Quantitative analysis of a selection of chemicals was performed including 25 PFAS and 35 polar pesticides. The PFAS selection is based on previously found pollutants in Antarctic seawater [5, 6]. It is expected that empiric trends will concur with previously observed results as reported by Casas et al. 2023. The study showed PFOA, PFNA, and PFBS as the most frequent PFAS in seawater surrounding the Antarctic peninsula [6]. Furthermore, the selection of 35 currently used pesticides was inspired by previous studies as well. Zhang et al. 2022 demonstrated the presence of currently used pesticides such as tebuconazole even in remote areas of the Southern Ocean [7]. However, very limited data is available regarding the analysis of currently used pesticides in Antarctica. Consequently, currently used polar pesticides e.g., atrazine and metribuzin were investigated to close the knowledge gap.
In order to provide a more detailed picture of the chemical profile of Antarctic seawater, high-resolution quadrupole time of flight mass spectrometric suspect screening was applied. Compound libraries stemming from the NORMAN network were used including a multitude of search categories

e.g., persistent, mobile, and toxic substances. Successfully identified chemicals will be communicated using established confidence levels [8,9] and will be discussed in the context of chemical regulation focusing on recommendations by Cousins et al. 2019 [4].

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Prioritisation, Identification, and Quantification of Emerging Contaminants in Recycled Textiles Using Non-Targeted and Suspect Screening Workflows by LC-ESI-HRMS

Drew Szabo 319 - Prioritisation, Identification, and Quantification of Emerging Contaminants in Recycled Textiles Using Non-Targeted and Suspect Screening Workflows by LC-ESI-HRMS, Meeting Room 110, August 20, 2024, 11:03 - 11:22

The production and use of recycled textiles is an important part of circular economy principles and will directly address the United Nations Sustainable Development Goals by reducing raw materials, waste, water use, land use, and carbon emissions to the atmosphere. The European Union is implementing policies to decrease textiles sent to landfills and promote the development of recycled textile processes. However, the risk of chemical contamination has not been addressed and it is not known if hazardous chemicals are used, produced, degraded or concentrated by current recycling processes. This study aims to develop, validate, and apply a high-resolution mass spectrometry (HRMS) workflow for detecting, identifying as well as assessing the quantity and risk of chemicals extracted from recycled textiles.

Methods

The method utilizes liquid chromatography coupled with high-resolution mass spectrometry and data analysis with patRoon R-package accessed through RStudio. XCMS was used for feature extraction and alignment followed by filtration based on intensity and relative standard deviation. Peak quality was assessed with NeatMS, and componentization with CAMERA preserved [M+H]+ and [M-H]- adducts. MS2 peak lists were extracted using the mzR package. Molecular formulas were generated with SIRIUS CSI:FingerID and structural matching was performed with SIRIUS, MetFrag, and MassBank. Textile-related and persistent, mobile, and toxic (PMT) substances were found by performed suspect screening. The concentration, ecotoxicity and risk were assessed based on SIRIUS fingerprints with MS2Quant and MS2Tox employed.

Novel Aspect

Developing a workflow to prioritize chemicals in consumer recycled textiles, assessing environmental risk and regulatory compliance.

Preliminary Data or Plenary Speaker Abstract

This workflow was successfully validated for 38 known substances with a wide range of molecular weight (112.09 to 916.10 g mol-1) and hydrophobicity (logP = -2.7 to 6.9). Through the detection, identification and prioritization of 20,119 LC-HRMS features, we have demonstrated that there may be restricted or potentially harmful substances present in recycled textiles. Suspect screening revealed six features that were confidently identified as PMT chemicals present on the EU REACH regulatory list. An additional 43 features were matched with other textile-related substances reportedly produced or imported to Sweden by the Swedish Chemicals Agency (Kemi). SIRIUS fingerprints were calculated for a total of 768 features such that the toxicity and concentration could be predicted to establish a priority score. The top 10 ranked features for positive and negative mode contained substances that have commonly reported uses in textile manufacture, including dyes, auxiliary and finishing chemicals. None of the top-ranked features were present in all samples, suggesting that there are differences in the manufacturing process for each textile sample. For example, the confident detection and identification of dinoterb (Level 2b), a toxic pesticide, detected in 69% of recycled textile samples was prioritized with the application of relative aquatic toxicity

predictions using machine learning techniques. Further investigation is required to determine the sources of chemicals. Due to the high predicted aquatic toxicity and high concentrations, some substances may be approaching concentrations that may cause adverse impacts to humans or the environment. The continued development and manufacture of recycled textiles is an integral part of the principles of the circular economy and will significantly contribute to the reduction of raw materials, waste production and carbon dioxide emissions to the atmosphere. These goals must be met with equal importance to the chemical health of the environment and should not be the cost of progress.

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The Coupling of a High-efficiency Aerosol Collector with Electrospray Ionisation/Orbitrap Mass Spectrometry for the Real-time Chemical Characterisation of Aerosol Particles

Yik-Sze Lau 412 - The Coupling of a High-efficiency Aerosol Collector with Electrospray Ionisation/Orbitrap Mass Spectrometry for the Real-time Chemical Characterisation of Aerosol Particles, Meeting Room 110, August 20, 2024, 11:22 - 11:41

The chemical characterisation of aerosol particles plays a vital role in understanding their chemistry in the atmosphere. To date, real-time chemical speciation of aerosol particles on a molecular level remains challenging due to the complex nature of the particles. The present study aims to develop an instrumental system that can provide real-time information on the chemical composition of aerosol particles. The proposed system is coupling a high-efficiency aerosol collector with an electrospray ionisation/Orbitrap mass spectrometer. Preliminary results showed that the coupled system is capable of real-time chemical characterisation of aerosols at atmospheric relevant concentrations. The products identified in particles formed upon α-pinene ozonolysis agreed with results in previous studies.

Methods

The high-efficiency aerosol collector (HEAC) continuously collects aerosol particles into a working fluid. This is done by first growing them into water droplets in a condensational growth chamber and then collecting the droplets in a wetted wall cyclone (vortex collector). The cyclone is wetted by a working fluid, which can be any solvent compatible with electrospray ionisation mass spectrometry (ESIMS). Compounds dissolved in the working fluid are continuously injected into an ESI-Orbitrap-MS for their chemical characterisation. The sensitivity and detection limit of the coupled system were investigated by nebulising a few selected chemical compounds relevant to atmospheric chemistry. The system's capability to analyse complex sample matrix was assessed by analysing the reaction products of α-pinene ozonolysis.

Novel Aspect

The HEAC-ESI-Orbitrap-MS system is a robust analytical technique for the chemical characterisation of aerosol particles in real-time.

Preliminary Data or Plenary Speaker Abstract

The sensitivity and detection limit study of the HEAC-ESI-Orbitrap-MS system was done by nebulising a single chemical compound at concentrations ranging from 0.1 to 30 µg m3. Several chemical compounds were used, including adonitol, erythritol, sucrose, trehalose, vanillic acid, methanesulfonic acid (MSA) and tricarballylic acid. Their sensitivities using Milli-Q water as the working fluid ranged from 3.2 x 104 (MSA) to 0.46 x 104 (trehalose) counts m3 µg-1, while the detection limits ranged from 0.9 (erythritol) to 49 (MSA) ng m-3. These results are of the same order of magnitude as similar analytical techniques, such as extractive electrospray ionisation mass spectrometry. Three working fluids, including Milli-Q water, methanol and acetonitrile (ACN), were used to determine the effect of working fluid on the sensitivity and detection limit. Using ACN as the working fluid resulted in the highest sensitivities while using Milli-Q water resulted in the lowest detection limits for the analysed compounds, followed by Milli-Q water and methanol.
The capability of the HEAC-ESI-Orbitrap-MS system to analyse more complex samples was assessed by a set of α-pinene ozonolysis experiments. The mass concentration of particles generated by α-pinene ozonolysis was monitored by a scanning mobility particle sizer (SMPS), and the chemical composition of the particles was determined by the proposed system. Results showed that the HEAC-ESI-Orbitrap-MS system could capture a large number of α-pinene ozonolysis products, including

monomeric and dimeric compounds. Moreover, the proposed system captured most of the reaction products reported in the literature. Another highlight of the proposed system is that all reaction products, including dimers, can still be identified in the mass spectrum under low mass concentrations of particles (< 2 µg m-3 This result emphasises the ability of the proposed system to chemically characterise aerosol particles at atmospheric-relevant mass concentrations.

Professor Quan Jason Cheng[1], Ms Bochao Li, Dr Daniel Stuart
[1]University Of California - Riverside

Improving Cytotoxicity Study with Single Cell Lipid Profiling of Microalgae and Bacterial Cells by Microchip-MALDI-MS

Quan Cheng 242 - Improving Cytotoxicity Study with Single Cell Lipid Profiling of Microalgae and Bacterial Cells by Microchip-MALDI-MS, Meeting Room 110, August 20, 2024, 11:41 - 12:00

Lipids play an important role in structural and physiological functions of a cell, and lipid profiling has become an effective approach to investigate cytotoxicity for a range of cellular systems. Current methods have been limited to LC-MS methods where extensive sample preparation and lipid enrichment is needed. New methodologies allowing fast assessment of lipid profiles without an enrichment step can facilitate the research by providing time-sensitive information for toxicity studies.

Methods

We report here a single cell platform based on the microchip-MALDI-MS method that utilizes chip's plasmonic property to enhance cell localization and ionization efficiency. MALDI-MS data were collected on microchips with intact cells where stimulants were applied to generate a stressed condition. The method was applied to study toxicity aspects of two systems: herbicide exposure of aquatic microorganisms and antibiotic-treated bacteria for understanding antimicrobial resistance property.

Novel Aspect

The results clearly show the high potential of the platform in rapid assessment of cytotoxicity at single cell level.

Preliminary Data or Plenary Speaker Abstract

Identification of lipids and attainment of lipid profiles without a purification/enrichment step allowed rapid analysis of lipid alterations in Chlamydomonas reinhardtii and Selenastrum capricornuium exposed to 3 herbicides (atrazine, clomazone, and norflurazon). Statistical analysis including PCA and volcano plot has been performed to identify specific response pattern of functional lipids including DGDG, TAG and MGDG. Machine learning algorithms were applied to improve classification of herbicide impact and help identify lipid species affected by the chemical exposure. Colistin-treated E. coli provided bacterial lipidomic study in a similar fashion where 68 lipids could be reproducibly detected from intact E. coli cells for evaluation of antibiotic exposure.

747

Professor David Wishart[1]
[1]University of Alberta

Automated, Massive-Scale, Quantitative Metabolomics Using Mass Spectrometry

Keynote: Professor David Wishart University of Alberta 747 - Automated, Massive-Scale, Quantitative Metabolomics Using Mass Spectrometry, Plenary 3, August 20, 2024, 15:00 - 15:25

Metabolomics researchers can be divided into three camps: 1) those who perform untargeted metabolomics; 2) those who perform non-quantitative targeted metabolomics and 3) those who perform absolutely quantitative, targeted metabolomics. The vast majority of metabolomics researchers belong to the first two camps. This is because it is widely believed that it is far too challenging and much too expensive to perform quantitative analyses via mass spectrometry (MS). However, without quantitation metabolomics, especially MS-based metabolomics, risks becoming progressively more irreproducible and progressively less relevant. Indeed, quantification is absolutely required if metabolomics assays are going to be transferred to clinical, forensic or environmental applications. In this presentation I will describe several approaches that my laboratory has developed to implement absolutely quantitative metabolomics assays. These assays have been implemented on both low-resolution triple-quadrupole and ion trap MS instruments as well as high resolution Orbitrap MS platforms. I will also discuss several novel isotopic labelling schemes that we have developed to allow isotopic standards to be inexpensively prepared. I will also highlight several innovative 96-well plate protocols that we have developed to enable high throughput sample processing along with new, web-based software that uses machine learning to automate metabolite identification and quantification of hundreds of metabolites in just a few seconds. I will also show how these methods are being combined, “kitified” and integrated into custom robotic environment that allows up to 1300 metabolites to be quantitatively measured in a single 50 uL sample in a fast, affordable and fully automatic fashion.

478

Dr Vinzenz Hofferek[1]
[1]The University Of Melbourne

Use of anion chromatography with modified chemical suppression for broad coverage HRMS metabolomics studies on the parasitic protists

Vinzenz Hofferek 478 - Use of anion chromatography with modified chemical suppression for broad coverage HRMS metabolomics studies on the parasitic protists, Plenary 3, August 20, 2024, 15:25 - 15:44

Parasitic protists, such as Leishmania spp, cause a range of neglected tropical diseases that pose a significant threat to global health. The limited availability of effective and affordable treatments highlights the urgent need for novel drug targets. Metabolomics can provide valuable insights into Leishmania's survival mechanisms and reveal potential vulnerabilities. However, current metabolomic methods often lack the necessary throughput and/or comprehensive coverage of metabolite classes. Here, we present a high-throughput method for Leishmania metabolomics using anion chromatography with a modified anion chemically regenerated suppressor (ACRS) coupled with mass spectrometry detection. The modified ACRS enables superior retention of a wide range of phospho-metabolites and organic acids, as well as zwitterionic compounds like amino acids.

Methods

A Dionex ICS-6000 ion chromatography system equipped with an AS11-HC column and a modified ACRS was employed. The ACRS was modified by flushing a 1 M ammonium sulfate solution for 24 h, followed by continuous regeneration with a 200 mM ammonium sulfate solution at pH 9.3 during analysis. Cultured Leishmania parasite stages (10^7) were extracted in MTBE:methanol:water using automated liquid handling robot and polar metabolites analyzed using the optimized IC-MS method with multi mass range switching (MRS).

Novel Aspect

A modified anion chemically regenerated suppressor enabled the detection and retention of amino acids in addition to other polar metabolites.

Preliminary Data or Plenary Speaker Abstract

The modified ACRS significantly improved the peak shape and sensitivity for zwitterionic compounds, such as amino acids, which are often lost in standard anion chromatography methods. The use of ammonium sulphate and ammonium hydroxide in the regenerative flow enhanced the efficiency of the suppression process and allowed for the detection of a wide range of metabolites. The method demonstrated excellent linearity for most metabolites up to a cell concentration of 6×10^7 cells per sample. The optimized IC-MS method enabled the identification and quantification of over 100 metabolites across various classes, including amino acids, nucleotides, sugar/inositol-phosphates and organic acids. Preliminary data revealed significant differences in the metabolic profiles of Leishmania promastigotes under different growth conditions, providing new insights into the parasite's adaptability and potential drug targets.

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A/prof Darren Creek[1], Dr Dovile Anderson, Dr Christopher A MacRaild, Dr Katy Jeppe, Dr Joshua Morrow, Dr Christopher Barlow
[1]Monash University

Combining metabolite standards cocktails with IDEOM v24 to enable routine semi-targeted metabolomics

Darren Creek 456 - Combining metabolite standards cocktails with IDEOM v24 to enable routine semi-targeted metabolomics, Plenary 3, August 20, 2024, 15:44 - 16:03

Metabolite identification is a key bottleneck in metabolomics studies. LC-MS/MS platforms based on high resolution mass spectrometry provide opportunities for high throughput metabolite identification in untargeted metabolomics. However, confirmation of metabolite identification requires spectral or chromatographic comparison with authentic metabolite standards, which is often expensive and time consuming.

Methods

We have developed a semi-targeted metabolomics approach combining untargeted high-resolution LC-MS-based metabolomics with a recently-updated chemical library of standards to rapidly identify common metabolites in central pathways. The key advance with this approach is to combine over 500 authentic metabolite standards into 8 cocktail mixtures that allow unambiguous retention time assignment by avoiding co-injection of isomers and other combinations that generate interfering peaks. This enables the routine and automated determination of accurate mass and retention time for all 500 standards for each analytical batch.

Novel Aspect

This approach is broadly applicable to a wide range of metabolomics studies, as exemplified in our studies of drug mechanisms.

Preliminary Data or Plenary Speaker Abstract

This batch-specific accurate mass and retention time, along with in-house MS/MS libraries, provides a subset of high-confidence targeted metabolomics data within an unbiased metabolomics analysis that still allows for discovery of unique metabolites. A data analysis workflow is provided within the latest version of the open-source IDEOM software (v24), which includes enhanced compatibility with alternative peak-picking algorithms, and uses the batch-specific standard retention times to predict retention times for over 100,000 compounds in the database, thereby improving the accuracy of putative identification of metabolite features in the untargeted analysis.

681

Dr. Per Andrén[1]
[1] Uppsala University, Science for Life Laboratory (SciLifeLab)

Spatial metabolomics reveals region-specific alterations induced by parkinsonism and L-DOPA-induced dyskinesia

Per Andrén 681 - Spatial metabolomics reveals regionspecific alterations induced by parkinsonism and L-DOPA-induced dyskinesia, Plenary 3, August 20, 2024, 16:03 - 16:22

L-DOPA (L-3,4-dihydroxyphenylalanine) is the most effective medication for treating Parkinson's disease (PD). However, with disease progression, chronic use of L-DOPA can lead to complex motor complications known as L-DOPA-induced dyskinesia (LID). The full molecular mechanisms underlying both PD and LID are not yet fully understood. We employed matrix-assisted laser desorption ionization mass spectrometry imaging (MALDI-MSI) to comprehensively map different molecular species, such as neurotransmitters, neuropeptides, metabolites and lipids in specific brain regions. Using brain samples from an experimental PD model (MPTP) with LID, we previously observed abnormal elevations of L-DOPA and its metabolite, 3-O-methyldopa, in the whole brain of LID animals (Sci Adv. 7(2):eabe5948). This resulted in increased dopamine and downstream metabolites in all brain regions, except putamen and caudate. We also found that the abundance of selected neuropeptides was associated with L-DOPA concentrations in the putamen, emphasizing their sensitivity to L-DOPA (NPJ Parkinson's Dis. 8(1):41). Here we used MALDI-MSI to also visualize brain region-specific alterations of metabolites in coronal sections of the same PD and LID brains.

Methods

We used FTICR MALDI-MSI for the comprehensive mapping of metabolite networks in specific brain regions. The reactive MALDI matrix (FMP-10) facilitated the covalent charge-tagging of molecules containing phenolic hydroxyl and/or primary or secondary amine groups, including dopaminergic and serotonergic neurotransmitters and their associated metabolites (Nat Methods. 2019, 16:1021-1028, Nat Protoc. 2021, 16(7):3298-3321). We developed a method to image multiple neuropeptides and dual polarity ionization mode was used to thoroughly image numerous lipids (NPJ Parkinsons Dis. 9(1):118). A spatial omics approach that combines histology, MALDI-MSI and spatial transcriptomics was developed to facilitate precise measurements of mRNA transcripts and low-molecular-weight metabolites (Nat Biotechnol. 2023, doi.org/10.1038/s41587-023-01937-y). These approaches provide a further level of multimodality when studying small molecules in a tissue context.

Novel Aspect

Multi omics imaging of Parkinson’s and L-DOPA-induced dyskinesia

Preliminary Data or Plenary Speaker Abstract

In this study, we identified distinct lipid alterations associated with Parkinson's disease (PD) and L-DOPA-induced dyskinesia (LID), involving specific lipid classes such as plasmalogen phosphatidylcholines (PCs), polyunsaturated fatty acid (PUFA)-containing glycerophospholipids (GPLs), and various sphingolipids. Notably, PC plasmalogens were found to be depleted in LID compared to non-LID animals within specific brain regions such as the internal segment of the globus pallidus (GPi), claustrum (Cl), and precentral gyrus (PrG). Conversely, these PC plasmalogens were more abundant in non-LID than in MPTP-treated animals. This depletion correlated with the severity of LID, and levels of L-DOPA and dopamine in these regions.
Additionally, three general trends were observed in PUFA-containing GPL changes across different animal groups within the GPi and external segment of the globus pallidus (GPe). First, certain PUFA-GPLs were elevated in LID animals and reduced in non-LID animals compared to MPTP-treated animals. Secondly, more saturated PUFA-GPLs displayed an opposite trend. Lastly, certain PUFA-GPLs were decreased in MPTP animals compared to control animals, particularly in the GPi and GPe areas. These changes showed correlations with the animals’ LID scores and neurotransmitter levels.

We also explored changes in sphingolipid species, specifically hydroxylated hexosylceramides (SHexCers) and non-hydroxylated sphingomyelin (SM), noting that certain sphingolipids were depleted in MPTP-treated animals compared to controls. A targeted analysis of these sphingolipids in LID versus non-LID animals revealed a pattern of depleted hydroxylated SHexCers and increased non-hydroxylated SHexCers. Furthermore, the ratios of these sphingolipids varied distinctly between control, MPTP, non-LID, and LID animals, indicating significant shifts in sphingolipid metabolism associated with disease and treatment conditions.

This research provides significant insights into the complex metabolite dynamics underlying PD and its pharmacological treatment, emphasizing the role of lipid changes in disease progression and treatment responses.

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Dr Fan Yang[1]
[1]Uppsala University

Untargeted mass spectrometry-based metabolomics workflow optimization for chronic and autoimmune chronic pancreatitis biomarker discovery

Fan Yang 16 - Untargeted mass spectrometry-based metabolomics workflow optimization for chronic and autoimmune chronic pancreatitis biomarker discovery, Plenary 3, August 20, 2024, 16:22 - 16:41

Chronic pancreatitis (CP) is a progressive and irreversible disease. CP has been related to several factors including age, obesity, alcohol consumption, diabetes, etc. Currently, the diagnosis of CP relies on symptoms and imaging features. Autoimmune chronic pancreatitis (AIP) is a rare form of CP. It is challenging to identify unique biomarkers to distinguish AIP from CP. Untargeted metabolomic analysis using ultra-performance chromatography mass spectrometry (UHPLC-MS) is a powerful approach for discovering metabolite biomarkers. However, untargeted metabolomics acquisition generates high-throughput and complex mass spectrometry data, which are time-consuming. Therefore, we are optimizing untargeted metabolomic data processing to accelerate feature annotation using in-house and open-source spectra libraries. We aim to discover potential biomarkers for CP and AIP.

Methods

Dry fecal samples were weighed and dissolved. 200 µl of supernatant was collected after sonication and centrifugation. Then supernatants were freeze-dried before reconstitution in the mobile phase. The UHPLC-QTOF analysis was performed with a Maxis II ETD Q-TOF mass spectrometer using an electrospray ionization (ESI) source coupled with an Elute UHPLC system with a UPLC HSS T3 column (1.8 mm, 100 x 2.1 mm). HRMS was acquired in both positive and negative modes at a mass range of m/z 50-1200, MS2 was acquired in Data-dependent acquisition (DDA) mode. The converted raw data were preprocessed with XCMS using R script. The features were screened against in-house and external databases for structural elucidation of metabolites of interest.

Novel Aspect

We discovered unknown biomarkers for chronic pancreatitis in human fecal samples through an improved workflow for mass spectrometric metabolomics analysis.

Preliminary Data or Plenary Speaker Abstract

To acquire these biomedically important metabolites, an untargeted metabolomics workflow for automated data processing and analysis has been developed in R. This workflow combines feature detection using XCMS, spectral screening against in-house and online experimental spectral libraries using MetaboAnnotation, and KEGG pathway analysis. Over 1000 reference standards were purchased from chemical suppliers or synthesized in the laboratory. The standard mix solutions were injected into the UHPLC-QTOF system under the same acquisition condition as the samples. Therefore, the in-house library enables the mapping of the features with retention time (rt), accurate precursor m/z, and MS2 spectra. Meanwhile, external spectra databases, such as HMDB and MoNA are also used to elucidate structure. The matching score is calculated by similarity scores of pairwise mass spectra after normalization based on the highest fragmentation mass peak(s). Our build-up pipeline has been applied for the discovery of CP metabolite biomarkers with 52 fecal samples of individuals with AIP and CP. The statistic studies were performed in MetaboAnalyst, and the significant features to distinguish AIPs and CPs were selected by P value < 5 % and fold-change (FC) > 2. The identified and important metabolites were of diverse compound classes including fatty acids, and microbiome-derived metabolites. The authentic standards were co-injected with samples to validate the structure. Four potential biomarkers were annotated with confidential level 1 in both positive and negative ionization modes.

714

Dr. Soumen Kanti Manna[1]
[1]Saha Institute Of Nuclear Physics

Effect of wearing face mask on cardiopulmonary parameters and salivary metabolome.

Soumen Kanti Manna 714 - Effect of wearing face mask on cardiopulmonary parameters and salivary metabolome, Plenary 3, August 20, 2024, 16:41 - 17:00

Masks have become an integral part of public life and exposome in the post-pandemic era. However, the understanding of the effect of wearing mask on physiology, which is essential to inform public health advisories amid lack of conclusive evidence on efficacy of masks in preventing the viral infection, remains poor. While some earlier studies have indicated that that wearing mask may affect cardiopulmonary parameters, their biochemical impact remains to be characterized in detail. Given that saliva is constantly exposed to breath , salivary metabolomic analysis can be used to non-invasively gauge the biochemical impact of mask use.

Methods

The study was implemented in two phases. In the first phase, impact of short-term N95 mask use was examined in young healthy volunteers. Cardiopulmonary parameters (heart rate, pulse rate, respiratory rate and SpO2) were recorded before and after 30 minutes of wearing N95 masks. Uninduced saliva samples were collected for metabolomic analysis before and after mask use. In the second phase, effect of longer duration of mask use was examined in healthcare workers. Cardiopulmonary parameters, blood pressure and body temperature were recorded and saliva samples were collected before and after 3 hours of wearing surgical or N95 mask or no mask (control) under similar condition (without consumption of any food or water). Changes in metabolome were analyzed using GCMS.

Novel Aspect

Even in absence of any change in cardiopulmonary parameters, N95 mask may be associated with changes related to microbial metabolism.

Preliminary Data or Plenary Speaker Abstract

Short-term (30 minutes) N95 mask use did not cause any significant change in heart rate, pulse rate or SpO2 in the ten young healthy volunteers participating in the pilot study. Three independent data normalization approaches were used to analyze changes in metabolomic signature along with correlation analysis. The individuality of overall salivary metabotypes was found to be unaffected by mask use. However, a trend of correlated increases in abundances of L-fucose, 5-aminovaleric acid, putrescine and phloretic acid was indicated irrespective of the normalization method. Targeted quantitative analysis confirmed increases in concentrations of these metabolites upon mask use in paired saliva samples amid high inter-individual variability. Results showed that while there was no change in measured physiological parameters and individual salivary metabotypes, mask use was associated with correlated changes in these metabolites plausibly originating from altered microbial metabolic activity. Putrescine and phloretic acid might also explain changes in odour perception reported to be associated with mask use. On the other hand, L-fucose has been implicated in host-microbiome interaction. A sample set from twenty-seven young healthy volunteers, further corroborated increases in their salivary abundances in paired samples despite no significant change in cardiopulmonary parameters. In order to examine if these observations were consistent and progressive, impact of longer duration of mask use was examined. In healthcare workers comprising a wider distribution of age range and health conditions, no significant change in cardiopulmonary parameters or blood pressure or body temperature were observed after 3 hours of mask use. The analysis of changes in metabolome is currently ongoing. While further analysis of impact of life-style and demographic factors as well as long-term consequences are due, these results indicate that use

of face mask may be associated with unwarranted biochemical changes and, thus, warrant requisite cost-benefit analysis before mandating mask use en masse.

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Ruijun Tian[1]
[1]Southern University Of Science And Technology (sustech)

Exploring functional proteome in spatially resolved and high-throughput fashion

Keynote: Professor Ruijun Tian Southern University of Science and Technology 182 - Exploring functional proteome in spatially resolved and high-throughput fashion, Meeting Room 105, August 20, 2024, 15:00 - 15:25

Spatial multi-omics has drawn tremendous attention due to its powerfulness for unbiasedly and systematically discovering biomolecules from biomedical samples with high heterogeneity. Comparing with genomic approaches, current proteomic approaches have been largely lagged behind due to the requirement for processing limited amount of samples.

Methods

We developed a fully integrated proteomics sample preparation technology SISPROT which could efficiently process nanogram level formalin-fixed, paraffin-embedded (FFPE) tissue slice sample for removing the staining dyes, protein digestion, peptide desalting, and even TMT labeling. By further combining with multi-color immunohistochemical imaging investigation with single-cell resolution at centimeter scale and automated laser microdissection, we achieved high-sensitive spatially visualized proteomic analysis of various clinical tissue samples, such as pancreatic tumor. Furthermore, we developed a fully automated version of the SISPROT (autoSISPROT) by combining the all-in-tip sample preparation capabilities of SISPROT with the programmable and automated liquid handling equipment.

Novel Aspect

Tumor heterogeneity and drug targets now can be explored at the proteome level in spatially resolved and high-throughput fashion.

Preliminary Data or Plenary Speaker Abstract

By incorporating the high-sensitive SISPROT technology into spatial proteomic analysis, we systematically revealed the spatial proteome changes of different phenotypes of neoplastic cells and immune cells in the pancreatic tumor microenvironment utilizing only 60-100 single cells dissected from 4 μm-thick FFPE tissue slices of pancreatic tumor, and with more than 5,000 proteins identified. We also found increasing expression of numerous proteins as the tumor progressed, along with a distinct composition of immune cells in the tumor microenvironment and lymph node. Moreover, the autoSISPROT platform enables the simultaneous processing of 96 samples in less than 2.5 hours. Leveraging its all-in-one tip operation, the autoSISPROT facilitated fully automated integration of proteomics sample preparation and TMT labeling. We combined autoSISPROT with DIA-based thermal proteome profiling (TPP) to identify the known targets and potential off-targets of a panel of 20 kinase inhibitors, affording over a 10-fold improvement in throughput compared to classical TPP.

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Professor Christopher M. Overall[1]
[1]University of British Columbia, Vancouver

Multiplex MS Profiling of SARS-CoV-2 3CLpro/Main Protease Cleavage Kinetics for Ranking Interactors as Substrates and Designing Optimal Peptide Assay Formats

Christopher Overall 413 - Multiplex MS Profiling of SARS-CoV-2 3CLpro/Main Protease Cleavage Kinetics for Ranking Interactors as Substrates and Designing Optimal Peptide Assay Formats, Meeting Room 105, August 20, 2024, 15:25 - 15:44

SARS-CoV-2 3C-like main protease (3CLpro/Mpro) excises proteins from the viral polyprotein. 3CLpro drug development focused on the catalytic non-prime (P) side, but the importance of the prime side (P') for cleavage and drug development remains underappreciated. Moreover, high throughput screening for follow-up inhibitors is hampered by poor solubility and handling properties of current quenched fluorescent peptide substrates. Terminal Amino Isotopic Labeling of Substrates (TAILS) proteomics has discovered >150 host cell substrates of 3CLpro. We reasoned that orthogonal methodology, e.g., Inactive Catalytic Domain Capture ICDC-MS/MS, would identify candidate substrates from the 3CLpro human cell interactome. However, when validating cell proteins of enormous size >500kDa or for which recombinant proteins are unavailable, high-throughput secondary screens are problematic.

Methods

To identify substrates within the interactome of 3CLpro, we used PICS, the first MS approach that simultaneously identifies both P and P' cleavage site specificities from database searchable cleaved-peptide libraries. Amino acid preferences were confirmed by synthetic peptide substrates followed by positional peptide libraries, with molecular docking simulations used to unveil key P' structure-activity relationships impacting 3CLpro-substrate interactions. Candidate substrates were then predicted in the interactome from these cut site preferences and ranked by enzyme kinetics measured by a new multiplex LC-MS/MS peptide assay format scaled to accommodate up to 250 cleavage sites. From this, we designed two highly soluble 3CLpro quenched-fluorescent peptide substrates for improved FRET monitoring of 3CLpro activity with 15x greater sensitivity over current assays.

Novel Aspect

New MS-based peptide assay formats enabled high throughput identification of cleavage sites and multiplex cleavage site kinetics of 250 cut-sites.

Preliminary Data or Plenary Speaker Abstract

3CLpro cleavage occurred at canonical P1-Gln and after non-canonical P1 Met and His sites. These also displayed P2 and P3’ amino acid cooperativity differences versus P1-Gln. Essential h-bonds between the N-terminal Ser1 of protomer B in 3CLpro dimers and P1-His are formed, but not with P1-Met. Nonetheless, MAP4K5 is cleaved after Met456. As reactive oxygen species are elevated in SARS-CoV-2 infection, we compared peptide-cleavage rates of P1-Met versus P1-ox-Met, which was greatly enhanced. Molecular simulations showed that P1-Met now forms a h-bond with Ser1. Hence, oxidised-Met is a biologically relevant modification of intracellular proteins.

We measured enzyme kinetic specificity constants for 139 3CLpro cut-sites in 43 interactors, which were predicted from the new cleavage specificities we determined. To do so, we developed a multiplex LC-MS/MS assay to simultaneously measure the cleavage kinetics in up to 250 peptides. We used the kinetic specificity constants to rank the candidate substrates for follow-up assays. Thereby, we confirmed the cleavage of 13 interactors as 3CLpro substrates in vitro in SARS-CoV-2-infected human lung cells and verified that five substrates were depleted in COVID-19 post-mortem

lungs. Over half of the 259 3CLpro interactors were associated with the cytoskeleton that suggested infection led to cell shape changes, which we confirmed by confocal microscopy. Surprisingly, SARS-CoV-2 infection rapidly generated Tunneling NanoTubes (TNTs) within 2 h post-infection and employed 3CLpro substrates, e.g., infection-induced 3CLpro cleavage removal of nuclear localization sequences or cytosolic retention sequences leading to the translocation of NUMA1 and TRIM28 to epithelial cell adherens junctions and YAP1 to the nucleus, respectively. These intercellular channels are conduits for the SARS-CoV-2 virus to promote immune-protected “stealth” infection of nearby cells, which may provide additional therapeutic avenues to treat COVID-19.

Haoyun Fang[1,2], Dr Alin Rai[1,2,3], Dr Kevin Huynh[1], Mrs Hsiao-Chi Liao[2], A/Prof Agus Salim[1,2], Dr David Greening[1,2,3]
[1]Baker Heart And Diabetes Institute, [2]University of Melbourne, [3]La Trobe University

Deciphering subcellular proteomic niches of mouse heart using label-free DIA-MS and machine learning

Haoyun Fang 448 - Deciphering subcellular proteomic niches of mouse heart using label-free DIA-MS and machine learning, Plenary 3, August 20, 2024, 15:44 - 16:03

Heart is a highly structured organ which biological functions are dependent on coordinated activities of specialised cardiac organelles. Malfunctioning cardiac organelles with perturbed inter-organelle communications have been well-reported in myriad cardiomyopathies. To understand perturbed cardiac organelles at molecular level, mass spectrometry (MS)-based proteomics profiling has been well-established to investigate the protein compositions of a single organelle or a target subcellular niche of interest using biochemical enrichment or proximity-labelling. However, it remains an unmet obstacle to understand the protein compositions and dynamics of subcellular proteome at a systemic and spatial level in heart.

Methods

In the past decade, proteomics workflows such as protein correlation profiling (PCP), localisation of organelle proteins by isotope tagging (LOPIT), dynamic organellar maps (DOMs) and SubCellBarCode have combined biochemical fractionation, quantitative MS and ML-assisted subcellular assignment to investigate proteome-wide subcellular features from varies cell and tissue types. Here, we streamlined a proteomics and bioinformatics pipeline for studying the subcellular protein compositions of frozen heart from mouse. For subcellular proteomics, we applied 10-step differential centrifugation-based fractionation, SP3-based proteomic sample preparation, label-free data-independent acquisition (DIA) MS from an orbitrap-based instrument. For subcellular protein assignment, we have curated cardiac-specific subcellular protein markers and applied support vector machine (SVM), random forest (RF) and Xgboost for subcellular protein assignments.

Novel Aspect

This resource and pipeline complement the understanding of cardiovascular pathophysiology and facilitate the development of organelle-specific targets at spatial level.

Preliminary Data or Plenary Speaker Abstract

Overall, our proteomics pipeline has demonstrated great depth, high subcellular resolution, and strong intra- and inter-day reproducibility from as little as 30-40 mg of mouse heart tissue. To assess the quality of data, we commenced two independent inter-day experiments, each compromise three biological replicates. From 11 differentially centrifugated fractions of mouse heart tissue, we report 5134 protein groups with quantitative subcellular profiles. Our data demonstrates high reproductivity with average Pearson correlation of 0.96 between intra-day replicates and 0.95 including inter-day replicates. At proteome level, our data demonstrated strong subcellular resolution with distinctive protein distributions across the 11 fractions gradient. Hierarchal clustering analysis revealed proteins residing in larger organelles (i.e. sarcomere, mitochondria) are enriched in low g-force fractions. Endomembrane residing proteins (i.e. endoplasmic reticulum, plasma membrane) are enriched in mid-range g-force fractions. Protein complexes (i.e. ribosome, proteasome) and cytosolic proteins are enriched in high g-force and supernatant fractions.

Based on protein fractionation profiles, we have curated a cardiac tissue subcellular marker list comprised 450 proteins distinguishing 10 subcellular features in parallel, including cell surface, cytosol, endo/sarcoplasmic reticulum, Golgi apparatus/endolysosome, mitochondria, nucleus, peroxisome, proteasome, ribosomes and ECM/sarcomere. Using SVM, RF and Xgboost-based ML, we have mapped around 50% of mouse heart proteome with confidence as confirmed by gene ontology

cellular compartment (GOCC)-based functional enrichment analysis. Further, by overlapping our data with previously reported proteins from specialised cardiac sub-organellar niches (i.e. junctional sarcoplasmic reticulum, intercalated discs, transverse tubules), our work has complemented the previously reported subcellular protein entities, and provided a list of highly confident proteins that exert crucial roles in cardiac-specific functions.

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Bente Siebels[1], Manuela Moritz[1], Dr. Olga Shevchuk[2], Lars Widera[2], Prof. Dr. Daniel Robert Engel[2], Prof. Dr. Hartmut Schlüter[1], Dr. Jan Hahn[1]
[1]Section for Mass Spectrometry and Proteomics, Center for Diagnostics, University Medical Center Hamburg-Eppendorf, [2]Department of Immunodynamics, Institute for Experimental Immunology and Imaging, Medical Research Centre, University Hospital Essen

Spatial resolved mass spectrometric proteomics analysis facilitated by infrared laser-based sampling of infected murine bladders

Bente Siebels 343 - Spatial resolved mass spectrometric proteomics analysis facilitated by infrared laser-based sampling of infected murine bladders, Meeting Room 105, August 20, 2024, 16:03 - 16:22

To fully understand the underlying cellular processes during infection with uropathogenic E. coli (UPEC) a spatially and in-depth resolved proteome analysis of the bladder is required, as different cell layers are associated with different molecular functions and proteomic profiles. Three-dimensional nanosecond infrared laser (NIRL)-based tissue sampling, a soft tissue ablation technology that generates a homogenized aerosol of tissue molecules, has previously been shown to enable spatially resolved proteomics. Here, we aim towards a cell layer-resolved proteome analysis to decipher the molecular profiles of different bladder wall layers, for the first time directly from the infected and non-infected tissue.

Methods

We ablated eight consecutive tissue layers of about 20 µm directly from PFA-fixed mouse bladders targeting the urothelial side. The aerosols were collected separately, antigen retrieval was performed followed by digestion with trypsin. Measurement was performed on a nano UHPLC (Vanquish Neo, Thermo Fisher) coupled to an Orbitrap Mass Spectrometer (Exploris 480, Thermo Fisher) by label-free LC-MS/MS in DIA mode followed by data base search in Proteome Discoverer and CHIMERYS algorithm. Consensus clustering was performed to distinguish the bladder layers on the proteomic level. Quantitative protein abundances were compared between different clusters and significantly differential abundant proteins between healthy and infected mice were determined.

Novel Aspect

Spatially resolved differential proteomic analysis of consecutive cell layers of healthy and UPEC infected murine bladders.

Preliminary Data or Plenary Speaker Abstract

Consensus clustering of NIRL-ablated tissue layers ablated from mouse bladder revealed different molecular profiles. Identified layers corresponded to the molecular profiles of the predefined cellular bladder layers, including: urothelium, submucosal layer, detrusor, and adventitia. This was further indicated by the abundance distribution of cell type-specific proteins, including uroplakin, elastin, and myosin. The data was further confirmed with histological data derived from the human protein atlas and based on our results, a proteomic profile of cellular bladder layers was generated. For the analysis of the influence of infection on cell layer proteome profiles, differential quantitative protein abundances of the different bladder layers from infected and non-infected murine bladders were analyzed. Pathway analysis revealed inflammation processes differing between the different layers of the bladder and gives the opportunity to identify potential biomarkers for urinary tract infections.

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Dr. Kermit Murray[1], Kelcey Hines[1], B. Chisom Egbejiogu[1], Neda Fiezi[2], Dr. Touradj Solouki[2]
[1]Louisiana State University, [2]Baylor University

Laser Ablation Mass Spectrometry of Native Proteins

Kermit Murray 563 - Laser Ablation Mass Spectrometry of Native Proteins, Meeting Room 105, August 20, 2024, 16:22 - 16:41

Mass spectrometry is a powerful method for localized sampling and imaging of proteins from tissue sections; however, localized information about native protein configurations and protein interactions is difficult to obtain due to the harsh conditions often encountered in the sampling and imaging procedures. We are developing methods for localized detection of proteins in tissue while preserving their native structure using 1) pulsed laser ablation for region selected sampling off-line mass spectrometry and 2) laser ablation electrospray on-line mass spectrometry. We have demonstrated that the laser ablated proteins maintain their native structures and are adapting this to sampling and imaging mass spectrometry.

Methods

Laser ablation sampling was carried out using an infrared optic parametric oscillator (OPO) at 2940 nm wavelength or an ultraviolet excimer laser at 193 nm wavelength. The laser is focused to spot size of 10 to 100 µm and ablates tissue from selected regions of sections mounted on microscope slides. The ablated material is captured in a buffer solution and analyzed by off-line native ion mobility mass spectrometry and broadband collision induced unfolding (CIU) with a Waters-Synapt G2-S mass spectrometer. On-line mass spectrometry by laser ablation electrospray was accomplished using a solid state tunable optical parametric oscillator (OPO) DUV laser source tunable from 193 to 210 nm coupled to the Waters-Synapt G2-S instrument.

Novel Aspect

Laser ablation of proteins from surfaces maintaining native conditions for mass spectrometry sampling and imaging.

Preliminary Data or Plenary Speaker Abstract

Two general approaches are being developed for native mass spectrometry of proteins in tissue: offline sampling and online imaging. The offline approach uses a pulsed laser to ablate tissue material from selected regions of a thin tissue section which is collected in a buffer solution. The solution is electrosprayed under native conditions. The online approach uses a pulsed laser to ablate tissue which is captured in an electrospray comprising native solvent. The proteins in the ablated material are captured in the spray droplets and ions are formed by droplet evaporation in the standard electrospray process. Offline native electrospray has been demonstrated with pulsed infrared and ultraviolet lasers and protein standard native deposits. A reflective objective assembly was used for infrared irradiation; the DUV was focused with a single lens element. The ablated material was captured in 20 µL of native solvent in wells below the sample slide. Off-line static nanoESI of ablated materials was performed with a Waters Synapt G2-S mass spectrometer. Protein charge state distribution and broadband CIU plots were used as indicators of protein native structure and it was observed that in both UV and IR laser ablation the native structures of the ablated and captured proteins were preserved. Online laser ablation with electrospray post-ionization was carried out with a wavelength-tunable solid state DUV OPO. The laser was operated at 206 nm wavelength and directed through a quartz microscope slide to ablate the sample material which merges with the electrospray source of the mass spectrometer. The sample slide was translated with respect to the laser and electrospray to maintain signal intensity for protein standards and provide the capability for tissue imaging. The sampling and imaging methods are being developed together for region selected native protein surface analysis and imaging.

Dr Colleen Maxwell[1,2], Dr Jatinderpal K Sandhu[1,2], Dr Thong H Cao[1,2], Professor Gerry P McCann[2], Professor Leong L Ng[1,2], Professor Donald JL Jones[1,2,3]
[1]van Geest multiOmics Facility, University of Leicester, [2]Department of Cardiovascular Sciences, University of Leicester, [3]Leicester Cancer Research Centre

The Edge Effect in High-Throughput Proteomics: A Cautionary Tale,

Colleen Maxwell 13 - The Edge Effect in High-Throughput Proteomics: A Cautionary Tale, Meeting Room 105, August 20, 2024, 16:41 - 17:00

In order for LC-MS/MS to continue to grow as a platform for clinical and translational proteomics on par with immunoassays and aptamer-based platforms, careful consideration must be given to quality control and throughput. To meet these requirements multiplexed, targeted LC-MS/MS assays are increasingly paired with sample preparation and analysis in multiwell plates. However, as throughput increases, large scale MS-based proteomics studies are often plagued by batch effects: sources of technical variation acquired throughout sample preparation or MS analysis which can confound biological signal and compromise data quality. Here we present an intra-plate batch effect termed the edge effect arising from temperature gradients in multiwell plates, not previously reported in a bottom-up proteomics setting.

Methods

Plasma was collected from healthy donors with informed consent and prepared following the bottom-up plasma proteomics protocol described by Mbasu et al. (2016) with volumes adjusted for use in multiwell plates. Peptide intensities across the plate were assessed for 46 human peptides by LC-MS/MS multiple reaction monitoring using a Waters Acquity LC coupled to the Xevo TQ-XS mass spectrometer. The edge effect was characterised by assessment of relative standard deviation (RSD) in peptide intensities across the multiwell plate. Thermal images were obtained after each heating step to measure temperature gradients using an FLIR SC600 series infrared camera. The extent of the edge effect was assessed for a variety of multiwell plates, plate sealing techniques, and plate heating techniques.

Novel Aspect

An intra-plate batch effect not previously reported in proteomics. We assess additional heaters/plates not included in our recent publication.

Preliminary Data or Plenary Speaker Abstract

We found that the edge effect, extensively reported in preclinical cell culture studies, is also a potential pitfall of using multiwell plates in LC-MS/MS based bottom-up proteomics. For deep well plates heated in an oven incubator, total peak areas of the 46 peptides exhibited unacceptably high intra-plate variation with an average RSD across the 96 wells for all peptides of 38.7%. An assessment of plate sealing and heating techniques coupled with thermal imaging of the plates confirmed that temperature gradients during the reduction and tryptic digestion step were causing variable peptide abundances, with particularly high variation in the edge and corner wells. We assessed the extent of the edge effect for a variety of multiwell plates and commonly used heaters including standalone incubators, heat blocks, and heaters integrated with liquid handling systems. The use of PCR-style plates and heaters which contact each well individually significantly ameliorated the edge effect. The additional incorporation of surrogate standards spiked into the sample prior to digestion to normalise for digestion inefficiencies further reduced average RSD across the 46 peptides to < 5% across the plate.
There is an opportunity for LC-MS/MS to be a dominant analytical technique in translational and clinical proteomics. It is a high-throughput, multiplexable technique that has been demonstrated to be highly reproducible over many hundreds of injections. However, every aspect of the work-flow – from sample preparation to analysis – must be adequately assessed for issues which may compromise data quality and reproducibility. We propose that all laboratories performing high

throughput bottom-up proteomics carefully consider conditions for heating multiwell plates (and indeed other vessels) and call for vendors to supply plates and heaters which have been assessed for even heating in bottom-up proteomics applications.

Food, Nutrition and Agriculture B

764

Sulfur compounds in wine: the good, the bad and the unknown

Keynote: Professor Bruno Fedrizzi The University of Auckland 764 - Sulfur compounds in wine: the good, the bad and the unknown, Meeting Room 106, August 20, 2024, 15:00 - 15:25

Volatile sulfur compounds (VSC) are commonly found in food and fermented foods and beverages; they play a critical role in the products overall quality, which can lead to either consumer acceptance or rejection.

Yeast, a key ingredient in the food industry, can play a key role in altering the final concentration of these molecules. Labelled-isotope tracing experiments using 13C-,15N-, 34S-labelled sulfur-containing putative precursors, coupled with mass spectrometry, has shown to be very effective to study the pathways leading to the formation of these molecules. Similarly, storage and technological approaches influence the formation of these molecules.

Most recently our attention has been devoted to the most volatile and most deeply studied VSC: hydrogen sulfide (H2S). The intrinsic volatility and reactivity of this molecule, highlights the possibility that this molecule has a key role both from a molecular and technological point of view.

From this research new non-volatile polysulfurylated molecules were identified in wine. Our recent finding that yeast (Saccharomyces cerevisiae) can produce these polysulfanes has shed new light on the impact of fermentation and technology on the level of polysulfanes.

Danica Mostoles[1], Carla Egido[1], Andrea Mara[2], Professor Gavino Sanna[2], Professor Javier Saurina[1,3,4], Dr. Sònia Sentellas[1,3,4], **Professor Oscar Nuñez**[1,3,4]
[1]Department of Chemical Engineering and Analytical Chemistry, University of Barcelona. Martí i Franquès 1-11, 08028, [2]Department of Chemical, Physical, Mathematical and Natural Sciences, University of Sassari, Via Vienna 2, 07100, [3]Reserach Institute in Food Nutrition and Food Safety, University of Barcelona. Av. Prat de la Riba 171, Edifici Recerca (Gaudí), 08921, [4]Serra Húnter Fellow, Departament de Recerca i Universitats, Generalitat de Catalunya, Via Laietana 2, 08003

High-throughput LC-LRMS and FIA-LRMS fingerprinting and polyphenolic profiling for the geographical characterization and authentication of honey

Oscar Nuñez 35 - High-throughput LC-LRMS and FIA-LRMS fingerprinting and polyphenolic profiling for the geographical characterization and authentication of honey, Meeting Room 106, August 20, 2024, 15:25 - 15:44

Honey is a natural product produced by bees from nectar and other non-floral secretions. It is highly appreciated by society as a natural sweetener and for its important health benefits, mainly due to the presence of bioactive substances such as polyphenolic antioxidants. The great diversity of botanical varieties and countries of production has given rise to products with a disparity in quality and prices, also increasing fraudulent practices. In this line, the development of methods capable of characterizing honey and authenticating and certifying not only its botanical varieties but also its geographical origin, is essential in order not to generate distrust in society or economic losses in the beekeeping sector.

Methods

A C18 reversed-phase LC-LRMS fingerprinting methodology using a QTrap mass analyzer in full scan acquisition mode (m/z 100-550) and an LC-LRMS polyphenolic profiling methodology by monitoring 53 polyphenols in MRM acquisition mode were proposed to address geographical characterization and authentication of honey with chemometrics. In addition, high-throughput FIA-LRMS fingerprinting and polyphenolic profiling methodologies were also evaluated for fast sample screening.
184 honey samples produced in different countries, including Spain and Italy (in these cases incorporating multi-floral, eucalyptus, and rosemary botanical varieties) as well as Portugal, France, The Netherlands, Serbia, Japan, China, Costa Rica, Australia, and New Zeeland, were analyzed after a simple sample treatment (1 g of honey dissolved in 10 mL of water and diluted 1:1 with methanol).

Novel Aspect

Novel and reliable FIA-MS screening and LC-MS confirmatory fingerprinting and polyphenolic profiling methods for geographical characterization and authentication of honey.

Preliminary Data or Plenary Speaker Abstract

Raw data was treated using MSConvert and mzMine 3.0.11 software and the subsequent LC-LRMS and FIA-LRMS fingerprinting and polyphenolic profiling data matrices (samples × peak signal intensity values as a function of m/z) were subjected to partial least squares regression-discriminant analysis (PLS-DA) using Solo 8.6 chemometrics software from Eigenvector Research. Very acceptable honey geographical characterization and authentication were accomplished with all the evaluated methodologies showing, in general, sensitivity, specificity, and classification rates higher than 90%. The performance was 100% when addressing honey discrimination between the different production continents, the different production countries within a specific continent, and the different botanical varieties in Italian and Spanish honey samples. PLS-DA models were also validated by employing 70% of the samples (randomly selected) as calibration sets, and the remaining 30% as the prediction (unknown sample) sets, obtaining, in general, sensitivity and specificity values higher than 90%, with classification errors below 10%.

In addition, the capability of the proposed LC-LRMS and FIA-LRMS fingerprinting and polyphenolic profiling methodologies to detect and quantify honey adulterations was also evaluated. With that aim, several adulteration cases based on blended adulterated honey produced in two different countries were also evaluated by partial least squares (PLS) regression. For that purpose, calibration models were built with adulterant-honey contents of 0, 20, 40, 60, 80, and 100%, while 15, 25, 50, 75, and 85% adulterant-honey contents were employed for prediction purposes. Good linearity, with calibration and prediction errors, in general, below 15%, were accomplished.

The obtained results demonstrate the feasibility of FIA-LRMS fingerprinting and polyphenolic profiling methodologies as high-throughput screening strategies to discriminate honey samples that may be fraudulent in relation to their production region and botanical variety, which can then be analyzed with the proposed LC-LRMS fingerprinting and polyphenolic profiling confirmatory methodologies.

Ms Yada Nolvachai[1], Mr Kevin Ghavalas[1], Ms Sherlyn Ardison[1], Dr Snehal Jadhav[1], Dr Dan Dias[1], Prof Robert Shellie[2], Professor Russell S.J. Keast[1]
[1]Consumer Analytical Safety Sensory (CASS) Food Research Centre, School of Exercise and Nutritional Sciences, Deakin University, [2]Centre for Food Safety and Innovation, Tasmanian Institute of Agriculture, University of Tasmania

Enhancing food safety and authenticity: GC–MS analysis of adulterated edible oils and early detection of foodborne pathogens

Yada Nolvachai 165 - Enhancing food safety and authenticity: GC–MS analysis of adulterated edible oils and early detection of foodborne pathogens, Meeting Room 106, August 20, 2024, 15:44 - 16:03

The assurance of food safety and authenticity is paramount in the food industry to safeguard public health and maintain consumer confidence. However, conventional workflows often fall short in high-throughput testing environments such as food testing, where they prove to be relatively time- and labour-intensive. Recognising the significance of early detection, implementing such processes becomes imperative to safeguard the integrity of the supply chain and mitigate potential risks to consumers.

Methods

The current research explores the application of gas chromatography–mass spectrometry (GC–MS) in combination with univariate and multivariate statistical analyses to develop analytical workflows for detecting adulteration and microbial contamination in foods. Specifically, it explores the application of this analytical approach in discerning adulteration in hop oil, while also showcasing its utility in early identification of foodborne pathogens, with Salmonella as a representative bacterium.

Novel Aspect

The research demonstrates GC–MS as a versatile tool for ensuring authenticity and enabling early detection of microbial contaminants in foods.

Preliminary Data or Plenary Speaker Abstract

For hop-oil authenticity analyses, this methodology integrates GC–MS analysis, following the Adams' essential oil method, and adheres to the International Organization for the Flavor Industry (IOFI) guidelines for quantification using relative response factors. The optimised method enables detection of adulteration levels of up to 10% in hop oil samples, (p-value ≤ 0.05).

For detection of microbial contamination, the developed workflow employed a headspace solid-phase microextraction (HS-SPME)-GC–MS technique to detect non-polar metabolites from spiked samples contaminated with Salmonella Typhimurium (spiking load 1 cfu/mL). The developed workflow allows the analysis to be completed within 1.5 days (i.e., 24-h enrichment, 1.5-h extraction, and 0.75-h GC–MS analysis), which is significantly more rapid than the gold standard culture-based methods that may take up to one week.

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Jessica Prenni[1], Dr. Melanie Odenkirk, Tracy Shafizaheh, Chi-Ming Chien, Steve Watkins, Selena Ahmed
[1]Colorado State University

The Periodic Table of Food Initiative

Jessica Prenni 573 - The Periodic Table of Food Initiative, Meeting Room 106, August 20, 2024, 16:03 - 16:22

What is in our food? Conventional food composition databases typically report up to 160 food components (50 components on average). Taking advantage of advances in mass spectrometry technology, the Periodic Table of Food Initiative (PTFI) is a global, non-profit effort dedicated to providing global partners with standardized analytical tools to enable the generation of a comprehensive biomolecular catalog of the world's edible biodiversity. The goal of the PTFI is to establish a public database of food composition using standardized mass spectrometry-based methods to profile compounds including metabolites, lipids, and minerals. Here, we present the results for 500 foods and discuss how this information can be utilized to address grand challenges in both human and planetary health.

Methods

500 food samples were processed through lyophilization, homogenization, and extraction for analysis using various analytical platforms. Standardized methods for each compound class were developed by PTFI in collaboration with partner laboratories. Nontargeted metabolite profiling by LC-MS included the use of a novel PTFI internal retention time standard (IRTS) reagent, standardized chromatographic conditions (reverse phase and HILIC), and detection by high-resolution mass spectrometry (Q-TOF or Orbitrap). Data were processed using a novel PTFI workflow for peak picking, alignment, and annotation. Targeted lipid profiling included custom PTFI lipid internal standards, and analysis was performed using a SelexION DMS-MS. Ionomics, targeting 26 elements, was performed following microwave digestion of samples using ICP-MS.

Novel Aspect

Characterizing the biomolecular composition of 500 commonly consumed foods using standardized analytical methods with the Periodic Table of Food Initiative.

Preliminary Data or Plenary Speaker Abstract

The standardized PTFI methods were utilized to characterize the biomolecular composition of 500 foods, showcasing a wide array of diversity spanning 250 unique species and 56 unique food ontologies from Actinidiaceae to Zingiberaceae. These 56 ontologies can be categorized into six FoodOn categories, a globally harmonized food ontology system describing food from production to consumption. The 500 foods included 46 plant species (10 vegetables and 36 fruits), 19 animal species, algae, fungi, bacteria, prepared meals, and processed foods. Foods originate from 11 distinct countries and encompass various production methods (e.g., organic vs. conventional) and preparation techniques (e.g., raw vs. cooked).
Using a custom PTFI internal standard mix, non-targeted metabolite data was retention time aligned. Detected features were filtered based on consensus detection across three independent laboratories. Annotation was assigned based on matching features to the PTFI library, which includes both authentic standards and known-unknowns (molecular formula and aligned retention index). Data processing led to the detection of over 12,450 confident metabolites, represented by a normalized relative abundance. Solanaceae was found to be the organism family with the greatest diversity of unique metabolites followed by Leguminosae, Rosaceae, and Poaceae. The lipidomics assay identified approximately 1,800 lipids, spanning multiple lipied classes. Lipid concentrations (absolute quantitation) were normalized using the custom PTFI lipid standard mix, which includes

multiple lipid standards per class. Ionomics analysis detected 26 elements in each food, with concentrations varying over 6 orders of magnitude between elements and across food types. The overall chemical profiles were evaluated across and between ontological families, highlighting the chemical diversity across foods in terms of both qualitatively annotated or detected compounds and quantitative differences. These data collectively demonstrate the chemical diversity across foods and provide a valuable resource for future studies to understand the relationship between food composition and human and planetary health.

Doctoral Student Takumi Fujiki[1]
[1]Hokkaido University

Varietal differences in distribution of soluble carbohydrates and organic acids in strawberry fruits visualized using quantitative MALDI-TOF MS imaging

Takumi Fujiki 484 - Varietal differences in distribution of soluble carbohydrates and organic acids in strawberry fruits visualized using quantitative MALDI-TOF MS imaging, Meeting Room 106, August 20, 2024, 16:22 - 16:41

The taste of strawberry fruits is affected by soluble carbohydrates and organic acids. Not only total content but also distribution of these compounds in a fruit can affect the taste. MALDI-TOF MS imaging (MSI) is available for visualizing distribution of such compounds in a tissue specimen. A disadvantage of MSI is that it was difficult to compare two or more specimens, since ion intensity did not reflect the absolute content of the compound. To conquer this difficulty and to make comparison of fruits from different cultivars possible, we used five consecutive specimens, one for MSI and four for HPLC quantification. Then, each MS image was corrected by incorporating the quantified value with the total ion intensity of MSI.

Methods

Matured fruits of six strawberry cultivars (‘Saga i9’, ‘Mouikko’, ‘Yumenoka’, ‘Suzuakane’, ‘HS-138’, and ‘Natsunoshizuku’) were used. Three fruits/cultivar were treated in the same manner. Four consecutive longitudinal sections (100 µm in thickness each) were cut from a fruit using cryo-microtome. A specimen mounted on a ITO-coated glass slide was sprayed with matrix (10 mg/mL 1,5-Diaminonaphthalene in 70% acetonitrile) at a density of 250 µL/cm², then analyzed using a MALDI-TOF MS instrument (ultrafleXtreme, Bruker Daltonics) equipped with a Nd:YAG laser(335 nm) at a irradiation interval of 350 µm in negative ion mode. Other specimens were used for quantifying soluble carbohydrates, organic acids and ascorbic acid, respectively. Incorporation of quantified value with the MS image was performed using statistical software R(version 4.1.0).

Novel Aspect

Distribution of organic acids, soluble carbohydrates and ascorbic acid in strawberry fruits was quantitatively visualized using MALDI-TOF MSI.

Preliminary Data or Plenary Speaker Abstract

The molecular ion peaks of 179.06 m/z = [hexose−H]−, 341.11 m/z = [sucrose−H]−, 133.01 m/z = [malic acid−H]−, 191.02 m/z = [citric acid−H]− and 175.02 m/z = [ascorbic acid−H]− could be confirmed in the mass spectrum. The peak related to dehydroascorbic acid could not be detected, since a fragment ion peak of the matrix had overlapped. The hexose peak might be originated from both fructose and glucose, since they were detected from the specimen at a concentration similar to each other using HPLC.
On the distribution of organic acids in MS image of the fruit, malic acid was mainly localized at the pith of receptacle. This tendency was clearly observed in ever-bearing cultivars rather than June-bearing cultivars. By contrast, accumulation of citric acid was confirmed in the cortex of receptacle regardless of the cultivar. As for soluble carbohydrates, hexose concentration was high in the pith, whereas sucrose concentration was high in the inner tissue of cortex, at just outside of the pith. Ascorbic acid was detected from almost all region of the fruit flesh, but the concentration was relatively high at the pith. In these cases, differences in distribution of the compounds among fruits from the same cultivar was small. However, several varietal differences could be observed: ascorbic acid was localized at the top of fruits in ‘Natsunoshizuku’; hexose concentration at the pith was quite high in ‘HS-138’; sucrose accumulation was observed at the top of fruits in ‘Saga i9’; total level of sucrose was low in ‘HS-138’.

In conclusion, it will become easy to compare the distribution of phytochemicals in fruits using visual data constructed by quantitative MALDI-TOF MSI, which can be available for breeding new cultivars with good taste fruits.

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Miss Arundhati Singh[1], Professor Joshua Mylne[1], Dr Ayalsew Zerihun[1], Mr Mark Gibberd[1], Mr Jordi Muria-Gonzalez[1]

[1]Centre for Crop and Disease Management, Schol of Molecular and Life Sciences, Curtin University

Arundhati Singh 447 - Mass spectrometry to unveil the foliar distribution of fluxapyroxad within fungicide seed-treated barley, Meeting Room 106, August 20, 2024, 16:41 - 17:00

Barley is the fourth most cultivated cereal globally, playing an important role in the agricultural sector. However, protecting barley from diseases, especially during seed and early growth stages, challenges growers. These challenges compromise barley yield and affect the quality of malt and beer, emphasising the critical role of fungicides in crop protection. Fluxapyroxad is a seed dressing fungicide commercially available as Systiva®. It is used to manage leaf- and soil-borne diseases and is a game changer for barley. Therefore, the study aims to determine the distribution and concentration of fluxapyroxad in Systiva-treated barley, focusing on its efficacy against foliar diseases.

Methods

This study employs an Orbitrap Exploris-120 to quantitatively assess the distribution of fluxapyroxad within plant tissues in Systiva®-treated barley. The samples were collected at different barley time points and extracted using the original QuEChERs, followed by the dispersive solid phase extraction method. They were then analysed using tandem mass spectrometry with the help of the C18 column and acetonitrile with 0.1% formic acid as the mobile phase, focusing on single ion monitoring to enhance selectivity and sensitivity.

Novel Aspect

This research aims to pinpoint optimal fungicide application times, enhancing its efficacy, minimising environmental impacts, and ensuring sustainable barley production.

Preliminary Data or Plenary Speaker Abstract

Our results conclude variable fluxapyroxad concentrations within and between leaf layers, with higher concentrations in tips and the older leaves. This observation is evidence of the translocation of fluxapyroxad through the xylem system in planta. These variations in fungicide concentration question the common belief that fungicide protection is available throughout the plant.

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Professor Perdita Barran[1]
[1]The University of Manchester

Visualizing Molecules: The role of Ion Mobility Mass Spectrometry where m/z selection is not enough

Keynote: Professor Perdita Barran The University of Manchester 759 - Visualizing Molecules: The role of Ion Mobility Mass Spectrometry where m/z selection is not enough, Meeting Room 109, August 20, 2024, 15:00 - 15:25

The availability of the technique of ion mobility mass spectrometry has increased substantially over the past 20 years. Once the preserve of a few academic groups with home-built instruments, there are now many commercial offerings that have allowed a far wider uptake of this hybrid method. Whilst it has undoubted importance in many workflows bringing increased sensitivity, reduced chemical noise and confirmation of chemical identity, IM-MS has had the largest impact in providing rotationally averaged collision cross sections of m/z selected ions. This talk will focus on its use where m/z data is not sufficient and provide an outlook for its future use in light of improved predictive methods.

Methods

In a typical ion mobility mass spectrometry experiment, a narrow pulse of ions enter a cell filled with an inert buffer gas which they are propelled through with electric fields whilst being retarded by collisions with the gas. The time spent in the cell is measured and can be related to the rotationally averaged, temperature dependent collision cross section of m/z selected ions. We have applied this technique to many different molecular species including proteins, lipids and supramolecular molecules with a range of ionisation and sample transfer methods. For non-covalent complexes gentle transfer is most effective in retaining aspects of structure from the solvated state.

Novel Aspect

Use of IM-MS to examine conformational distributions of proteins and supramolecules and isobaric distributions of lipids.

Preliminary Data or Plenary Speaker Abstract

Our research with IM-MS has focussed on measurements where high accuracy mass spectrometry does not provide all of the information possible from a gas phase measurement on the molecule(s) of interest. This talk will consider such cases. It will describe the use of IM-MS to measure the conformational landscapes adopted by intrinsically disordered proteins and proteins with intrinsically disordered regions, lipids and supramolecular complexes all of which are challenging to examine with other structural characterisation methods. It will consider the advantages and biases of using IM-MS and also provide an outlook on the integration of AI developed computational approaches trained on IM-MS to discern complex conformations.

Hayden Thurman, Atena Tajaddodi, **Professor Alexandre Shvartsburg**[1]
[1]Wichita State University

Novel Differential Ion Mobility Approaches Based on the Macromolecular Dipole Alignment

Alex Shvartsburg 568 - Novel Differential Ion Mobility Approaches Based on the Macromolecular Dipole Alignment, Meeting Room 109, August 20, 2024, 15:25 - 15:44

Ion mobility spectrometry (IMS) has become a powerful tool for separations prior to MS. Nonetheless, linear IMS capturing the ion mobility (K) in generally moderate electric field (E) is tightly correlated with MS because of the mobility/mass dependence. Differential IMS exploiting the high-field mobility increment is much more independent of ion mass and provides superior isomer resolution at equal resolving power. Most differential or field asymmetric waveform IMS (FAIMS) methods rely on the field-dependent ion heating. However, many proteins, complexes, and other macromolecules are strong dipoles, aligned by sufficient field without significant collisional heating. The resulting low-field differential (LOD) IMS can characterize the dipole magnitude and ion aspect ratio, and distinguish the conformers by those properties largely orthogonal to mobility.

Methods

While the optimum FAIMS waveforms are rectangular, most systems adopt the bisinusoidal profile for the engineering constraints of high-voltage electronics. The much lower voltages needed for LODIMS (~1 kV) permit arbitrary rectangular waveforms, which we synthesized by digital key switching and combined with planar-gap cells for maximum resolution. The near-ideal waveforms with two field points double the resolution versus the bisinusoidal analogs and enable more accurate and robust dynamic modeling of measurements to extract the dipole properties. The curved-gap FAIMS units focus or defocus ions depending on the waveform polarity and second derivative of K(E) function defining the A/B/C ion types. This allows fractionating the protein conformers by that metric complementary to the first derivative that broadly governs FAIMS separations.

Novel Aspect

New IMS approaches based on dipole alignment in low field and ensuing focusing in inhomogeneous fields enable novel macromolecular separations.

Preliminary Data or Plenary Speaker Abstract

Locking dipoles in the nitrogen gas at ambient pressure and temperature fundamentally requires the moments over ~400 Debye. Those for proteins tend to increase with size and charge state, for the denatured conformations (produced by ESI) crossing that level at ~25 kDa. The alignment of larger proteins such as Alcohol Dehydrogenase (ADH, 37 kDa) and Bovine Serum Albumin (BSA, 66 kDa) in differential IMS is manifested by intense bands at compensation voltages (CVs) below zero which scale linearly with the waveform amplitude (dispersion voltage, DV) and extend to extreme absolute values.

The new LODIMS stage employing a rectangular waveform with flexible duty cycle and DV up to 1.2 kV is coupled to the Velos ion trap and Orbitrap MS platforms and evaluated for ADH, BSA, and further proteins including Concanavalin A in comparison with near-isobaric trypsin (25 kDa). As with the bisinusoidal waveforms, the aligned conformers emerge at CV < 0 with rotary species near bias. The onset threshold at negative CVs remains linear versus DV, but the slope about doubles expectedly in proportion to the <F3> quantities for two profiles. The ongoing assessment across the duty cycles will facilitate grasping this matter better and picking the best waveform for LODIMS applications.

Same proteins were examined using the Dome-geometry cell (comprising the cylindrical and hemispherical regions) in FAIMS and LODIMS regimes. We see the ion transmission with both negative and (not previously observed) positive waveform polarities. The selected conformer subpopulations apparently differ, establishing new IMS approach based on the ion focusing

properties in inhomogeneous field which reflect the local curvature of K(E) functions rather than their increment controlling the CVs. This capability is now advanced by adding the rectangular waveforms to Dome cell for both improved separation and stronger focusing.

Therese M. Fulloon[1,2], Dr. Venkateswara R. Narreddula[1,2], Dr Samuel Brydon[1,2], Dr Berwyck Poad[1,2], **Associate Professor Tim Causon**[3], Professor Stephen Blanksby[1,2]
[1]Central Analytical Research Facility, Queensland University of Technology, [2]School of Chemistry and Physics, Queensland University of Technology, [3]BOKU University, Department of Chemistry, Institute of Analytical Chemistry

The role of unsaturation in defining the 3-dimensional structure of ionised lipids in the gas phase

Tim Causon 545 - The role of unsaturation in defining the 3- dimensional structure of ionised lipids in the gas phase, Meeting Room 109, August 20, 2024, 15:44 - 16:03

The lipidome's complexity and structural diversity represents a frontier challenge for analytical technologies to separate and identify individual molecular components among many hundreds or thousands of similar structures. Ion mobility-mass spectrometry (IM-MS) is emerging as one means to address this challenge, with its ability to rapidly resolve ionised lipids based on differences in shape, charge and mass. Further, IM-derived collision cross sections (CCS) are increasingly being utilised to identify lipids and differentiate isomers. Despite this, the influence of primary molecular structure on lipid ion CCS is less-well-understood and thus less predictable when compared to other biomolecular classes. Herein we systematically explore the influence of position of unsaturation on alkyl chain on the structure(s) of ionised lipids in the gas phase.

Methods

A test set was generated comprising eleven different monounsaturated fatty acids with carbon-carbon double bond positions ranging from n-3 to n-15 (relative to the methyl terminus) and including a subset of cis- and $trans$-stereoisomers. These fatty acids were derivatized as 3-pyridylcarbinol amides (PA), 3-pyridylcarbinol esters (PE) and N-methyl-pyridinium-3-methanamines (N-MePA). Methanolic solutions of each compound were ionised by electrospray ionisation to form variously, $[M-H]^-$, M^+, $[M+H]^+$ and $[M+Na]^+$ ions. Accurate $^{DT}CCS(N_2)$ measurements were made for each ion using an Agilent Drift Tube Ion Mobility-Q-TOF Mass Spectrometer (DTIM-QTOFMS) while high-resolution ion mobility measurements were used to study the same precursors using both Waters Cyclic Ion Mobility (cIM-MS) and MOBLIon Structures for Lossless Ion Manipulation (SLIM-MS) instrumentation.

Novel Aspect

Combining accurate and high-resolution ion mobility achieves separation of isomeric unsaturated lipids.

Preliminary Data or Plenary Speaker Abstract

Previous gas phase ion spectroscopy measurements have highlighted the strong influence of the intramolecular interactions between protonated ammonium cations and carbon-carbon double bonds in defining the 3-dimensional structure of ionised lipids. Based on these insights, the $[M+H]^+$ cations from PE and PA derivatives of FA 18:1 isomers were investigated as a function of the carbon-carbon double bond position. When cis double bond stereochemistry was considered, $^{DT}CCS(N_2)$ values for the 3-pyridylcarbinol amide derivatives ranged from 198.3 Å² for PA 18:1n-3,cis through to 203.8 Å² for PA 18:1n-15,cis. This indicates a general trend of more compact structures wherein the double bonds are near the methyl terminus of the fatty acid, and more expanded structures when the double bond is proximate to the carbonyl moiety. The latter showed a greater similarity to saturated homologues with PA 18:0 ($^{DT}CCS(N_2)$ of 204.4 Å²). The significance of the interaction of the N-H proton and the double bond in defining the structure of these ions is supported by the much greater similarity in $^{DT}CCS(N_2)$ values for the same set of fatty acids when derivatized as a fixed charge N-Me moiety. While some of the measured $^{DT}CCS(N_2)$ differences are sufficiently large for unique identification, many were too small to be resolved on conventional IM-MS (e.g., the PA 18:1cis,n-7 and n-9 with 200.0 Å² and 200.2 Å², respectively,). However, investigations using high-resolution IM-

MS demonstrated that ionised lipids with CCS differences as small as 0.2 Å² could be resolved, indicating that (i) DTIM-MS CCS measurements are accurate despite the lower resolving power and (ii) small differences in 3-dimensional structure of lipid isomers could be exploited for resolution and identification of lipid isomers in complex mixtures. Computational modelling will be presented that provides insight into how the measured CCS differences of these ionised lipids are influenced by the position of unsaturation.

Ms Olivia Rusli[1], Mr Oscar Lloyd Williams[1], Mr Kevin Hes[2], Dr Sjors Bakels[2], Prof.dr. Anouk Rijs[2], Dr Nicole Rijs[1]
[1]School of Chemistry, UNSW Sydney, [2]Division of BioAnalytical Chemistry, Vrije Universiteit Amsterdam

Understanding the Coordinative Selectivity of Glyphosate and AMPA Toward Divalent Metals by IM-MS and IM-IRMPD-MS

Olivia Rusli 192 - Understanding the Coordinative Selectivity of Glyphosate and AMPA Toward Divalent Metals by IM-MS and IM-IRMPD-MS, Meeting Room 109, August 20, 2024, 16:03 - 16:22

Glyphosate (N-phosphonomethylglycine) is the most popular herbicide in the world. Both glyphosate and its primary metabolite, aminomethylphosphonic acid (AMPA), are zwitterionic and have multiple groups that can coordinate metals, behaving as ligands. Both glyphosate and AMPA form a myriad of metal complexes. Despite being ubiquitous, the structures of these metal complexes and their preferred speciation remain largely unknown. It has been shown that specific structures of these complexes dictate their characteristics and behaviours. Therefore, a detailed understanding of structure and speciation is desirable to better understand their environmental fate and potential indirect biological mechanisms.

Methods

Glyphosate and AMPA complexes were characterised using ion mobility mass spectrometry (IM-MS). Solutions with M = Mg2+, Ca2+, Sr2+, Ba2+, Mn2+, Co2+, Cu2+, or Zn2+ were ionised using electrospray ionisation and analysed using cross platform IM-MS to robustly characterise the complexes under different instrumental conditions. These included drift tube, trapped ion, travelling wave (TWIMS), and cyclic ion mobility spectrometry separations. Target [M+Glyphosate-H]+, [M+AMPA-H]+, [2Glyphosate+M-H]+ and [M+2AMPA-H]+ complexes were thoroughly investigated. A custom Photo-Synapt, which combines TWIMS with Infrared multiphoton dissociation (IRMPD) spectroscopy, was used to investigate the IR absorption in the 2700-3700 cm-1 region of the mass- and mobility-selected species of interest. DFT was employed to predict the gas-phase structures and vibrational frequencies of glyphosate and AMPA metal complexes.

Novel Aspect

IM-IRMPD-MS and cross-platform IM-MS definitively revealed that cations bind to the phosphonate group in glyphosate-metal complexes.

Preliminary Data or Plenary Speaker Abstract

Initial measurements of Glyphosate and AMPA complexes without mass selection show that [M+Glyphosate-H]+, [M+AMPA-H]+, [M+2Glyphosate-H]+ and [M+2AMPA-H]+ all have structural isomers well separated by their distinct CCS across all IM-MS platforms. Surprisingly, the isomers observed on the different IM-MS platforms are not always consistent which could be a result of varying ionisation sources and mobility types. Implementing pre-mobility mass selection proved to be important, however, confirming that for [M+Glyphosate-H]+ and M+AMPA-H]+, there are indeed multiple isomers, but for [M+2Glyphosate-H]+ and [M+2AMPA-H]+, there is only 1 structural isomer. Some of the isomers observed initially were a result of post-mobility dissociation of higher order assemblies, a result of the modular formation of glyphosate assemblies. Comparison between the experimental and calculated collision cross sections (CCS) revealed a “common” conformation favoured across the various metals investigated.
For [M+Glyphosate-H]+, deprotonation occurs on the phosphonate group, allowing the metal ion to bind. The metal ion is surrounded by glyphosate, maximising the interaction between the cation and the electron rich carboxylic acid and amine groups. A similar trend is also observed for [M+AMPA-H]+, where deprotonation also occurs at the phosphonate group and the metal cation sits centrally around AMPA allowing maximum interaction with the electron rich groups. Preliminary IRMPD spectroscopic results for the mass and mobility selected [Ba+Glyphosate-H]+ ion support this

conclusion. Two intense peaks at 3550 cm-1 and 3650 cm-1 were observed, agreeing with the theoretical IR spectrum of the previously described conformation. Other conformations yielded theoretical spectra where these peaks were not observed. This has eliminated several candidate conformations that could not previously be distinguished by the comparison of CCS values alone.

Dr. Yimin Wang[1], Dr Melvin Gay[3], Dr. Andres Bernal Escobar[1], Dr. Ruey Leng Loo[1,2]
[1]Australian National Phenome Centre, Murdoch University, [2]Centre for Computational and Systems Medicine, Health Futures Institute, Murdoch University, [3]Bruker Pty Ltd.

Simultaneous Polyphenol Profiling and Quantification with LC-TIMS-TOF-MS: An Application to Different Apple Matrices

Yimin Wang 298 - Simultaneous Polyphenol Profiling and Quantification with LC-TIMS-TOF-MS: An Application to Different Apple Matrices, Meeting Room 109, August 20, 2024, 16:22 - 16:41

Mass spectrometry (MS)-based metabolomic analysis of food has significantly contributed to food safety testing, nutritional assessment, food authentication, and identification of bioactive compounds. Liquid chromatography coupled with trapped ion mobility spectrometry and time-of-flight mass spectrometry (LC-TIMS-TOF-MS) provides unambiguous collision cross-section (CCS) data, along with information on retention time (RT), precursor ions, and fragments. While this technique has been successfully applied for identification and quantification of proteins, its application in small molecular analysis remains limited compared to LC-TOF-MS. The overarching goal of this study is to evaluate the feasibility of comprehensively performing chemical profiling and achieving absolute quantification of key polyphenols that have been linked to health benefits, using three apple products (whole apple, apple juice, and apple pomace) as exemplars.

Methods

Apples were de-cored. Half were used to prepare juice and pomace, while the other half remained whole apples (N=10 for each matrix). Whole apples and pomace were freeze-dried, while the juice was filtered. All samples were prepared using methanol-water for polyphenol extraction. LC-TIMS-TOF-MS and LC-TOF-MS analyses utilised the same LC setting and an HSS T3 LC column. Profiling and quantification of 10 polyphenols, incorporating internal standard (catechin-13C3) and calibration curves were conducted using the PASEF scan for LC-TIMS-TOF-MS and bbCID scan for LC-TOF-MS. MetaboScape 2024 was applied to extract information on m/z, RT, CCS, and peak intensity. Extracted profiles underwent principal component analysis (PCA), and the reproducibility was assessed using pooled quality control (PQC) samples. TASQ software facilitated polyphenols quantification.

Novel Aspect

The LC-TIMS-TOF-MS resolves polyphenol isomer ambiguities, enabling simultaneous quantification in diverse food matrices rich in polyphenols.

Preliminary Data or Plenary Speaker Abstract

The LC-TIMS-TOF-MS was applied to profile and quantify all apple matrices, demonstrating comparable results to the LC-TOF-MS. All apple matrices show numerous polyphenols presented as sugar conjugates, with quercetin 3-galactoside and quercetin 3-glucoside dominating. Quercetin 7-glucoside, quercetin-3-rutinoside, quercetin 3-rhamnoside, and quercetin 3-arabinoside were also detected. Separate PCA was constructed on each MS dataset, revealing three distinct clusters for each matrix, along with a tight cluster of PQC samples, demonstrating high reproducibility across both MS datasets.
Catechin and its cis configuration (epicatechin) were separated by RT (3.66 min, and 4.48 min, respectively). LC-TIMS-TOF-MS provided unambiguous identification through CCS values for catechin (156.8 Å²) and epicatechin (167.7 Å²) with negative ion polarity, a capability lacking in the LC-TOF-MS system. Notably, LC-TIMS-TOF-MS enabled the identification of three quercetin sugar conjugates: quercetin 3-galactoside (CCS 201.5 Å², RT 6.12 min), quercetin 3-glucoside (CCS 203.4 Å², RT 6.16 min), and quercetin 7-glucoside (CCS 213.0 Å², RT 5.67 min). Given the known variability in RT between batches and systems, CCS information offers a robust parameter and increased confidence for polyphenol identification or annotation.
Ten polyphenols (catechin, epicatechin, procyanidin B2, quercetin, quercetin 3-galactoside, quercetin 3-glucoside, quercetin 7-glucoside, quercetin-3-rutinoside, chlorogenic acid, and phloridzin) were

quantified using both MS systems, demonstrated good reproducibility based on the PQC samples (CV typically <20%). LC-TOF-MS shows lower limits of quantification for catechin at 0.056 µg/mL, enabling its quantification (CV 6.3%) but was not achievable with LC-TIMS-TOF-MS. Both MS systems showed comparable quantified polyphenol results between apple matrices; strong correlations ($R^2 > 0.9$) and deviations typically < 25%, e.g., phloridzin concentrations varied: whole apple 3.81 to 6.67 µg/mL, juice 1.83 to 3.29 µg/mL, pomace, 4.95 to 7.77 µg/mL via LC-TIMS-TOF-MS, and 4.33 to 7.00 µg/mL, 2.10 to 3.37 µg/mL, 4.94-8.71 µg/mL via LC-QTOF-MS.

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Dr Jackie Mosely[1], Dr Martin Palmer[2], Dr Adam King[2], Dr Steph King[2], Prof Mike Bowers[3], Dr Kalju Khan[3], Dr Eddie Clayton[4], Prof Jim Scrivens (deceased)[5]
[1]University of York, [2]Waters Corporation, [3]University of California Santa Barbara, [4]Consultant, [5]Teesside University

Structures for ion mobility resolved radical cations of benzocaine, and consequences for dissociation.

Jackie Mosely 565 - Structures for ion mobility resolved radical cations of benzocaine, and consequences for dissociation., Meeting Room 109, August 20, 2024, 16:41 - 17:00

Ion mobility has been shown to separate and characterise a number of molecules that form differentiated protomer structures. These structures are shown to have characteristic fragmentation processes that enable the site of protonation to be established. It is known that radical cations can also exist as a number of structural forms, based on the site of ionisation, and that these sites can direct fragmentation. Until recently it was not possible to isolate and study these species within the mass spectrometer. Now different structural forms of radical cation can be separated and individually studied by MS/MS, and dissociation mechanisms, supported by computational modelling, deduced.

Methods

A cyclic ion mobility separation-enabled quadrupole time-of-flight (Q-cIM-oaToF) mass spectrometer (SELECT SERIES Cyclic IMS) was equipped with Atmospheric pressure Solids Analysis Probe (ASAP) ionisation (Waters, Wilmslow, UK). The ion source region was kept free from all sources of protic solvent to ensure radical cations were generated.

Between 1-5 mg ethyl 4-aminobenzoate, otherwise known as the pharmaceutical ingredient benzocaine (Acros Organics, Thermo Fisher Scientific, US) was dissolved in 1 mL methanol. 2 µL aliquots were deposited on to the probe for analysis. Instrument parameters were tuned for optimal signal intensity and duration.

Computation was achieved by combining density fitting to avoid 4-centre e-repulsion integrals with orbital optimized MP2, MP3 or OLCD in aug-cc-pVTZ basis implemented in Psi4.

Novel Aspect

Ion mobility separation of radical cations; effect of radical cation structure on dissociation mechanism and complementary computational approaches

Preliminary Data or Plenary Speaker Abstract

After 15 passes through the cyclic device ($\Omega/\Delta\Omega = 250$) the radical cation resolved into two distinct components, estimated to have rotationally averaged collisional cross-section (CCS) measurements of 134 and 136 $Å^2$. These structures were found to have significantly different stabilities and dissociate to give significantly different product ion spectra.

Fragmentation profiles generated over a range of collision energies shows that the more compact structure dissociates primarily to m/z 150 through loss of CH_3 radical, yet this is not a simple mechanism to explain. Analysis of D5-ethyl 4-aminobenzoate radical cations also mobility-separated into two structures, and MS/MS of the more compact form has shown a way forward to explain the likely dissociation mechanism, supported by computational modelling and study of the fragmentation profile of protonated 5-aminophthalide.

Fragmentation profiles generated over a range of collision energies shows that the more elongated structure dissociates primarily to m/z 137 and 120 through loss of C_2H_4 and C_2H_5O respectively.

Analysis of the elongated radical cation of D5-ethyl 4-aminobenzoate shows how the ethyl group interacts with the aromatic ring, and that H/D scrambling occurs as a function of residence time in the cyclic track and is independent of collision energy. In further experiments, the elongated form is seen to convert to the compact form, yet there is a clear barrier preventing the reverse action.

Computational modelling and CCS measurements for the different product ions have been used to explore potential transition states for proposed dissociation mechanisms, leading back to original radical cation structures.

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Prof. Isabelle Compagnon[1]
[1]University of LYON

Interrogating structure and dynamics of molecular ions from the IR to the XUV domain for fundamental Physical-Chemistry and Analytical applications.

Keynote: Professor Isabelle Compagnon University of Lyon, France 767 -Interrogating structure and dynamics of molecular ions from the IR to the XUV domain for fundamental Physical-Chemistry and Analytical applications, Meeting Room 110, August 20, 2024, 15:00 - 15:25

Mass Spectrometry and Laser Spectroscopy have worked hand in hand in the past 20 years to bring the best of both worlds and push the limits of Molecular Sciences. The recent development of stable table-top High Harmonic Generation laser sources allows for the study of XUV irradiation of ions of unprecedented complexity, as well as ultrafast time-resolved experiments. On the other hand, the integration of IR spectroscopy into MS schemes has never been so seamless thanks to compact and high repetition rate commercial mid-IR sources, offering an additional level of structural characterization to a variety of Analytical Chemistry workflows. A selection of novel ion spectroscopy schemes and recent results will be presented.

A selection of cutting-edge performance of ion spectroscopy will be presented, ranging from ultrafast charge dynamics and nuclei rearrangements in proteins to the detection of minute structural disruptions in polysaccharides.

Methods

Due to the low density of ions in a mass analyser, ion spectroscopy challenges traditional spectroscopic schemes and new observables must be considered. Static and time-resolved photoinduced measures compatible with Mass Spectrometry will be presented, as well as their interpretation in terms of molecular properties.

Preliminary Data or Plenary Speaker Abstract

A selection of cutting-edge performance of ion spectroscopy will be presented, ranging from ultrafast charge dynamics and nuclei rearrangements in proteins to the detection of minute structural disruptions in polysaccharides.

Prof. Chi-Kit (Andy) Siu[1]
[1]City University of Hong Kong

Impact of Solvent Reorganization on Disulfide Bond Cleavage in Hydrated Electron Clusters

Chi Kit (Andy) Siu 706 - Impact of Solvent Reorganization on Disulfide Bond Cleavage in Hydrated Electron Clusters, Meeting Room 110, August 20, 2024, 15:25 - 15:44

Hydrated electron, e−(aq) is a powerful reducing agent in organic synthesis, atmospheric and radiation chemistry with noteworthy biological implications. Its formation and properties have sparked debate among scientists, yet with few insights into its reactions in solvents. To date, reaction mechanism involving e−(aq) remains as an unsolved puzzle. Given its intrinsic structural variability, interaction of e−(aq) with vicinal chemical environment likely depends on its solvation structure and interplay with reorganization of water molecules. Investigating the solvent reorganization associated with a charge transfer from e−(aq) to neighboring chemical species offers valuable insights into its underlying reaction mechanism.

Methods

Reaction dynamics of a chemical model comprising a dimethyl disulfide, the prototypical molecule containing a S—S bond, and a hydrated electron cluster with six water molecules, [CH3SSCH3(H2O)6•–], at 100 K (comparable with the temperature in a previous FT-ICR mass spectrometric study) has been examined using density functional theory based molecular dynamics (DFT-MD) simulations performed with the CP2K Quickstep module. The revPBE functional with restricted open-shell formalism and a triple-ζ basis set with two polarization functions were used. Reaction energy profile was constructed using constraint DFT-MD simulations.

Novel Aspect

Importance and details of solvent reorganization and its influence toward reaction dynamics of hydrated electron has been theoretically revealed.

Preliminary Data or Plenary Speaker Abstract

Intracluster electron transfer in [CH3SSCH3(H2O)6•–] gives [CH3SSCH3•–(H2O)6], in which the reduced CH3SSCH3•– anion is greatly stabilized by the water cluster. Our DFT-MD simulations show that this process is barrierless and highly exothermic (ΔH = −166 kJ mol–1) and exergonic (ΔG = −100 kJ mol–1), which is comparable with the value of −113 ± 13 kJ mol–1 determined by Beyer and co-workers based on the nanocalorimetry approach performed with a FT-ICR mass spectromter. The substantial decrease in reaction entropy (TΔS = −66 kJ mol–1) in this electron transfer process is unexpected! This results from both inner and solvent reorganizations, which involve formation of a hydrogen bond between the hydrated electron cluster and a sulfur atom, followed by a rapid elongation of the S—S bond and subsequent solvent reorganization. This reaction period spans from hundreds of femtoseconds to a few picoseconds. Three distinctive stages of solvent reorganization have been identified: (1) Solvent relaxation to recover a frustrated solvent cavity originally occupied by the hydrated electron; (2) solvent migration to facilitate formation of hydrogen bonds in the vicinity of the S—S bond; (3) further solvent reorganization to establish an improved solvation shell around the anionic 2-center-3-electron S—S•– hemibond.

Professor Frantisek Turecek[1], Jiahao Wan[1], Hongyi Zhu[1], Kim Vu[1], Professor Karel Lemr[2], Marianna Nytka[2]
[1]University of Washington, Seattle, [2]Palacky University

Nitrile Imines as Photochemical Crosslinkers in Gas-Phase Peptide Ions: Reactivity and Action Spectroscopy

Frantisek Turecek 89 - Nitrile Imines as Photochemical Crosslinkers in Gas-Phase Peptide Ions: Reactivity and Action Spectroscopy, Meeting Room 110, August 20, 2024, 15:44 - 16:03

Photochemical crosslinking in gas-phase ions and peptide-DNA complexes has been introduced as a method to study biomolecular structures and dynamics. Emphasis up to date has been on carbene-based crosslinking induced by photodissociation of diazirine-tagged ions. A new approach to be presented utilizes nitrile-imine based crosslinking in gas-phase ions as a promising strategy to ion structure analysis that offers high efficiency and has the potential for wide ranging applications.The features that characterize gas-phase crosslinking include (1) well defined stoichiometry of the complexes due to mass-selective isolation, (2) facile reaction monitoring and yield determination, and (3) post-crosslinking structure analysis by tandem mass spectrometry that has been combined with hydrogen-deuterium exchange, UV-vis action spectroscopy, and ion mobility.

Methods

Photodissociation of gas-phase cations produced by electrospray ionization was performed at 250 and 214 nm, using a Bruker amaZon ion trap and Orbitrap Ascend Tribrid mass spectrometers, respectively. Peptides were tagged with a 2,5-diaryltetrazole group at the lysine side chain amine or cysteine thiol group. UV-vis action spectra were taken in a CID-UVPD-MS3 mode on the Bruker instrument at 210-700 nm. The light beam was provided from an NL301G (Altos Photonics) Nd-YAG laser and an PG142C unit that incorporated a 3rd-harmonic generator and an OPO coupled with with an optional 2nd harmonic generator. The laser pulse energies were measured at each wavelength and used to calibrate the action spectra.

Novel Aspect

Nitrile imines represent new transient intermediates that undergo efficient covalent coupling to peptides, resulting in crosslinking.

Preliminary Data or Plenary Speaker Abstract

Nitrile imines produced by photodissociation of
2,5-diaryltetrazoles were found to undergo cross-linking reactions with amide groups in peptide-tetrazole (tet-peptide) conjugates and a tet-peptide-dinucleotide complex. Tetrazole photodissociation in gas-phase ions was found to be efficient, achieving ca. 50% conversion with 2 laser pulses at 250 nm. The formation of cross-links was detected by CID-MS3 that showed structure-significant dissociations by loss of side-chain groups and internal peptide segments. The structure and composition of cross-linking products were established by a combination of UV−vis action spectroscopy, accurate mass measurements, and cyclic ion mobility mass spectrometry (c-IMS). The experimental absorption bands were found to match the bands calculated for vibronic absorption spectra of nitrile imines and cross-linked hydrazone isomers. The calculated collision cross sections (CCS-th) for these ions were related to the matching experimental CCS-exp from multipass c-IMS measurements. Loss of N2 from tet-peptide conjugates was calculated to be a mildly endothermic reaction with ΔH0 = 80 kJ mol−1 in gas phase ions. The excess energy in the photolytically formed nitrile imine drives endothermic proton transfer, followed by exothermic cyclization to a sterically accessible peptide amide or side-chain group. The exothermic nitrile imine reaction with peptide amides and side-chain groous is promoted by proton transfer and may involve an initial [3 + 2] cycloaddition followed by cleavage of the oxadiazole intermediate. Nitrile imine cross-linking to 2'-deoxycytidylguanosine was found to be >80% efficient and highly specific in targeting guanine. The

further potential for exploring nitrile-imine cross-linking for biomolecular structure analysis will be discussed.

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Professor Han Bin Oh[1]
[1]Sogang University

A combination of genetic code expansion and free radical-initiated peptide sequencing mass spectrometry

Han Bin Oh 166 - A combination of genetic code expansion and free radical-initiated peptide sequencing mass spectrometry, Meeting Room 110, August 20, 2024, 16:03 - 16:22

Free radical-initiated peptide sequencing mass spectrometry has been shown to be a powerful technique for fragmenting peptides and, in some cases, small proteins via a radical activation mechanism. This method involves the generation of a radical site through thermal activation at the radical precursor. Subsequently, the incipient radical can abstract a hydrogen atom to transfer the radical site to adjacent peptide backbone locations, leading to peptide backbone dissociation. In essence, this process enables the precise localization of peptide backbone cleavage sites, providing valuable insight into the cleaved site's location relative to the incipient radical site. This principle was successfully demonstrated for a small protein, revealing the proximity of certain residue areas to the N-terminal radical precursor site.

Methods

Instead of introducing a free radical only at the N-terminus, we vary the position deploying it in different sites to evaluate radical directed fragmentation as a function of radical sites aiming to elicit the gaseous geometry of the protein ion. We took advantage of the genetic code expansion technology that allows unnatural amino acids to be incorporated at specific sites. We incorporate a Aacetophenylalanine(AcF) to affibody to provide an affibody mutant followed by the conjugation of p-TEMPO-Bn-alkoxyamine to AcF generating p-TEMPO-Bn-ON-affibody-mutant (AFT(Mu)). To generate reactive benzyl radical at the mutant site, thermal activation of AFT(Mu) induces homolytic cleavage between benzylic carbon and the oxygen of the TEMPO. Further thermal activation of isolated benzyl radical ion induces radical-directed fragmentation.

Novel Aspect

A new FRIPS approach with the genetic expansion strategy enriches the toolkit available for studying gaseous protein structure.

Preliminary Data or Plenary Speaker Abstract

We address the question, does the three-helix bundle of affibody remain compact in the gas phase like the native structure? To relate the proximity between radical site and cleavage sites, calculated length of the isolated radical moiety is considered as a scale to compare with the native structure, assuming it is nearly maintained upon mutation. Structurally sensitive fragmentations are observed to vary throughout due to the varied sites of the radical suggesting collectively that the gaseous structure of affibody ion with the lowest charge state may remain compact as the native structure.

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Mr Oscar Lloyd Williams[1], Ms Tracy To[1], Ms Olivia Rusli[1], Dr Berwyck Poad[2], Dr David Marshall[2], **Dr Nicole Rijs**[1]
[1]UNSW Sydney, [2]QUT

Directing Molecular Trams on the Picoscale! Perturbing Structural Outcomes of Self-Assembly Monitored by Ion Mobility Mass Spectrometry

Nicole Rijs 190 - Directing Molecular Trams on the Picoscale! Perturbing Structural Outcomes of Self-Assembly Monitored by Ion Mobility Mass Spectrometry, Meeting Room 110, August 20, 2024, 16:22 - 16:41

Coordination driven self-assembly is a powerful supramolecular approach to generate chemical complexity and diversity. Ligands and metals subunits come together in a modular fashion into ordered structures that open the door for functional materials, enhanced catalysis, and sensors. The primary challenge of self-assembly is controlling the structural outcome of the systems. To moderate the structures formed, small molecules are often added to a dynamic ensemble of components, with the intent they will “template” to guide the self-assembly to desirable outcomes or “modulate” to introduce targeted defects. Decoding the mechanism of templating and modulating agents presents an analytical challenge. The systems are inherently complex and non-covalent interactions govern the reactivity and selectivity.

Methods

Ion mobility- mass spectrometry enabled analysis of a large combinatorial library of bis-β-diketonate ligands, metal cations and small molecule agents. A novel workflow established in 2023 by our group enables high throughput ion mobility categorisation of structure. Using automated experiment generation, it was possible to perform many cyclic 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.

Novel Aspect

Mechanistic aspects of templating and modulating agents are revealed for the first time via high throughput ion mobility- mass spectrometry.

Preliminary Data or Plenary Speaker Abstract

Through this approach it was possible to directly observe the impact of templating agents by tracking the “up regulation” or “switching off” 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. Mechanistically, it is clear that the long standing controversy about the mechanisms of such small molecule agents is warranted, as no one type of mechanistic pathway is feasible, and indeed different templates and modulators function differently.

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Prof Caroline Dessent[1], Dr Kelechi Uleanya, Ms Ambar Shaik, Dr Brett Sallach
[1]University Of York

Mapping the Photodegradation Products of Antibiotics Using Laser Interfaced Mass Spectrometry

Caroline Dessent 574 - Mapping the Photodegradation Products of Antibiotics Using Laser Interfaced Mass Spectrometry, Meeting Room 110, August 20, 2024, 16:41 - 17:00

Antiobiotic use by humans and animals has increased dramatically over recent decades, resulting in signifcant mass transfer of antibiotics into the natural environment. Currently, there is only a limited understanding of their degradation pathways, leading to concerns relating to the identity of their transformation products. Here, we apply laser interfaced mass spectrometry to map the photodegradation pathways of three common antibiotics, which are studied in vacuo within the mass spectrometer. Photodepletion cross sections for the antibiotics are acquired across the key UVA/UVB spectral range, and photodegration products are identified via mass spectrometric analysis. We observe a rich variation in the photolytic breakdown pathways for the three antibiotics, and present results to link the gas phase work to solution measurements.

Methods

Experiments are conducted using laser interfaced mass spectrometry. The mass spectrometric instrument is a commercial Bruker amaZon mass spectrometer, which has been custom adapted for performing laser spectroscopy on mass selected ions within the ion trap. The laser employed in the current experiments is a Continuum Horizon OPO. Laser photodissociation is performed at selected wavelengths, and photodepletion spectra acquired along with photofragment ions at each of the photoexcitation wavelengths. The gas-phase measurements are complemented by solution-phase on-line mass spectrometric photolysis. Higher energy collisional dissociation is also conducted for each of the three antibiotics to aid the interpretation of the laser photodissociation data, and to provide information on the molecular photodynamics

Novel Aspect

This work represents the first application of laser interfaced mass spectrometry to study photolytic breakdown of antibiotic molecules.

Preliminary Data or Plenary Speaker Abstract

We have acquired a full set of results (UVA/UVB photodepletion spectra, photofragmentation spectra - both mass spectra and wavelength scanned production spectra - and HCD data for each of the three antibiotics we have studied. These are Penicillin, Metroformin, and Oxytetracycline, all of which are common antibiotics, with each being studied here in vacuo as its protonated form. We will present the three sets of data, highlighting significant differences in the number and nature of the photoproducts produced from these three antiobiotics. In addition, we will present the results from on-line photolysis measurements of solutions of the antibiotics, and highlight similarities between the gas-phase and solution-phase data. Overall, these results illustrate the challenge in predicting photodegradation pathways for common pharmaceuticals, leading to a significant knowledge gap in assessing environmental impact of such compounds in the natural environment.

WEDNESDAY

Plenary - IMSF Curt Brunnée Award lecture, Plenary 3, August 21, 2024, 08:30 - 09:00

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Dr. Jens Soltwisch[1]
[1]University of Münster

MALDI with Laser Postionization and its benefits for mass spectrometry imaging

Matrix-assisted laser desorption/ionization (MALDI) is one of the most widely used techniques in the analysis of intact biomolecules. By design, it utilizes a focussed laser to ablate and ionize material from a solid making it a perfect match for mass spectrometry imaging (MSI). Ion yields in MALDI, however, are inherently low and somewhat restrict the analytical space to abundant and easily ionisable species. This becomes especially apparent at the minute sample amounts available for ever decreasing pixel sizes in MSI. Here, a technique based on laser postionization coined MALDI-2 can help to increase ion yields for a number of analyte classes and to improve the analysis of complex samples in MSI at high spatial resolution.

Methods

The analytical benefit of MALDI-2 for a number of analyte classes such as lipids, glycans, small metabolites, and drug-like compounds has been described in a number of publications. In parallel to its application, its underlying mechanisms have been systematically studied with regard to employed laser parameters such as wavelength, fluence and pulse duration, but also investigating delay times and ion source pressure. Since its introduction in 2015, the technique has been implemented on a number of MALDI platforms and is now also commercially available. Almost since its beginnings, it has been used to help pushing the limits of spatial resolving power in MALDI-MSI.

Novel Aspect

MALDI-2 increases the analytical depth of MALDI analysis and is especially valuable in the context of MALDI-MSI.

Preliminary Data or Plenary Speaker Abstract

MALDI-2 is based on a multi-step process that is initiated by the material ejection of the MALDI-process. The evolving plume, consisting of mainly neutral matrix and analyte molecules, is slowed down and confined by a background pressure of typically a few mbar inside the ion source and remains relatively dense for up to a few ten microseconds. In MALDI-2 a second laser pulse with a wavelength of typically 266 nm is guided into the dense plume about 10 µs after the initial MALDI event and induces resonance enhanced multiphoton ionization (REMPI) of a large number of matrix molecules in the gas phase. In secondary processes, collisions within the dense plume promote charge transfer reactions based on differences in proton affinities. Different to a direct REMPI analysis, this intermediate step facilitates the postionization of a large variety of analyte classes independent of their absorptivity or optical properties. MALDI-2 is especially powerful in the analysis of complex and unseparated mixtures. Here, the large excess of additional charges made available inside the plume helps to circumvent notorious ion suppression effects and to increase the depth of analysis. It is therefore not surprising that a major field of application of MALDI-2 lays in MSI analysis where sample processing and separation before analysis are very limited and samples are very complex. In this field the technique has been used to increase signal intensities and analytical depth in the spatially resolved analysis of lipids, metabolites, glycans and drug-like compounds. In

combination with transmission mode illumination, these benefits have also helped to decrease the employed pixel size to 1 µm² and below, paving the way towards single cell analysis.

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Highlights from the journey towards spatial omic measurements

Spatial biology bridges genotype and phenotype by providing high throughput mapping of transcripts, proteins, and metabolites. Proteins (and metabolites) are of particular interest in establishing cellular phenotypes because they are the primary effectors (and products) of biological processes, and their abundance or modification state cannot be inferred from transcripts. While current approaches allow for comprehensive and high throughput mapping of transcripts, accessing proteins (or metabolites) still represent a significant analytical challenge. This presentation will highlight the advancements and capabilities of various FTMS platforms for spatially resolved omics at (near) cellular resolution. Specifically, we will report on recent advances in spatial proteomics, metabolomics and lipidomics enabled by custom instrumentation including MALDI-Q Exactive™ UHMR mass spectrometer and hybrid FTMS systems featuring an FTICR analyzer operating at 21T, employed in MALDI, nanoDESI, or LCMS mode. Due to slower reduction in resolving power with increasing m/z for Orbitrap MS compared to FTICR MS, MALDI UHMR HF Orbitrap offered better performance and duty cycle for proteoform imaging, while 21T FTICR hybrid provided exceptional mass resolving power in lower m/z range as exemplified by isotopic fine structure measurements of metabolites and lipids across tissue sections. Finally, the newest Orbitrap – 21T FTICR hybrid, which combines the two highest performance analyzers available within one system, takes advantage of the optimal m/z regime for each FTMS method. This configuration allows for unique MS experiments, including parallel measurements with both analyzers. Exemplary applications include metabolomics and lipidomics with concurrent high resolution MS1 and DIA MS2 measurements in the FTICR and Orbitrap analyzers, respectively. Herein, we emphasize the superior performance and flexibility of these advanced FTMS platforms for comprehensive high-resolution spatial omics profiling of selected human, plant, and microbial systems.

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Dr. Tae-Hun Hahm[1], Ms. Wanyue Emily Wang[1], Dr. Maxime Siegler[1], Mr. Dalton Brown[1], Mr. Cole Johnson[1], Dr. Caitlin M. Tressler[1], Dr. Alison J. Scott[2], Dr. Scot C. Kuo[1], **Dr. Kristine Glunde**[1]
[1]Johns Hopkins University School of Medicine, [2]University of Maryland School of Dentistry

Multimodal Imaging: FluoMALDI and RaMALDI

Keynote: Professor Kristine Glunde Johns Hopkins Medical Institutions 757 - Multimodal Imaging: FluoMALDI and RaMALDI, Plenary 3, August 21, 2024, 10:00 - 10:25

We recently developed the FluoMALDI pipeline (Yang et al, Adv. Sci. 2023), which integrates slide-scanning fluorescence microscopy (SSFM) with matrix-assisted laser desorption/ionization (MALDI) imaging. FluoMALDI is based on the discovery that MALDI matrix co-crystallization with fluorophores significantly enhances fluorophore brightness, which enables the amplification of tissue autofluorescence and fluorescent dyes upon MALDI matrix deposition. In a parallel study, we developed RaMALDI, a streamlined, integrated, multimodal imaging workflow of Raman microscopy and MALDI imaging (Yang et al, Biosens. Bioelectron. 2023). FluoMALDI and RaMALDI imaging workflows are performed on a single tissue section with one sample preparation protocol. They leverage the discovery that MALDI matrices do not disturb, and in many cases even enhance, Raman and fluorescence signals from tissues.

Methods

We investigated the effects of MALDI matrix-fluorophores interactions on fluorescence enhancements in solid-phase and liquid-phase using various pairings of widely used fluorophores with commonly used MALDI matrices. MALDI matrix–fluorophore co-crystals were analyzed with single-crystal X-ray crystallography. We systematically compared sublimation versus spraying as methods for MALDI matrix deposition onto tissues, examining their respective impacts on (auto)fluorescence enhancements and MALDI imaging. The spatial distribution of metabolites and lipids was evaluated by Raman and MALDI imaging. Matrix crystal morphology was assessed by Scanning Electron Microscope (SEM). We also tested new FluoMALDI and RaMALDI imaging applications in mouse brain, kidney, spleen, and lungs, and studied cell types which naturally produce bright autofluorescence across the visible light range within their tissue context.

Novel Aspect

FluoMALDI and RaMALDI imaging allow for spatially targeted biomolecular discovery on a single tissue section with one sample preparation protocol.

Preliminary Data or Plenary Speaker Abstract

To gain deeper insights into the FluoMALDI effect, we tested the interactions of various fluorophores, including Rhodamine-B, Alexa dyes, and Protoporphyrin-IX (PPIX) with commonly used MALDI matrices in a pairwise manner. In solid-phase experiments, we observed that PPIX, an endogenous fluorophore causing tissue autofluorescence, displayed significantly enhanced fluorescence intensities in the red and far-red channels when co-crystallized with 9-Aminoacridine (9AA) matrix as compared to PPIX alone. Increasing the matrix density by doubling the layers of robotically sprayed matrix onto PPIX fluorophore spots significantly enhanced its fluorescence brightness. X-ray crystallography revealed that π-π stacking in 9AA-PPIX co-crystals correlated with this increased PPIX fluorescence brightness. In liquid-phase experiments, higher matrix concentrations did not enhance the fluorescence, but increased fluorophore concentrations did. We are currently investigating additional fluorophore-matrix pairs in solid- and liquid-phase. Comparative analysis between sublimation and spray deposition of MALDI matrices onto tissues revealed distinct autofluorescence enhancement profiles, with many matrices displaying consistent enhancements for both matrix

application methods. SEM confirmed smaller crystal sizes for sublimation as compared to spray deposition. Our findings demonstrated the efficacy of autofluorescence-guided MALDI imaging post-sublimation and post-spraying of matrix, thereby significantly improving structural and anatomical insights. Capitalizing on the bright autofluorescence of phagocytes in mouse infection models, we effectively utilized FluoMALDI imaging to characterize the immune response by spatially targeting fluorescence-enhancing phagocyte-enriched regions for detailed molecular analysis by MALDI imaging. RaMALDI imaging from the same kidney tissue section revealed clear spatial correspondence of anatomical structures including renal cortex, medulla, and capsule, which were apparent in various molecular Raman and MALDI images. In RaMALDI images of brain tissue sections acquired at 5-µm spatial resolution, the molecular distributions of lipids, proteins, and nucleic acids clearly showed the molecular layers of the cerebellum. FluoMALDI and RaMALDI workflows enable rapid, simple multimodal tissue imaging for spatially targeted biomolecular discovery.

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Dr. Stefania Alexandra Iakab[1], Dr. Florian Keller[1], Lars Gruber[1], Dr. Stefan Schmidt[1], James-Lucas Cairns[1,2], Jasjot Singh[4], Frank Fischer[4], Richard Schneider[4], Rüdiger Rudolf[1,3], Carsten Hopf[1,2,3]
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Molecular Snapshots of a Colon Cancer 3D Cell Culture Model

Stefania Lakab 311 - Molecular Snapshots of a Colon Cancer 3D Cell Culture Model, Plenary 3, August 21, 2024, 10:25 - 10:44

Matrix assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) is the go-to technique for describing in situ the molecular composition of biological samples. Organoids and spheroids are attractive samples for studying biological phenomena in human cells, and are becoming vital for drug screenings and clinical studies in terms of time and cost efficiency. Therefore, molecular snapshots provide valuable information for applications such as cell classification, biomarker discovery, and drug screenings. Here we developed a platform that allows high throughput and reliable detection of small molecules (in 3D) from spheroid models.

Methods

Spheroids were generated in house and flash frozen in a gelatin cryo-mold using HPMC-PVP as embedding medium. Sections were obtained at 20 µm thickness with a cryostat, and mounted on ITO coated slides. NEDC matrix was sprayed on the sections prior to MS acquisition. Bruker's timsTOFfleX was used in tims ON mode, at 20x20 µm2 pixel size. Data were preprocessed, analyzed and exported using SCiLS Lab (Bruker Daltonics), M2aia software was used to obtain 3D reconstructions and volume visualizations, and in house scripts were used in R language to further analyze the data.

Novel Aspect

High-throughput spatial multi-omics data acquired from micron-scale samples reveal more than two cell-specific metabolic fingerprints in a biculture model.

Preliminary Data or Plenary Speaker Abstract

Our in-house designed and manufactured 3D-printed metal casting molds enabled the embedding of several 3D culture samples in a precise and reliable sterical arrangement. Thus, sample preparation including harvesting, embedding and freezing of the spheroids has become reproducible, high throughput and, most importantly for 3D- reconstructions and multimodal applications, reliable for obtaining consecutive sections. High quality imaging data was acquired in the ranges of small molecules (m/z 50-600 Da) and lipids (m/z 300-1200 Da), from which we were able to filter over 200 spheroid-associated molecules, which have been annotated using Metaboscape. Unsupervised multivariate data analysis (segmentation and principal component analysis) revealed multiple different spectral patterns, presumably corresponding to fibroblasts and cancer cells, as well as regions with mixed molecular fingerprints. PCA results suggested that the positioning of a specific cell type within a spheroid changed that cell type's molecular composition. Significant values were selected in volcano plots, by setting the adjusted p value threshold to 0.01 and the fold change threshold to 0.5. After annotating cell specific features, we obtained the stacked images from ~ 50 consecutive 20 µm thick sections. Region-specific features were the following: m/z 498.26 for the exterior layer, m/z 835.56 for the cancer cells and m/z 885.55 for the fibroblasts. Our 3D visualization workflow further proved that specific metabolites can be distinguished based on cell type and cell layer within a 3D cell culture model. The potential of our platform to identify markers for biological mechanisms unique to each cell type could be valuable for studying specific cellular signaling or metabolic interaction between cancer and fibroblast cells.

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Dr Esther Cheow[1], Dr U-Ming Lim[1], Dr Matthew Choo[1], Dr Nikhil Tulsian[1], Dr Tze Khee Chan[1], Dr Brian Henry[1], Dr Aaron Zefrin Fernandis[1]
[1]MSD International Gmbh (Singapore)

Spatial Multi Omics Strategy to Advance Target Biology and Biomarker Discovery for Pulmonary Fibrosis

Esther Cheow 547 - Spatial Multi Omics Strategy to Advance Target Biology and Biomarker Discovery for Pulmonary Fibrosis, Plenary 3, August 21, 2024, 10:44 - 11:03

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive lung disease characterized by progressive scarring of lung tissue, resulting in impaired respiratory function and eventual respiratory failure. Despite extensive research, the underlying molecular mechanisms driving IPF progression remain elusive. Omics technologies, notably mass spectrometry-based tools, play a vital role in modern drug discovery and development. These advanced methodologies offer invaluable insights into the mechanisms of action associated with novel therapeutic targets, thereby addressing significant unmet medical needs. Here, we highlight the unparalleled potential of multi-omics spatial mass spectrometry tissue imaging coupled with laser capture microdissection (LCM)-LC-MS/MS technologies to identify key drivers of fibrosis progression, enabling target identification and therapeutic invention strategies for IPF.

Methods

Fresh-frozen lung tissue sections (10 µm) were mounted on IntelliSlides. Sequential MALDI-MSI analysis was performed using 1,5-DAN matrix for metabolite/lipid analysis and CHCA matrix for HiPlex-IHC (Ambergen) intact protein analysis. High-resolution (20 µm) images were acquired on a timsTOF fleX MALDI-2 (Bruker) instrument. SCiLS lab software (Bruker) was utilized for data visualization and analysis. Image segmentation algorithms embedded within QuPath and SCiLs lab were then applied to refine regions of interest (ROI) selection and ensure precise isolation of target areas for downstream analysis. LCM was performed using the LMD7 system (Leica) to isolate these ROIs for in-depth proteomic characterization via LC-MS/MS (timsTOF fleX MALDI-2 coupled with EvoSep). Spectronaut software (Biognosys) with the built-in directDIA™ algorithm was employed for data analysis.

Novel Aspect

Sequential multi-omics MALDI imaging LCM-LC-MS/MS characterization, reveals key interactions and localized proteomics in IPF, surpassing single-omics approaches for comprehensive insights.

Preliminary Data or Plenary Speaker Abstract

Existing spatial omics studies in IPF have analyzed single omics layers, limiting their ability to capture the complex interplay between biomolecules. Our innovative approach utilizes sequential multi-omics MALDI imaging, which enables us to concurrently analyze and integrate information from untargeted metabolites and lipids alongside targeted proteins within a single tissue section. Multi-omics analysis revealed co-localization of increased inflammatory metabolite expression with macrophages, suggesting a potential link between these mediators and macrophage activity in IPF pathogenesis. This approach unveils co-localization patterns and potential pathway interactions critical for understanding IPF progression.

Furthermore, our methodology incorporates laser capture microdissection (LCM) for targeted isolation of fibrotic regions, guided by a multi-modal approach. First, we utilize MALDI-IHC to identify regions of interest, using established protein marker for fibrosis. The reliability of MALDI-IHC with these markers in determining fibrotic regions is verified using pentachrome staining, a gold standard for characterizing fibrotic foci. This two-step approach ensures that the most relevant areas were isolated for further in-depth proteomic characterization via LC-MS/MS. Preliminary data from this approach reveals dysregulation in key pathways associated with IPF pathogenesis. This localized

analysis provides a more granular understanding of the molecular makeup within fibrotic regions, surpassing the limitations of bulk tissue analysis.

By simultaneously visualizing and analyzing the spatial distribution of metabolites, lipids, and proteins, we can potentially identify novel co-localization patterns and inter-omic relationships that may be missed by single-omic analyses. This comprehensive data can shed light on the underlying molecular pathways driving IPF progression and ultimately lead to the development of more targeted therapeutic strategies.

Dr. Charles Schurman[1], Dr. Diane Hu[2], Nannan Tao[3], Dr. Tamara Alliston[2], Dr. Ralph Marcucio[2], Dr. Peggi Angel[4], Dr. Birgit Schilling[1]
[1]Buck Institute For Research On Aging, [2]University of California San Francisco, [3]Bruker, [4]Medical University of South Carolina

Spatial Proteomics via Extracellular Matrix Imaging of Bone Fracture Callus Reveals Delayed Transition of Osteochondral Remodeling with Age

Charles Schurman 358 - Spatial Proteomics via Extracellular Matrix Imaging of Bone Fracture Callus Reveals Delayed Transition of Osteochondral Remodelling with Age, Plenary 3, August 21, 2024, 11:03 - 11:22

Delayed fracture healing in the elderly is a common challenge that may lead to bone infection, nerve damage, and amputation. In fracture healing, stem cells differentiate into chondrocytes which produce a cartilage matrix that transforms into bone via endochondral ossification. This process is marked by the transition of several matrix proteins, including a progression from Collagen Type 2, through Collagen Type 3, and eventual replacement by Collagen Type 1. It is unknown whether spatiotemporal dysregulation of extracellular matrix (ECM) proteins is associated with age-related delays in fracture healing. An ECM-targeted Matrix-Assisted Laser Desorption/Ionization (MALDI) mass spectrometry imaging (MSI) approach identifies for the first-time decreased presence of collagen and other ECM peptides in fractures of aged mice compared to young controls.

Methods

Fracture calluses were collected from unfixed fractures of the femur of young (3 months, N=4) and aged (24 months, N=4) c57B6 mice. Tissues were formalin-fixed, paraffin embedded, and sectioned onto glass slides. Slides were sequentially treated with PNGase F and collagenase III to target the ECM. Spectra of the fracture were acquired on a timsTOF fleX (Bruker) mass spectrometer with a laser step size of 20 micrometers. Untargeted analyses of MS1 mass spectra were processed using the Bruker SCiLS Lab software. Following MSI, tissue sections were stained with hematoxylin/eosin (H&E) to identify different callus regions histopathologically. Statistically regulated peptides were identified via AUROC discriminating feature analysis in SCiLS and validated through independent t-tests comparing aged to young tissue sections (N=4).

Novel Aspect

The first MALDI-MSI of fracture calluses of ECM proteins identified a transition where aged bone exhibited reductions in healing capactiy.

Preliminary Data or Plenary Speaker Abstract

High resolution (20 micrometers) MS imaging of fracture calluses from young and aged mice revealed spatial and temporal differences in ECM proteins. In young animals, a clear separation was observed at the transition zone (TZ) between the newest cartilage-like callus and the matured mineralized bone. This boundary was defined by the almost mutually exclusive distribution of peptides for COL1A1, GPAGEEGKRGARGEP (1 HYP) at m/z 1483.72, and COL3A1, GLAGTAGEPGRDGNPGSDGLP (2 HYP) at m/z 1926.88, where COL3A1 was most abundant in the newest cartilage-like callus regions and the COL1A1 peptide abundant in the matured mineralized bone regions. In fracture calluses from aged animals, this specific peptide of COL1A1 is absent, with no transition from COL3A1 to COL1A1 apparent resulting in fracture healing in aged animals to appear stalled or very delayed. This is consistent across the entire young and aged cohort (N =4 ea.) where the average peak intensity for this COL1A1 peptide is significantly lower in aged fracture calluses compared to young animals ($p=0.007$). Area Under the Receiving Operator Curve (AUROC) discriminating feature analysis was used to create candidate m/z features that would discriminate fracture calluses from young and old animals. 41 unique m/z features from over 500 identifiable m/z peaks were found with AUROC > 0.75. Of these, 34 features were specific to calluses from young animals, while only 7 were specific to aged tissue. Of the 41 features identified, 34 features remained significantly different ($p<0.05$)

between calluses from young and aged bone in t-tests. The significant downregulation of several ECM proteins from aged bone identified a point during fracture healing where aged tissues experience an interruption in healing and failed to progress forward in osteochondral remodeling. Knowledge of the regulation of the ECM during callus remodeling may allow for more targeted therapies to overcome disruptions in age-related fracture healing.

Mr Jayden Mckinnon[1], Dr. Rachelle Balez[1], Dr. Reuben Young[1], Mikayla Brown[2], Dr Jeremy Lum[2], Liam Robinson[2], Prof Lezanne Ooi[2], Sara Tortarella[3], Prof Todd Mitchell[2], A/Prof Shane Ellis[1]
[1]Molecular Horizons and School of Chemistry and Molecular Bioscience, [2]Molecular Horizons and School of Medical, Indigenous, and Health Science, [3]Mass Analytica

Unveiling Single Cell Small Metabolite Distributions via an Oversampling approach enabled by MALDI-2-MSI

Jayden Mckinnon 260 - Unveiling Single Cell Small Metabolite Distributions via an Oversampling approach enabled by MALDI-2-MSI, Plenary 3, August 21, 2024, 11:22 - 11:41

Given the metabolic heterogeneity that can occur both within heterogeneous biological tissues and cell populations, it is pivotal to develop techniques capable of mapping the distribution of metabolites at high spatial resolution. MALDI-MSI has made tremendous strides in advancing spatial metabolomics, however, is still constrained by poor sensitivity towards many small metabolites because of poor ionisation efficiencies, which in turn can limit the practical spatial resolution. We have addressed these limitations by the coupling of MALDI-2 with an oversampling method that allows pixel sizes smaller than the laser spot. The benefits of this approach are demonstrated for both the high spatial resolution metabolic imaging (8 µm pixel size) of spinal cord tissues and for single-cell metabolomics analysis of iPSC-derived astrocytes.

Methods

Liver homogenate, mouse spinal cord tissue and pluripotent stem-cell derived astrocytes were thawed to room temperature under vacuum prior to matrix application. Following this, an organic matrix consisting of napthyl ethylenediamine dihydrochloride (NEDC) dissolved in 70% MeOH:Water was applied to the surface of the samples using a HTX TM-Sprayer. The samples were analysed using a Spectroglyph intermediate pressure dual MALDI/ESI source coupled to an Orbitrap Elite. MALDI-2 was enabled by using a frequency-quadrupled laser emitting at 266 nm. Resulting datasets were analysed using the PyxisMSI data analysis pipeline.

Novel Aspect

MALDI-2 combined with oversampling improves the metabolite coverage and spatial reoslution of MSI experiments performed on biological tissues and cells

Preliminary Data or Plenary Speaker Abstract

To assess improved spatial resolution achievable for small metabolites using MALDI-2 coupled with oversampling, MALDI and MALDI-2 datasets were generated from line scans across liver homogenate tissue at step sizes ranging from 20 µm to 6 µm, with a laser spot size of ~13 µm. Under non-oversampling conditions, MALDI-2 notably enhances signal intensities and metabolite coverage compared to conventional MALDI at 20 µm pixel sizes, encompassing sugars, amino acids ([Taurine-H]-, [Glutamate-H]-, [Histidine-H]-), and Purines ([Xanthine-H]-). Despite signal intensity decrease with increasing oversampling, MALDI-2 partially compensates, facilitating enhanced metabolite coverage at pixel sizes down to 8 µm, approximately half the nominal laser spot size.

Leveraging oversampling to enhance spatial resolution for small metabolite imaging, spinal cord tissue was imaged at 8 µm pixel size, spatially resolving regions of interest including dorsal, lateral, and ventral horns, grey commissure, central canal, and motor neurons. Notably, isobaric masses m/z 259.0127 (glucose-6-sulfate) and m/z 259.0223 (glucose-6-phosphate) were specifically distributed to white matter and gray matter, respectively, showcasing MALDI-2 coupled to an Orbitrap's ability to separate isobaric features at single cell resolution.

The approach's utility was demonstrated in single cell metabolomics, a domain with limited exploration. iPSC astrocytes at 8 µm pixel sizes were analyzed post-fixation, staining, and microscopy, with data processed using PyxisMSI. Various cell segmentation methods based on MSI data were

explored. Critically, numerous metabolite signals were detected and localized within astrocytes. For instance, signals at m/z 134.0465 (adenine) and m/z 328.0433 (cyclic adenosine monophosphate) localized to the cell center (likely nucleus), while others such as m/z 346.0537 (adenosine monophosphate), m/z 323.0267 (uridine monophosphate), and m/z 500.2783 (lysophosphoethanolamine 20:4) were distributed throughout the cell body.

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Haruki Uchino[1], Hiroshi Tsugawa[1,2,3,4], Dr. Makoto Arita[1,4,5,6]
[1]Laboratory for Metabolomics, RIKEN Center for Integrative Medical Sciences, [2]Department of Biotechnology and Life Science, Tokyo University of Agriculture and Technology, [3]RIKEN Center for Sustainable Resource Science, [4]Cellular and Molecular Epigenetics Laboratory, Graduate School of Medical life Science, Yokohama City University, [5]Division of Physiological Chemistry and Metabolism, Graduate School of Pharmaceutical Sciences, Keio University, [6]Human Biology-Microbiome-Quantum Research Center (WPI-Bio2Q)

Sex-dependent changes in renal spatial lipidome revealed by MALDI-2-TIMS-MS imaging

Haruki Uchino 672 - Sex-dependent changes in renal spatial lipidome revealed by MALDI-2- TIMS-MS imaging, Plenary 3, August 21, 2024, 11:41 - 12:00

Lipids are essential compounds for living organisms that play multifunction e.g. cellular membrane components, signaling molecules, and energy storage. It is known that various diseases including nervous system diseases, cancer, and chronic kidney disease (CDK) were caused by or related to lipidome alteration. Our previous study revealed that renal lipidome was greatly subject to sex dependency at the bulk tissue level, which would be relevant to the different incidences of kidney diseases including CDK against males and females. In this study, we aimed to investigate the spatial lipidome in male and female mouse kidneys by MALDI-MS imaging whose sensitivity was enhanced by post-ionization (MALDI-2) and trapped ion mobility spectrometry (TIMS).

Methods

Male and female mouse kidneys were embedded in 2.5% carboxymethyl cellulose (CMC) and cut with a thickness of 10 µm. 5 mg/mL in 70% ethanol with 0.1% trifluoroacetate of 2,5-dihydroxy acetophenone (DHAP) and 5 mg/mL in 70% methanol of diaminonaphthalene (DAN) were sprayed on the tissue sections by HTX M5 Sprayer (HTX technologies, NC, USA). Spatial lipidomics was performed using a timsTOF flex MALDI-2 system (Bruker, Bremen, Germany) equipped with a secondary UV laser (266 nm). MSI data were analyzed by SCiLS Lab software and MS-DIAL 5 software. For unbiased data mining of MALDI-MSI data, we performed segment and component analyses using bisecting k-means and probabilistic latent semantic analysis (pLSA), respectively.

Novel Aspect

Our study revealed the lipidome signatures of mouse kidneys were spatially regulated by sex dependency.

Preliminary Data or Plenary Speaker Abstract

Our spatial lipidomics system detected over 5000 ion signals containing unknown peaks in positive and negative ion mode, respectively, and from the data we annotated over 200 lipid species in total including fatty acyls, phospholipids, glycelolipids, sphingolipids, and sterol lipids. The spatial distributions of ion peaks were annotated by integrating the tissue staining images of the analyzed samples and those of the sequential sections. As a result, while some lipid species were mapped on the whole tissue area, some species showed characteristic distributions reflecting the renal region such as renal cortex, outer and inner strip of the medulla, inner zone, and renal papilla. And the distribution differed according to its structural properties including lipid subclass, acyl-chain length, the degree of unsaturation, and functional groups as well.

To grasp the spatial lipidome difference between male and female tissue in an unbiased manner, we utilized ion feature lists containing annotated and unknown peaks for the following two data mining. First, the result of the segmentation analysis suggested that ion image signatures from the tissues were mainly distinguished in outer strip of renal medulla and medullary rays. And pLSA analysis classified the patterns of ion images into 10 components, some of which reflected the renal regions described above. Of importance, several components showed clear distributional differences

between male and female tissues. By extracting the main factors forming these components, we confirmed that sulfatides were enriched in the medulla of male tissue but much less in the female tissue, which was consistent with our bulk analysis. Furthermore, although ether-linked phosphatidylcholine (EtherPC) and phosphatidylethanolamine (EtherPE) with highly unsaturated moiety were abundant in the outer strip of renal medullar of the two sexes, but also uniquely enriched in the medullary rays of male tissue.

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Dr Da Ren[1]
[1]Biotherapeutics Solutions

Trends of MS Applications in Biopharmaceutical Industry

Keynote: Dr Da Ren Biotherapeutics Solutions 766 - Trends of MS Applications in Biopharmaceutical Industry, Meeting Room 105, August 21, 2024, 10:00 - 10:25

Mass spectrometry (MS) is one of the most important analytical tools that has been widely used in the biopharmaceutical industry. Recent trends of using various liquid chromatography-mass spectrometry (LC-MS) tools for research, development and manufacturing of biotherapeutics will be reviewed in this presentation. The comparison of MS applications in academia and in biopharmaceutical industry will also be covered.

Novel Aspect

Overview of novel applications and industry trends of using LC-MS based analytical methodologies in the biopharmaceutical industry

Preliminary Data or Plenary Speaker Abstract

As part of the Pharmaceutical Quality for the 21st Century Initiative, the United States Food and Drug Administration (FDA) introduced the Quality by Design (QbD) concept to guide the pharmaceutical industry. The focus of QbD is that quality should be built into a drug product with a thorough product and process understanding. One critical element of QbD is the identification and control of potential critical quality attributes (CQAs). A CQA is a physical, chemical, biological, or microbiological property that should be within an appropriate range to ensure the desired product quality. For biotherapeutics, CQAs are often chemical modifications on various amino acid residues that can impact immunogenicity, efficacy, and pharmacokinetics profile.

Due to its high specificity, sensitivity, and accuracy, LC-MS has become a crucial analytical tool for the identification and quantitation of CQAs in the biopharmaceutical industry. Traditionally, most LC-MS applications have been limited to product characterization in early-stage development. But with the introduction of the Multi-Attribute Method (MAM) in 2015, the industry has seen increasing applications of LC-MS in cGMP environments. US Pharmacopeia (USP) is on track to publish a general chapter in 2024 to provide the industry with principles and practical guidance regarding the LC-MS-based MAM for biotherapeutics characterization and release/stability testing in Current Good Manufacturing Practice (cGMP) environments (Chapter <1060> Mass Spectrometry-Based Multi-Attribute Method for Therapeutic Proteins). Trends of other MS applications in biopharmaceutical industry and considerations for implementation will be discussed.

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Prof. Daniele Fabris[1]
[1]University Of Connecticut

A Mid-Down Strategy for the Characterization of Non-Coding and mRNAs

Daniele Fabris 340 - A Mid-Down Strategy for the Characterization of Non-Coding and mRNAs,
Meeting Room 105, August 21, 2024, 10:25 - 10:44

The characterization of nucleic acid (NA) biotherapeutics by traditional genomics approaches, which rely on the analysis of amplification products or library copies of the original sample, is hampered by the utilization of non-canonical nucleotides to improve pharmacological activity, stability, and bioavailability. Mass spectrometry (MS) approaches offer valid alternatives by virtue of their ability to recognize the presence of man-made and natural variants from characteristic mass and fragmentation signatures. However, intrinsic size limitations continue to hinder their adoption for the characterization of the larger NA biotherapeutics, such as mRNA vaccines. To address these limitations, we have been exploring different strategies that combine bottom-up and top-down approaches to enable the mid-down analysis of progressively larger NA strands, such as non-coding and mRNAs.

Methods

All NA samples, enzymes, and reagents employed in the study were purchased from commercial sources, or prepared in house by standard recombinant techniques. When necessary, extensive desalting was carried out by ultrafiltration against a 150 mM ammonium acetate solution. Strand-cleavage reactions were performed in the same environment at different temperatures and time intervals, as discussed in the presentation. All samples were analyzed by direct infusion nano-flow electrospray (i.e., static nanospray) ionization without front-end separations. All analyses were carried out in negative ion mode on either a timsTOF Pro or a solariX XR FT-ICR, both from Bruker. Data interpretation was accomplished by using the SeqRead software developed by RiboDynamics.

Novel Aspect

novel mid-down sequencing for nucleic acids, novel data interpretation strategy

Preliminary Data or Plenary Speaker Abstract

The presentation describes a novel mid-down approach based on the ability to obtain larger hydrolytic products from limited digestion and alternative cleavage reagents, and the support of enhanced interpretation techniques for fragmentation data produced by larger precursor ions. In one direction, typical endonuclease used in the bottom-up analysis of larger strands, such as the 364-nt HIV-1 5'-UTR or the 758-nt eGFP mRNA, produce hundreds of small products consistent with the high-frequency of susceptible nucleotides in the sequence. We have shown that limiting the digestion efficiency by reducing reaction time/temperature leads to larger products, which are more sequence-informative and less prone to ambiguities associated with isomeric products of identical base composition and thus mass. We have shown that the nanospray emitter can be used as reaction vessel to record the entire time-course of the digestion and capture products of the desired size. We finally demonstrated that custom-made deoxyribozymes can be designed to target specific sequence motifs rather than individual nucleotides, which allows one to control the size of ensuing products. In the other direction, we explored the factors that limit to 25-30 nt the practical size of strands that can provide full sequence information upon direct gas-phase fragmentation (i.e., top-down sequencing). We have demonstrated that this limitation stems from the increasing production of internal fragments as a function of precursor size, which are traditionally shunned as source of reliable sequence information. We developed ad hoc software capable of combining the information provided by both internal and terminal fragments to achieve full sequence coverage for strands up to 125 nt. These concomitant developments have allowed us to propose a mid-down strategy in which progressively larger NA strands consisting of hundreds to thousands nt could be effectively hydrolyzed into 100-150 nt products that are readily amenable to gas-phase sequencing.

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In-depth Characterization of DNA-Encoded Chemical Libraries using Native Mass Spectrometry: The Impact of DNA-tags on Binding Affinities

Philipp Bittner 208 - In-depth Characterization of DNAEncoded Chemical Libraries using Native Mass Spectrometry: The Impact of DNA-tags on Binding Affinities, Meeting Room 105, August 21, 2024, 10:44 - 11:03

The discovery and development of novel drugs against targets of pharmacological interest is still a highly challenging and expensive process. Recently, DNA-encoded chemical libraries (DELs) have been proven to be a fast and cost-effective platform for the discovery of novel small molecules. These libraries consist of thousands to billions of diverse chemical compounds produced by combinatorial chemistry, each encoded by a unique DNA sequence, which serves as a "barcode" to be later read out by next-generation DNA sequencing methods. DELs are used in affinity-based selections against a target protein where only a few binders are separated from the millions of non-binding molecules. These few lead candidates are then usually re-synthesized for a detailed characterization.

Methods

Because it remains unclear how binding constants are influenced by the molecule’s DNA-tag, we have therefore set out to investigate the binding properties of small molecules with various DNA-tags. We apply native nano electrospray ionization mass spectrometry (nESI-MS), which can preserve non-covalent protein–ligand interactions and allows to determine dissociation constants (Kd) of DNA-tagged ligands using a titration approach. Temperature-controlled nano electrospray ionization (TCnESI) in combination with ion mobility mass spectrometry (IM-MS) further allows one to compare the solution versus the gas phase stabilities of protein–ligand complexes. Both methods are expected to reveal possible steric or electrostatic DNA–protein interactions, which may already be present in solution and contribute to the observed binding affinities.

Novel Aspect

Label free on-DNA hit validation and characterization of DELs against protein targets by native IM-MS and TCnESI.

Preliminary Data or Plenary Speaker Abstract

It has been shown that many Kd values of small molecules to proteins determined by native nESI-MS titration agree with solution-phase binding properties. In this comparative study, we are investigating the effect of different DNA-tags such as single- or double stranded DNA tags on multiple protein targets. Preliminary results have already shown significant differences in the binding affinities of single- versus double stranded tagged ligands against human carbonic anhydrase II (hCAII), bromodomain containing protein 4 (BRD4) and Bruton's tyrosine kinase (BTK). This effect was observed for multiple binders with Kd values in the nM to µM range, depending on the type of DNA-tag and its protein target. To understand these differences, we performed TCnESI-MS, which revealed a higher melting or dissociation point in solution for a protein in complex with a ssDNA-labeled ligand compared to its dsDNA-labeled variant. The results indicate a synergistic binding effect of the ssDNA-tag itself to the protein in solution, which is not being observed for the dsDNA-tag variant. In contrast, both protein–ligand complexes show a similar gas phase stability upon performing collision induced dissociation (CID) with increasing collision energies.
Our approach further allowed us to perform “pull-down” assays from unpurified ligand material, revealing, e.g., binding events of precursor molecules, which are correctly DNA-encoded but failed in the final synthesis step of the ligand itself. This not only allows for the identification of false positive binders but also provides insights into which interacting sites of the molecule are important for

binding, which is crucial information for hit discovery. In a later stage, this is expected to facilitate “off-DNA” synthesis steps of an identified library hit, because non-binding sites of the molecule can be excluded.

Devin Makey[1], Marion Emmert[2], Hang Hu[2], Erik Regalado[2], Rodell Barrientos[2], Brandon Ruotolo[1]
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Cyclic Ion Mobility-Mass Spectrometry for Rapid Protein Structure and Stability Assessment During the Development of Next-Generation Antibody Therapeutics

Devin Makey 235 - Cyclic Ion Mobility-Mass Spectrometry for Rapid Protein Structure and Stability Assessment During the Development of NextGeneration Antibody Therapeutics, Meeting Room 105, August 21, 2024, 11:03 - 11:22

Antibody-drug conjugates (ADCs) represent a rapidly expanding therapeutic approach for cancer treatment, leveraging the specificity of monoclonal antibodies (mAbs) and the cytotoxicity of small molecule drugs. Optimization of the mAb, linker, and payload is crucial for the development of safe and effective ADCs. However, the process of drug conjugation can introduce structural changes within the ADC. This presentation will discuss the development of improved cyclic ion mobility-mass spectrometry (cIM-MS) methods to characterize mAb and ADC higher-order structure and stability. We have applied improved cIM-MS workflows to reveal subtle the effects of the LP structure (size, shape, hydrophobicity, etc.) on the higher-order structure of site-specific and interchain cysteine-linked ADCs, highlighting the utility cIM-MS as a high-throughput screening tool during ADC development.

Methods

Site-specific ADCs were generated by reacting an excess of maleimide LP with a mAb containing two available conjugation sites (cysteine residues engineered in the Fc region). For interchain cysteine-linked ADCs, the interchain disulfide bonds of a mAb were reduced using tris(2-carboxyethyl)phosphine. The reduced mAb was then reacted with various maleimide LPs, producing a heterogeneous mixture of drug-to-antibody ratio (DAR) states ranging from 0 to 8, depending on the extent of reduction. Samples were directly infused using nanoelectrospray ionization gold-coated capillaries produced in-house. Experimental data were acquired using an ultrahigh mass range hybrid quadrupole-orbitrap mass spectrometer (Thermo) and a cyclic ion mobility-mass spectrometer (Waters). Data analysis utilized CIUSuite 3, UniDec 5.2, MassLynx 4.2 (Waters), and IMSCal19 (Waters).

Novel Aspect

Next-generation cIM-MS and CIU methods to investigate linker-payload effects on ADC higher-order structure and stability.

Preliminary Data or Plenary Speaker Abstract

Cyclic ion mobility (cIM) offers benefits over linear IM instruments for biotherapeutic characterization, such as improved IM resolution and the ability to produce data of greater depth and information content using timed ion selection with multiple stages of ion activation and IM separation. Collision cross section (CCS) measurement plays a crucial role in IM-MS proteins by providing valuable information about size, shape, and conformational dynamics, yet cIM CCS measurements have only been applied to basic cIM separations modes and typically utilize outdated power-law functions. We have applied and extensively validated a new high-accuracy, high-precision method for cIM CCS calibration over a variety of analytes (ranging in CCS from 140 to 11000 Å²) and experimental conditions. Collision induced unfolding (CIU) experiments enable the simultaneous assessment of both protein structure and stability when coupled with IM-MS. We will demonstrate that cIM-MS offers improved sensitivity, acquisition speed, and resolution in CIU experiments.
We have utilized cIM-MS to probe subtle changes in the overall conformation and stability of ADCs upon specific Fc-conjugation to LPs with different sizes, functional groups, and hydrophobicities. Preliminary results revealed significant increases in collision cross section (CCS) values compared to

the control mAb (1.2–3.6 nm^2). CIU transition voltages (CIU50s) varied between ADCs and the control. The first CIU transition (Fab unfolding) destabilized upon conjugation (CIU50-1 decreased by 2.5–4.5 V). The second CIU transition (Fc unfolding) exhibited both stabilizing and destabilizing effects, with decreases by 3.5 V and increases by 2.6–22.6 V depending on the LP. Finally, we observed changes in CIU of interchain cysteine-linked ADCs that varied depending on the LP and DAR state. We typically observed between 3–6 CIU features and global root-mean-square deviation between ADCs and the control varied from 20–40%. The ADCs exhibited significant increases in CCS values (2–8 nm^2).

Laura van der Vloet[1]
[1]M4i, Maastricht University

Visualizing antisense oligonucleotides and its biological impact in brain tissue using a multi-omics mass spectrometry imaging approach

Laura van der Vloet 540 - Visualizing antisense oligonucleotides and its biological impact in brain tissue using a multiomics mass spectrometry imaging approach, Meeting Room 105, August 21, 2024, 11:22 - 11:41

In recent years, the development of antisense oligonucleotides (ASOs) has gained wide interest as therapeutic agent for their potential in treating neurodegenerative diseases. ASOs are chemically modified oligonucleotides that are designed to bind complementary regions of RNA and thereby modulating the function of the targeted RNA by different mechanisms, leading to altered protein expression. In this study, we present a novel workflow that enables the detection and spatial visualization of ASOs within brain tissue, utilizing mass spectrometry imaging (MSI). This is followed by the investigation of the ASOs' effects on the lipidome and proteome, as well as their impact on neurotransmitter regulation.

Methods

Fresh frozen brain samples were collected and sectioned at 12 µm. All MALDI-MSI experiments were performed on a timsTOF flex instrument. To increase sensitivity for the detection of the ASO backbone, tissues were washed 30s with dichloromethane prior to matrix application. On a consecutive section, the spatial distribution of lipids was investigated in both ionization modes. Afterwards, matrix was removed, followed by a trypsin on-tissue digestion for spatial proteomics analysis. On a third consecutive section, a deuterated neurotransmitter internal standard mixture was sprayed on the tissue sections, followed by the application of the reactive matrix FMP10, enabling the visualization and quantification of neurotransmitters. Lastly, semi-quantification of the proteome was performed using LC-MS/MS analysis on a Q Exactive HF Hybrid Quadrupole-Orbitrap.

Novel Aspect

The visualization of ASOs and biomolecular compounds in brain facilitates deeper understanding of the molecular processes within the nervous system.

Preliminary Data or Plenary Speaker Abstract

Our study represents a newly developed MALDI-MSI method, enabling the visualization of the spatial distribution of the phosphorothioate backbone of two different ASOs in brain tissue. The method was optimized by introducing washing steps to enhance ASO intensity. The tested washing solutions include diethyl ether, ethyl acetate, dichloromethane, and chloroform followed by acetone of the ASO-dosed brain tissue. Tissue sections washed with dichloromethane for 30 sec resulted in the highest intensity of the ASO backbone fragments in brain tissue. Both ASOs were only detected in the ventricles of the brain, suggesting that we could detect the unbound ASOs in the cerebrospinal fluid, but could not detect the bound ASOs in brain tissue itself.
The biological effect of the ASOs on the lipidome, proteome and on neurotransmitters were evaluated next. We investigated the spatial distribution of lipids in brain tissue, for which no alterations were detected between ASO dosed and control group. On the same tissue slide, spatial proteomic analysis was performed. For both ASOs, multiple peptides were significantly altered due to ASO administration. At the moment, we are conducting semi-quantification LC-MS/MS analysis on whole brain tissue slides to identify the altered peptides that were found in the MSI data as well as the biological pathways that are up- or down-regulated due to ASO administration. Based on were the ASOs target, specific brain regions will be dissected using laser microdissection, followed by LC-MS/MS for proteomics analysis, allowing in-depth proteomics analysis to further evaluate the effect of ASOs within different brain regions.

Currently, we are quantifying and investigating the spatial distribution of neurotransmitters since the ASOs (in) directly target neurotransmitter receptors. Therefore, we expect alterations in neurotransmitter receptor composition or expression, leading to changes in neurotransmitter regulation.
We are currently optimizing a LC-fluorescence approach to quantify ASOs in brain tissue.

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Mr Janik Seidel[1], Dr Clifford Young[1], Dr Manuela Klingler-Hoffmann[1], Dr Leigh Donnellan[1], Dr Mark Condina[1,3], Dr Alok Shah[2], Dr Sri Ramarathinam[2], Dr Lee Xin Chong[2], Prof Peter Hoffmann[1]
[1]University of South Australia (UniSA), [2]CSL Ltd, [3]Mass Dynamics

An optimized DIA-MS workflow for HCP quantification in bioreactors to assess relationship in between processing conditions and critical quality attributes

Janik Seidel 255 - An optimized DIA-MS workflow for HCP quantification in bioreactors to assess relationship in between processing conditions and critical quality attributes, Meeting Room 105, August 21, 2024, 11:41 - 12:00

The production and purification of biopharmaceutical proteins like antibodies using cellular expression systems is challenging, due to the heterogeneity of the product and complexity of parameters influencing the production and purification processes.
Host cell proteins (HCPs), protein impurities introduced by the expression system, are challenging to monitor and to deplete, due to the similarity of physicochemical properties such as molecular weight and isoelectric points.
Mass spectrometry (MS) as a non-targeted approach could potentially allow identification and quantification of HCP population. This study aims to utilise MS to monitor HCP populations within the bioreactors to gain a better understanding of the influence of processing conditions on HCP distribution and quantity.

Methods

Cell Culture Harvest samples were precipitated using acetone, subjected to SMART Trypsin digest followed by C18 cleanup and peptide quantification using a fluorescence-based assay. Two different spiking strategies (protein and peptide-level) were also evaluated. 200 ng of peptides were separated on a C18 Waters nanoEase column using 20 min gradient with a total method duration of 30 min. DIA data was acquired using Sciex ZenoTOF 7600 using 65 variable windows. Data Acquired was searched using Spectronaut (Biognosys) v17.4 and further processed and visualised using in house Python and R scripts.

Novel Aspect

This work presents a novel optimized workflow and investigation of effects on process changes to HCP population during cell culture.

Preliminary Data or Plenary Speaker Abstract

In this study we have evaluated and optimised sample preparation and data acquisition parameters to characterise the HCP population within cell culture harvests. The optimised method was able to identify and quantify over 1000 HCPs from daily cell culture samples and harvest. Additionally, this enabled monitoring and reporting of 20 high-risk proteins known to impact biopharmaceutical process development.
The impact of various standard spike-ins and modifications to the data processing pipeline on semi-absolute hi3-peptide quantification-based approaches. Our findings underscore the imperative for standardizing these methodologies to facilitate broader utilization.
The optimised method was used to investigate changes and differences in HCP abundance during the time course of the production within a bioreactor, as well as in between bioreactor runs, where the feeding control strategies and media composition was altered. The method was able to successfully monitor relative change in host cell protein abundance across these samples.
An investigation of process parameter influences showed that the change of feeding strategy change from bolus feeding to a setpoint strategy resulted in only small changes of the HCP populations, whereas changes in the media lead to changes in relative expression of individual high-risk HCPs.

In a last step we measured the amino acid profile of the bioreactors during the culture and tried to correlate observed changes in HCPs to observed changes in the metabolic profile.
In summary, this work presents a case study to highlight the capabilities to use MS to investigate CPP and CQA relationships during the cell culture.

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Developing MS-cleavable Cross-linking Mass Spectrometry for Profiling Multimeric Interactions of Cellular Networks

Keynote: Professor Lan Huang University of California 765 - Developing MS-cleavable Cross-linking Mass Spectrometry for Profiling Multimeric Interactions of Cellular Networks, Meeting Room 106, August 21, 2024, 10:00 - 10:25

Protein-protein interactions (PPIs) are essential for the assembly of protein complexes, which are the active molecular modules for controlling various biological processes to maintain cell viability and homeostasis. Detailed analysis of PPIs at the systems-level will not only advance our understanding of cellular structures and functions as well as their associations with human pathologies, but also facilitate the exploration of novel interaction-based therapeutics. However, obtaining an authentic portrait of endogenous PPI networks has been a difficult task. In recent years, cross-linking mass spectrometry (XL-MS) has emerged as a transformative technology for elucidating the structure, interaction, and dynamics of proteins. In comparison to other methods, XL-MS is unique due to its capability of capturing native interactions from cellular environments, and uncovering their identities and connectivity simultaneously without cell engineering. New advancements in XL-MS technologies have enabled the generation of thousands of PPIs for delineating cellular networks at the systems-level1,2. In particular, diverse MS-cleavable cross-linking reagents have been developed to facilitate the detection and identification of cross-linked peptides, allowing us to elucidate the structural organization of proteomes from intact cells, subcellular organelles, tissues and clinical samples. While binary cross-links have been very effective in PPI mapping, multimeric cross-links can offer enhanced spatial resolution to facilitate the characterization of dynamic and heterogenous protein complexes. However, the identification of multimeric cross-links containing more than 2 peptide constituents remains extremely challenging, due to the enormous expansion of database search space. To this end, we have developed a novel trifunctional MS-cleavable cross-linker that targets three amino acid sites and enables their unambiguous identification. Importantly, we demonstrate that the new XL-MS platform is effective in mapping PPIs of protein complexes and cellular networks from living cells. The resulting trimeric interactions yield new structural details that cannot be easily uncovered by existing reagents, permitting in-depth description of PPIs for defining protein complexes. This work is supported by NIH grant R35GM145249 to L.H.

The development of a novel MS-cleavable XL-MS platform for mapping multimeric interactions.

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Miss Ashleigh Dale[1,2,3], Dr Dylan Harney[1,3,4], A/Prof. Mark Larance[1,3,4], Dr Stuart J. Cordwell[1,2,3,4]
[1]University Of Sydney, [2]School of Life and Environmental Sciences, [3]Sydney Mass Spectrometry, [4]School of Medical Sciences

Membrane and flagellar enrichment increase the depth of large-scale bacterial interactomics studies using ion mobility and cross-linking mass spectrometry (XL-MS)

Ashleigh Dale 633 - Membrane and flagellar enrichment increase the depth of large-scale bacterial interactomics studies using ion mobility and cross-linking mass spectrometry (XL-MS), Meeting Room 106, August 21, 2024, 10:25 - 10:44

The post-genome era has been driven by global technologies such as transcriptomics and proteomics that have enabled comparative analyses of cells, tissues and organisms under 'control' and 'test' conditions. This research however, while hugely significant, has generally ignored the dimensionality of protein biochemistry; while concentrating on the relative abundance of transcripts and proteins, the knowledge that proteins largely convey functions by forming transient or covalent interactions, such as protein complexes, has been mostly neglected. We aimed to exploit these knowledge gaps to provide genome-wide protein interaction maps of the bacterial pathogen Campylobacter jejuni, and to develop membrane- and flagellar-focused cross-linking strategies to define interactions associated with key virulence traits including motility and adherence.

Methods

We undertook cross-linking of total cell lysates (TCL) and membrane protein-enriched fractions (MPEFs) before membrane enrichment (XL-MEM) or post-membrane enrichment (MEM-XL). We developed a workflow to isolate and cross-link the flagellar apparatus by exploiting flagellar-enriched fractions (FGM-XL). We also investigated the use of different proteases, including trypsin, Glu-C, and Trypsin/LysC on complex and enriched protein samples. We next investigated different fractionation approaches, including offline SEC and online gas-phase fractionation with FAIMS, and then investigated how different digestion approaches and gradient lengths affect the success of these approaches in identifying cross-linked peptide species. A hybrid MS2-MS3 fragmentation strategy with or without an internal-stepping compensation voltage scheme with FAIMS was used, and data was searched using the XlinkX node in Proteome Discoverer.

Novel Aspect

In situ membrane protein and flagellar apparatus cross-linking revealed novel interactions associated with virulence.

Preliminary Data or Plenary Speaker Abstract

FAIMS with unfractionated cross-linked TCL significantly increases the identification of cross-links (XLs) compared to SEC by more than 38%, and greater than 2.7-fold compared to unfractionated samples without FAIMS ((-) FAIMS). A single 1 µg injection with FAIMS yields more comprehensive protein and cross-linked peptide information compared to any 1µg SEC fraction injection, and a single injection with FAIMS is commensurate with 3 injections without FAIMS in terms of cross-linked peptides. FAIMS and SEC are complementary approaches, with the combination of both methods covering >95% of all XL-MS identifications in this comparison. The addition of FAIMS reduces isolation interference and reporter ion intensities, yet reporter ion identifications increase 4.8-fold and 1.6-fold compared to (-) FAIMS and SEC, respectively. The benefit of FAIMS increases with sample complexity and longer gradients.

The MEM-XL approach resulted in the largest enrichment of membrane-associated proteins, and drastically increased protein-protein interaction (PPI) reproducibility across replicates (~60% PPIs in >1 replicate, compared to ~50% in TCL, and ~25% in XL-MEM). Significantly, 22% of MEM-XL PPIs

were identified in n=5 replicates, compared to 1% in TCL. ~55% of all interactions in TCL samples were between cytoplasmic proteins alone. The MEM-XL approach was successful in significantly reducing cytoplasmic protein interactions. Our FGM-XL approach revealed flagellin multimerisation and flagella conformational dynamics for the first time, including multimeric interfaces and filament stabilisation.

We found that Glu-C is the least efficient at cleaving cross-linked peptides, however is highly orthogonal to Trypsin and Trypsin/LysC which are complimentary, with 78% of the Glu-C cross-links being unique. Preliminary data on comparing the use of FAIMS and SEC with different protease approaches suggests that the types of cross-linked peptides yielded from each type of digestion respond differently to FAIMS ion-based separation across a number of physicochemical properties.

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Ms Liuyu Peng[1], Dr. Richard Lee[2,3,4], A/Prof Nichollas Scott[5], Prof. Aleksandra Filipovska[2,3,4], **Professor Gavin Reid**[1,6,7]

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A Co-Fractionation Mass Spectrometry-Based Method for Investigating Disease Associated Alterations in Lipid-Protein Interactomes

Gavin Reid 196 - A Co-Fractionation Mass SpectrometryBased Method for Investigating Disease Associated Alterations in Lipid-Protein Interactomes, Meeting Room 106, August 21, 2024, 10:44 - 11:03

Lipids, as fundamental components of cellular membranes, play crucial roles in maintaining plasma membrane and organellar structures, as well as regulating various signalling processes through functional interactions with integral or peripherally associated membrane proteins. Although the importance of many specific protein-lipid interactions have been elucidated, methods for the identification and functional characterization of the lipid-protein 'interactome' on a global scale are currently lacking. A co-fractionation mass spectrometry (CF-MS) technique employing size exclusion chromatography (SEC) coupled with mass spectrometry (MS) based proteome analysis has been established for the identification of protein-protein interactomes. Here, we apply this strategy to enable the mapping of membrane lipid-protein interactomes in a cardiolipin synthase-associated multi-system mitochondrial disease system.

Methods

Non-denaturing membrane protein-lipid complex solubilization was carried out with mild detergent prior to SEC co-fractionation. To confirm the efficiency of solubilization and stability of solubilized membrane protein complexes and lipid-protein complexes, blue native gel, and quantitative MS was utilized. Co-fractionated lipid-protein interactions were then identified using an integrated lipid and protein extraction method followed by parallel quantitative high field asymmetric ion mobility spectrometry (FAIMS) - "shotgun" lipidome and data-independent acquisition (DIA) proteome analysis.

Novel Aspect

This is a novel strategy for revealing alterations in membrane lipid-protein interactions in multi-system mitochondrial-disease.

Preliminary Data or Plenary Speaker Abstract

Recently published work identified cardiolipin synthase mutations trigger respiratory chain complex dysfunction, resulting in multi-system mitochondrial disease. This supports the importance of lipids for maintaining activity of mitochondrial membrane proteins and protein complexes.

Blue native gel electrophoresis confirms the stability of mitochondrial protein complexes under a mild detergent membrane solubilisation environment, and the stability of these protein complexes under buffer exchange and detergent removal conditions as being compatible with downstream SEC fractionation, and MS-based lipidome and proteome analysis.

CF-MS lipid-protein interaction analysis were conducted on wild-type (WT) and cardiolipin synthase knockout (CRLS1-KO) CAL51 cell lines. Results showed that intact mitochondria respiratory chain protein complexes and supercomplexes are co-fractioned with cardiolipin in WT cell lines.

Conversely, significant disassembly of complex III subunits and mild disassembly of complex I subunit from mitochondria supercomplexes and were observed in CRLS1-KO cell line, indicating altered lipid-protein interactions in cardiolipin defect.

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Venita Sitahal
[1]York University

Elucidating the Structural Dynamics of Binding Interactions in Regulator of G-Protein Signaling-1 (RGS1) using Hydrogen Deuterium Exchange Mass Spectrometry (HDX-MS)

Venita Sitahal 363 - Elucidating the Structural Dynamics of Binding Interactions in Regulator of G-Protein Signaling-1 (RGS1) using Hydrogen Deuterium Exchange Mass Spectrometry (HDX-MS), Meeting Room 106, August 21, 2024, 11:03 - 11:22

Regulator of G protein signaling 1 (RGS1) is directly related to G protein-coupled receptor (GPCR) signaling pathways and is thus implicated in a variety of physiological processes. Multiple studies have linked dysregulated RGS1 expression and function to the progression of cancers such as gastric and breast cancers. However, the conformational dynamics of RGS1 binding has gone largely unexplored in the literature, creating a gap in essential knowledge.

RGS1 is a GTPase activating protein (GAP) that acts on the Gαi1 subunit of the heterotrimeric G protein complex to accelerate the termination of GPCR signaling. However, the allostery of RGS1 interactions with Gαi1 and other proteins have not been structurally characterized.

Methods

RGS1 and Gαi1 were individually expressed in E. coli BL21 cells and protein was purified by metal affinity chromatography. Concentrations were determined by UV absorbance at 280 nm. The identity and purity of the proteins were assessed by SDS-PAGE.

5 µM samples of RGS1, Gαi1, and RGS1 with Gαi1 were prepared and subjected to HDX reactions in buffered D2O at timepoints of 15, 30 or 300 seconds before quenching. Quenched samples were digested using an Enzymate BEH Pepsin column, desalted and reverse separated prior to injection onto the Select Series Cyclic IMS. Peptide identification was carried out using ProteinLynx Global Server (PLGS), followed by HDX analysis using DynamX.

Novel Aspect

Characterizing the conformational dynamics of RGS1 interactions with HDX-MS reveals new information for future drug design and therapeutics.

Preliminary Data or Plenary Speaker Abstract

Experiments examining the binding of RGS1 with Gαi1 found clear evidence of weak but consistent binding regions in RGS1. There was increased deuterium uptake in the αI helix region, suggesting weak allosteric changes, and weak binding in the αI, αII and αVI helix regions where decreases were seen. However, these results were in the absence of GTP, which is needed to lock Gαi1 in the active conformation for RGS1 binding. Given that Gαi1 is an enzyme, it is likely very dynamic, and previous research has shown that GTP is necessary for Gαi1 and RGS1 to bind in the right conformation to undergo GTP hydrolysis. In contrast, RGS1 is less dynamic, and thus weak binding interactions were preserved. The absence of GTP thereby accounts for the weak signals present in RGS1 and the absence of noticeable changes in Gαi1.

Consequently, further experimentation will aim to examine the binding between RGS1 with Gαi1 in the presence of a GTP analogue which will allow us to elucidate allosteric changes in both RGS1 and Gαi1 when binding in the active conformation occurs. The allosteric changes and binding in both RGS1 and Gαi1 will reveal new information about their activity and structure.

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Mr Adam Cahill[1,2,3,4], Mr Benjamin Fenton[1,2,3], Dr Martin Walko[1,3], Prof Nikil Kapur[4], Dr Megan Wright[1,3], Dr Antonio Calabrese[1,2]
[1]University of Leeds, [2]School of Molecular and Cellular Biology, Faculty of Biological Sciences, University of Leeds, [3]School of Chemistry, Faculty of Engineering and Physical Sciences, University of Leeds, [4]School of Mechanical Engineering, Faculty of Engineering and Physical Sciences

Development of photoactivatable lysine reactive crosslinking reagents

Adam Cahill 322 - Development of photoactivatable lysine reactive crosslinking reagents, Meeting Room 106, August 21, 2024, 11:22 - 11:41

Cross-linking mass spectrometry (XL-MS) is a technique to study protein structure and interactions by covalently capturing non-covalent interactions through the addition of bifunctional molecules that react with amino acid sidechains to join proximal residues covalently. This method is widely utilised, but there are still many issues within the cross-linking workflow that limits the biological insights that can be obtained. A key issue is that photoreactive crosslinkers for temporally controlled cross-linking react slowly, non-specifically and with low yields, minimising the temporal resolution achievable and providing challenges for data analysis. Here we aim to address this challenge by developing new crosslinking head groups that incorporates ortho nitrobenzyl alcohol (oNBA) analogues that are photoactivatable, with fast reactivity and specificity towards lysine residues.

Methods

oNBA analogues were synthesised and we have characterised these molecules using a combination of intact mass, small molecule MS and UV/Vis spectroscopy. From this, we synthesised a range of crosslinkers, including different homo-bifunctional crosslinkers (containing two oNBA reactive groups) and hetero bifunctional crosslinkers (containing one oNBA and one commonly utilised NHS ester). Using model proteins, we performed SDS-PAGE gels and XL-MS experiments on both the Orbitrap Eclipse (Thermo Fisher) and TIMS-ToF Pro 2 (Bruker) to identify crosslinks and compare the data obtained to well established commercial crosslinkers. To analyse the data produced we have used a variety of software tools such as Proteome Discoverer, MeroX and MaxQuant.

Novel Aspect

Optimisation and validation of novel photoactivatable crosslinking reagents that specifically target Lysine residues.

Preliminary Data or Plenary Speaker Abstract

We have tuned the electronic properties of the oNBA functional group to develop oNBA analogues with improved properties for chemical crosslinking. Using these reagents, we have shown that oNBA containing reagents can be effectively utilised to profile solvent-accessible lysine residues. We have found that incorporating oNBA reactive groups into crosslinkers results in reagents that can produce yields of crosslinked material that are comparable to commercially available diazirine-containing crosslinkers (e.g. succinimidyl 4,4'-azipentanoate, SDA). Importantly, we have shown that oNBA crosslinkers react specifically with lysine sidechains, unlike UV activatable diazirine crosslinkers. We have also tuned the chemical properties of the oNBA group to improve the water solubility of the crosslinking reagents without affecting reactivity. To validate these analogues and their ability to crosslink proteins, we have developed and characterised a novel 365 nm UV LED irradiation platform that enables rapid, high yielding crosslinking reactions. In summary, here we have characterised oNBA analogues as lysine-reactive functional groups, which has allowed us to create both hetero-bifunctional and homo-bifunctional crosslinkers that provide high quantity and quality crosslinks for XL-MS. These reagents outperform commercially available crosslinkers and in combination with our novel UV LED irradiation platform have the added benefit of temporal control, with maximal yields of crosslinked material achieved in in under 10 seconds. We envisage that deploying this crosslinking strategy, using oNBA-containing reagents, will enable time-resolved XL-MS in vitro and in cells, to

afford novel insights into dynamic protein structures, transient interactions and conformational changes.

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Dr. Debasmita Ghosh[1], Prof. Michal Sharon[1]
[1]Weizmann Institute of Science

Mass Shifts Induction by Protein-Protein Interactions: A Novel Direct-MS Method

Debasmita Ghosh 337 - Mass Shifts Induction by Protein-Protein Interactions: A Novel Direct-MS Method, Meeting Room 106, August 21, 2024, 11:41 - 12:00

Direct-MS is a native MS (nMS) method pioneered by the Sharon group, offering a rapid and straightforward analysis of overproduced proteins without the need for prior purification. In this method, low-abundance proteins are effectively masked by the overproduced protein(s). This innovative approach preserves the natural environment and biological diversity, while saving considerable time and labor.
However, a challenge arises when applying this technique to human cells due to their typically lower protein production levels compared to bacterial cells. This can result in reduced signal-to-noise ratio, potentially masking the charge states of the target protein and compromising the method's advantages. To address this bottleneck in human cells, we have developed a methodology that enables the straightforward investigation of protein-protein interactions (PPIs).

Methods

Human expression systems are known for their superior capability in proper protein folding, assembly, and post-translational modifications compared to bacterial systems. However, this presents challenges when signals are distributed across multiple channels, as background peaks may mask the data. To address this issue, we are shifting the low-intensity peaks of the target protein to the less crowded high m/z region, thereby facilitating more efficient data analysis. To achieve this, we induce mass shifts by binding the target proteins with tandem split green fluorescent protein (GFP) tags. These tags consist of GFP_{11} and GFP_{1-10}, which auto-assemble into a complete GFP protein. We have fused one, three, and seven repeats of GFP_{11} to the C-terminus of the target protein.

Novel Aspect

This will offer unprecedented insights into the composition and structure of target protein from human cell lysates without prior purification.

Preliminary Data or Plenary Speaker Abstract

We applied direct-MS to the GFP_{11}-tagged kinase domain of Bruton's tyrosine kinase (BTK-1GFP_{11}), an important drug target in many diseases. BTK had a lower expression levels in human cells compared to E. coli cells. Hence, we employed BTK_KD as a model protein to implement the split GFP complementation method. Here the split GFP_{1-10} is capable of specifically bind with the target protein to selectively push its charge states to high m/z region. Following BTK-1GFP_{11} expression and lysis, this was characterized by direct-MS and confirmed by western blot. Subsequently, we initiated simultaneous co-expression of split GFP_{1-10} and target BTK-1GFP_{11} in HEK293T cells, which led to the observation of fluorescence signals, indicating the successful reassembly of split GFP and confirming non-covalent protein-protein interactions within the cells. Cell lysis and MS experiments were done in MS compatible 150 mM NH4OAc solution. Further characterization of the non-covalent complex was conducted using fluorescence microscopy and direct-MS. Analysis of HEK293T lysates revealed that the charge state distribution of endogenous proteins spanned from 2,000 to 3,500 m/z. Notably, the signal initially centered around m/z 3200 for the target protein alone, shifted to m/z 4500. This was confirmed by native MS/MS, which validated the presence of two split proteins. We then proceeded with tandem complementation using 3-GFP_{11} and 7-GFP_{11} fused BTK, resulting in an amplification of the fluorescence signal with both 3- and 7-GFP_{11}-tagged BTK. These non-covalent fluorescent complexes are currently undergoing further characterization through direct MS.

In summary, the MS signal has been distributed among multiple channels, and the migrated charge states are separated from the background signals. This makes them amenable to confident MS assignment. Also, this approach enables large flexibility in inducing the required mass shift, as various target protein systems can be used and combined, with a differing number of self-complementary tags.

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Dr Adam Cawley[1]
[1]Racing Analytical Services Ltd

Catching the cheats … explaining the innocent: Forensic perspectives for sports anti-doping.

Keynote: Professor Adam Cawley Racing Analytical Services Ltd 772 - Catching the cheats…explaining the innocent: Forensic perspectives for sports antidoping., Meeting Room 109, August 21, 2024, 10:00 - 10:25

Sports anti-doping is a multi-disciplined area of forensic science that aims to uphold integrity and protect the welfare of athletes. The continual evolution of pharmaceutical products derived from medical research to improve quality of life, coupled with the proliferation of illegitimate performance and image enhancement drugs, provide endless threats to sport. It follows from the proliferation of doping agents over time that a capability gap can exist between available drugs and reliable detection methods for them. To address this, untargeted screening methods are being developed to complement conventional targeted analysis strategies. High Resolution Mass Spectrometry (HRMS) technology and integrated software platforms can now provide the ability for anti-doping laboratories to identify abnormalities that can be further investigated.

Methods

Recent history and application of full-scan, data-dependent acquisition (DDA) and data-independent acquisition (DIA) in HRMS applied to the sports anti-doping and broader forensic context is presented. This is complemented with a “toolbox” of post-acquisition data analysis techniques that have the potential to combine conventional target-based analysis with reliable untargeted analysis. While the advantages of improved sensitivity and specificity from continued development of HRMS technologies to anti-doping is clear, chromatographic innovation has simultaneously played an important role for these parameters. Applications for these hyphenated methods include screening and identification of new psychoactive substances (NPS) and anabolic agents.

Novel Aspect

An intelligence-based framework for sports anti-doping using developments in mass spectrometry across multiple forensic applications.

Preliminary Data or Plenary Speaker Abstract

This presentation will explore how greater expertise in animal and human anti-doping can benefit from other forensic-related areas devoted to drug analysis using mass spectrometry. This provides the opportunity to develop an integrated drug intelligence and analysis model to support integrity and welfare in animal and human sports.

Miss Caitlin Jenkins[1,2], Fraser Powrie[3], Dr Celine Kelso[1,2], Dr Jody Morgan[1,2]
[1]School of Chemistry and Molecular Bioscience, University Of Wollongong, [2]Molecular Horizons, University of Wollongong, [3]NSW Ministry of Health, Centre for Population Health, Tobacco & E-cigarette Control Unit

CHEMICAL ANALYSIS OF ELECTRONIC CIGARETTES IN AUSTRALIAN SCHOOLS

Cailtin Jenkins 10 - Chemical Analysis of Electronic Cigarettes in Australian Schools, Meeting Room 109, August 21, 2024, 10:25 - 10:44

Disposable electronic cigarettes (e-cigarettes or vapes) have rapidly increased in popularity in Australia, particularly amongst the younger population. Despite regulations restricting the sale of nicotine containing e-cigarettes, these devices are still available illegally from a wide variety of over-the-counter 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.

Methods

This study chemically analysed 410 disposable e-cigarettes and e-liquids collected from Australian high school students. Identification and quantification of their chemical content 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 against the National Institute of Standards and Technology (NIST17).

Novel Aspect

This is the first study to chemically analyse e-cigarettes confiscated from Australian students, having implications for future Australian e-cigarette legislation.

Preliminary Data or Plenary Speaker Abstract

The majority of samples were fruity flavoured, disposable e-cigarettes from three main brands (IGET, HQD and Gunnpod). Nicotine was quantified in 97.3% of disposable samples with an average concentration of 40.0 mg/mL, always as the nicotine benzoate salt, while one refill e-liquid was found to contain freebase nicotine at a low concentration. Of the 21 samples with a labelled nicotine concentration, none were within ±10% of the stated value as required by the TGO110. Almost all samples contained the coolant WS-23 in relatively high concentrations compared to other flavouring chemicals present. Coolants may be added to reduce the throat irritation from high nicotine concentrations and flavouring chemicals, allowing for uptake by nicotine naive users. The observed concentrations of WS-23 may be unsafe based on published in vitro studies and therefore adversely impact the inhalational safety of these products. Acetal peaks were identified, confirming that the flavouring molecules were reacting in situ and forming products with relatively unknown toxicities. Chemicals prohibited under the TGO110 due to associated health risks were identified in 3.4% of the samples. This included the presence of ethylene glycol in moderately high concentrations (up to 13.2 mg/mL) which is a respiratory irritant and toxicological hazard.

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Ms Madysen Elbourne[1], Dr Adam Cawley[2,3], Mr Chris Bowen[4], John Keledjian[2], Anjali Gupta[1], Shanlin Fu[1]

[1]University Of Technology Sydney, [2]Australian Racing Forensic Laboratory, Racing NSW, , [3]Racing Analytical Services Ltd, [4]Shimadzu Scientific Instruments Australasia Pty Ltd

The indirect detection of dopaminergic manipulation in equine urine via an optimised routine and metabolomic-based LC-HRMS method.

Madysen Elbourne 543 - The indirect detection of dopaminergic manipulation in equine urine via an optimised routine and metabolomic-based LC-HRMS method, Meeting Room 109, August 21, 2024, 10:44 - 11:03

Current methods of urinary 3-methoxytyramine (3-MT) analysis for the indirect detection of dopaminergic manipulation in the equine industry are based on GC-MS and utilise a time-consuming sample preparation method featuring enzyme-hydrolysis, solid-phase extraction and derivatisation components. This study aims to develop a new analytical method to improve sample preparation efficiency and sensitivity by adapting a LC-HRMS method developed by Keen et al. (J Chroma B, 2023) for biomarkers in equine plasma. The adaption of this method was conducted for quantitative (3-MT and tyramine) and qualitative (phase I/II metabolite feature extraction) purposes, and "field-tested" using a pilot study involving the administration of dopaminergic Parkinson's disease medication, Stalevo 100 (25 mg carbidopa, 100 mg levodopa, 200 mg entacapone), in mares and geldings.

Methods

Urine (1mL) was combined with an acetate buffer solution and β-glucuronidase enzyme, pH adjusted to 5 – 5.5 and hydrolysed at 37°C overnight. The urine solution was aliquoted (200µL) with ACN and internal standard (10µL, 4µg/mL equiv. 3-MT-d4) in a microcentrifuge tube and vortexed, before micro-centrifuged (18000xg, 5 minutes). The supernatant was transferred to a glass test tube with methanolic HCl, and dried under nitrogen at 50°C. Reconstitution involved 20mM ammonium formate solution in 80% ACN. A C18 column guard was used with an Imtakt Intrada amino acid column (2.1x100 mm, 3µm), and samples analysed using electrospray positive mode on the Shimadzu LC-QTOF-MS 9030 instrument. Mobile phases were A:0.3% formic acid in 100% ACN, B:100mM ammonium formate in 100% water.

Novel Aspect

A fit-for-purpose LC-HRMS method has been developed for indirect detection of dopamine manipulation in equine urine via 3-MT and tyramine.

Preliminary Data or Plenary Speaker Abstract

The adapted Keen et al. method was further optimised for a urine matrix analysis, involving; the protein precipitation solvent type, addition of hydrolysis for conjugated compounds to follow current routine processes, and the reduction in column flowrate from 0.5 mL/min to 0.3 mL/min to account for analyte retention on the column and optimal peak shape. The quantitation of 3-MT and tyramine was conducted using a 2 – 8 µg/mL calibration range, spiked in a non-hydrolysed urine matrix. This range sits in line with the current urinary detection threshold for 3-MT in equine samples at 4 µg/mL. An adaptation of this untargeted method was optimised further to remove the need for hydrolysis and started by aliquoting a reduced volume of urine sample (100 µL) with ACN (300 µL) and internal standard (10 µL, 200 ng/mL equivalent of an IS mix) in a microcentrifuge tube (600 µL capacity). This metabolomic approach captured data using a data independent acquisition (DIA) method, and results were normalised and aligned in MS-Dial, before feature extraction/statistical processing in MetaboAnalyst 6.0. The metabolomic feature extraction of free and intact conjugated metabolites was analysed in both positive and negative mode to obtain other potential biomarkers for further investigation. Additionally, the identification of potential biomarkers may be validated with use of a reference population study in future to test their robustness. Results from a small pilot study analysis

show promising results for complementary use with current routine GC-MS processes in an equine racing context.

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Dr Kin-Sing WONG[1], Dr Hiu Wing CHEUNG[1], Ms Yung-Ching CHOI[1], Ms Ning-Sum TO[1], Dr Terence S.M. WAN[1], Dr Emmie N.M. HO[1]
[1]Racing Laboratory, The Hong Kong Jockey Club

Screening and confirmation of recombinant human follistatin in equine plasma for doping control purposes

Kin-Sing Wong 47 - Screening and confirmation of recombinant human follistatin in equine plasma for doping control purposes, Meeting Room 109, August 21, 2024, 11:03 - 11:22

Recombinant human follistatin (rhFST) is a potential performance enhancing agent owing to its stimulating effect on muscle growth. It functions as a homodimer and binds to members of the transforming growth factor β (TGF-β) family (e.g., myostatin), thus antagonising the TGF-β from binding to activin type II receptor which subsequently removes the inhibition to muscle growth. As a result, administration of rhFST to athletes is prohibited in human sports and horse racing. For an effective control of the misuse of rhFST in horses, methods for screening and confirmatory analysis are required.

Methods

High-throughput analysis of rhFST with a commercially-available enzyme-linked immunosorbent assay (ELISA) was used for the screening of equine plasma samples. Any suspicious finding would be subjected to confirmatory analysis as follows. rhFST in plasma was first immunocaptured by magnetic beads coupled with an anti-rhFST antibody. The extracted rhFST was then denatured, reduced and alkylated before trypsination. A proteotypic tryptic peptide, rhFST-T29, was then analysed by nano-liquid chromatography/high-resolution tandem mass spectrometry (nanoLC-MS/HRMS). Confirmation of rhFST was achieved by comparing the retention time and relative abundance of three characteristic product-ions of rhFST-T29 with those from the reference standard in accordance with the industry criteria published by the Association of Official Racing Chemists (AORC).

Novel Aspect

This is the first report of a comprehensive solution for the screening and confirmation of rhFST in equine plasma samples.

Preliminary Data or Plenary Speaker Abstract

The ELISA-based screening method had adequate specificity (0 % false hit out of 100 occurrences and 0 % false negative out of 51 occurrences) upon evaluation in a single-blind manner with a panel of blank and spiked plasma samples. The limit of detection [LoD, defined as the lowest concentration of rhFST spiked into multiple equine plasma samples (n = 15) that would be detectable for at least 95 % of occurrences] was estimated to be around 5 ng/mL. The high-throughput nature of the ELISA method together with its adequate specificity and sensitivity has provided a suitable screening method for the analysis of rhFST in equine plasma.

The selection of rhFST-T29 as the proteotypic tryptic peptide for the confirmatory analysis was based on its sequence uniqueness to rhFST and its highest signal-to-noise among other proteotypic tryptic peptides. As a result, the nanoLC-MS/HRMS-based confirmation method of immunocaptured rhFST had an estimated limit of confirmation (LoC) of 2.5 ng/mL. The estimated LoC was lower than the estimated LoD of the ELISA screen, thus providing a suitable follow-up method to confirm the presence of rhFST in an equine plasma sample (if any) that has been screened suspicious by the ELISA method. The method demonstrated adequate precision (12.9 %RSD for peak area and 0.03 %RSD for retention time) and recovery (55 %) as for confirmatory purposes.

The performances of the ELISA screening and nanoLC-MS/HRMS confirmation methods were fit for their intended purposes. The methods were successfully validated, and have been applied in the analysis of official equine plasma samples in the authors' laboratory since 2023.

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Emily Green[King's College London], Mr Steven Torney, **Dr Renee Webster**[1]
[1]CSIRO

Characterisation of stable isotopes in small molecules for chemical attribution signature determination using gas chromatography-high resolution accurate mass spectrometry

Renee Webster 364 -Characterisation of stable isotopes in small molecules for chemical attribution signature determination using gas chromatography-high resolution accurate mass spectrometry, Meeting Room 109, August 21, 2024, 11:22 - 11:41

Stable isotopes offer valuable insights into the origin, transformation, and fate of organic compounds in complex systems. As commercially available mass spectrometer technology improves, precise determination of isotope ratios can be achieved with accuracy in the sub-ppm range, and at trace concentrations without the need for a dedicated isotope ratio mass spectrometer instrument. By elucidating isotopic signatures of chemical compounds, this study aims to advance our understanding of chemical synthesis and degradation processes, and the identification of marker compounds which may serve as chemical fingerprints for source and/or synthetic route attribution. These can provide insights into interesting problems such as monitoring synthetic pathways, tracing chemicals of concern in complex matrices, and discerning signatures for verifying the provenance of products.

Methods

This study employs direct elution gas chromatography-high resolution accurate mass spectrometry techniques for the characterisation of stable isotopes in small organic molecules. Careful consideration and optimisation of the instrumental parameters, including mass resolution, acquisition rate, and chromatographic conditions is critical to ensure sufficient ions are transferred into the trap and requisite data are collected across peaks as they elute from the column. To determine isotope ratios, different scan ranges are applied to the mass filter in order to capture a) the predominant monoisotopic ion (M0) consisting of the lighter isotopes 1H, 12C, 32S as the base peak, and b) exclusion of the monoisotopic (M0) base peak, with a comparative increase in signal of the minor peaks and istoptologues.

Novel Aspect

Application of a new approach to stable isotopic analysis, for the determination of chemical attribution signatures and reaction monitoring.

Preliminary Data or Plenary Speaker Abstract

This study employs direct elution gas chromatography-high resolution accurate mass spectrometry techniques for the characterisation of stable isotopes in small organic molecules. Careful consideration and optimisation of the instrumental parameters, including mass resolution, acquisition rate, and chromatographic conditions is critical to ensure sufficient ions are transferred into the trap and requisite data are collected across peaks as they elute from the column. To determine isotope ratios, different scan ranges are applied to the mass filter in order to capture a) the predominant monoisotopic ion (M0) consisting of the lighter isotopes 1H, 12C, 32S as the base peak, and b) exclusion of the monoisotopic (M0) base peak, with a comparative increase in signal of the minor peaks and istoptologues.

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Mr Bruce Pui-Nam Yuen[1,2], Dr Kin-Sing WONG[2], Dr Yat-ming So[2], Dr Wai Him Kwok[2], Dr Hiu Wing Cheung[2], Dr Terence See Ming Wan[2], Dr Emmie Ngai-Man Ho[2], Prof Wing-Tak Wong[1]
[1]Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, [2]Racing Laboratory, The Hong Kong Jockey Club

Gene doping control analysis of human erythropoietin transgene in equine plasma by PCR-liquid chromatography high resolution tandem mass spectrometry

Bruce Pui-nam 645 - Gene doping control analysis of human erythropoietin transgene in equine plasma by PCR-liquid chromatography high resolution tandem mass spectrometry, Meeting Room 109, August 21, 2024, 11:41 - 12:00

Gene doping involves the misuse of genetic materials to alter athlete's performance, which is prohibited in both human and equine sports. Conventional approach in detection of transgene is accomplished by fluorescence-based real-time polymerase chain reaction (PCR) assay. Although sequencing techniques can be used to confirm the transgene sequence amplified by PCR, they are technically demanding and the necessary equipment may not be available in conventional doping control laboratories. Alternatively, mass spectrometry (MS) is recognised as a definitive technique and has been widely adopted for screening and confirmatory analyses by doping control laboratories. Therefore, it should be possible that the identification and detection of transgene by MS would facilitate the control of the misuse of transgene in humans and horses.

Methods

PCR was coupled with liquid chromatography high resolution tandem mass spectrometry (LC-HRMS/MS) for the confirmation of human erythropoietin (hEPO) transgene in equine blood sample. The method involved magnetic-glass-particle-based extraction of DNA from equine plasma prior to PCR. The targeted transgene was amplified by conventional PCR in the presence of 2'-deoxyuridine 5'-triphosphate (dUTP). Once amplified, the incorporated uracil bases throughout the amplicons were specifically cleaved by uracil DNA glycosylase (UDG) and hot piperidine treatments to generate LC-MS-detectable single-stranded DNA fragments. The detection of hEPO transgene was attained by monitoring a specific DNA fragment 5'-ATGGCTTCCTTCTGGGCTCCCAGAGCCCGAAGCAGAG-3' (R1) that corresponds to the exons 4/5 junction of the hEPO transgene.

Novel Aspect

This is the first report on a LC-HR/MSMS method coupled with PCR to confirm a transgene in equine blood sample.

Preliminary Data or Plenary Speaker Abstract

The method was validated in terms of estimated limit of detection (LoD), estimated limit of confirmation (LoC), specificity, and method precision. The LoD and LoC were both estimated to be 100 copies/mL of hEPO transgene in plasma, which was the lowest concentration prepared and evaluated. The specificity of the developed method was evaluated through in silico and in vitro analyses. A BLAST search against the human and horse genome databases displayed the in silico specificity of the R1 sequence to the hEPO transgene. In terms of in vitro specificity, no significant interference was observed from blank plasma matrices (n = 30) at the expected retention times of the targeted ion transitions. The specificity of this method for confirming the hEPO transgene in equine plasma arose from the PCR, silica-membrane-based purification, and LC-HRMS/MS analysis. The method exhibited adequate precision, with pooled relative standard deviations for relative abundances and retention times of 6% and 0.1%, respectively.

This method was successfully employed to confirm the presence of hEPO transgene in a blood sample collected from a gelding (castrated male horse) that had been administered the transgene. This work has presented an alternative approach to confirm the presence of a transgene via LC

retention times and characteristic product ions of a specific DNA fragment, in accordance with the industry criteria published by the Association of Official Racing Chemists (AORC).

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Professor Yasushi Ishihama[1]
[1]Kyoto University

Challenges toward ultrahigh-speed proteomics systems with high-sensitivity and high-depth

Keynote: Professor Yasushi Ishihama Kyoto University 688 - Challenges toward ultrahigh-speed proteomics systems with high-sensitivity and high-depth, Meeting Room 110, August 21, 2024, 10:00 - 10:25

Understanding proteins and protein complexes as life elements responsible for biological functions themselves is an essential step in understanding life homeostasis and diseases resulting from disturbances in life. However, while genomics and transcriptomics have reached technological maturity with the NGS advent, LC/MS/MS-based proteome sequencers, despite their rapid development, have yet to fully meet the expectations of the research community. What is required of next-generation proteome sequencers is (1) high sensitivity, (2) high depth to overcome the wide concentration dynamic range of proteome samples, and (3) high speed to enable high throughput measurements. These are in a trade-off relationship with each other, and further technological breakthroughs are required.

Methods

A Nexera Mikros (Shimadzu) pump was coupled online to a timsTOF Pro or timsTOF Pro 2 mass spectrometer (Bruker) in this study. The pump outlet was directly connected to a tee connector. A 50 µm ID × 150 mm capillary was used to connect the mixer to the sample injection valve. A home-made 1 µL sample loop was used in direct injection mode. The sample loop was kept in line during gradient elution. A 50 µm ID × 150 mm capillary was used to connect the sample injection valve to column inlet. Peptides were loaded onto a 15 cm monolithic silica capillary column (100 µm ID, self-pulled needle using home-made C18 monolithic silica column).

Novel Aspect

Ultrahigh-speed proteomics systems with 1000 samples per day were successfully developed with maintaining high sensitivity and depth of analysis.

Preliminary Data or Plenary Speaker Abstract

We have focused on speed-up and have investigated various ways to complete peptide sequencing by LC/MS/MS in about 1 min. To maximize the combination of fast MS/MS scans at the 100 Hz level and ion mobility separations at the 10 Hz level, we first studied reversed-phase HPLC systems under acidic conditions. In order to increase the speed of LC/MS measurements while maintaining the sensitivity of electrospray ionization under flow rates in nanoLC, a separation with an average peak with at half height of 1 s was achieved at flow rates of 7-10 µL/min on a 0.1 mm ID, 15 cm long capillary column with a 1-min linear gradient elution. On the other hand, more than 2.6 million peptide ions from proteome samples derived from human cultured cells were detectable on a 0.1 mm ID, 2 m long silica monolith capillary column with a 10-hour gradient elution at 500 nL/min, indicating that such a high depth of analysis is possible at the expense of high speed. To resolve this trade-off, we focused on increasing the depth and sensitivity of the above high-speed system, and through various studies, we have successfully developed an 1000 samples per day (1000 SPD) method in data independent acquisition mode that can achieve identification and quantitation of 3,139 protein groups from 100 ng HeLa cell digests and 2,145 protein groups from a sample of only 10 ng. In this talk, I will also show the robustness of this 1000 SPD method by analyzing 5000 samples within a week.

Associate Professor Laura Sanchez[1], Robert Shepherd[1], Austin Hopiavuori[1], Shaun McKinnnie[1]
[1]UC Santa Cruz

Trapped ion mobility spectrometry for high-throughput directed evolution screening of α-ketoglutarate dependent dioxygenases

Laura Sanchez 68 - Trapped ion mobility spectrometry for highthroughput directed evolution screening of αketoglutarate dependent dioxygenases, Meeting Room 110, August 21, 2024, 10:25 - 10:44

Recently, directed evolution (DE) technologies have become more sophisticated, enabling the creation of libraries of thousands of bacterial mutants to explore evolution in a laboratory setting with a desired biochemical result. While major advances have occurred in DE research, the time-consuming bottleneck of screening with specificity for certain chemical transformations, such as production of constitutional isomers, still persists. We have developed a new approach to directed evolution screening, implementing matrix assisted laser desorption/ionization-trapped ion mobility spectrometry-mass spectrometry (MALDI-tims-MS) directly from microbial colonies for high-throughput separation and identification of isomeric products generated in a DE screening campaign.

Methods

The kainic acid biosynthetic (Kab) enzyme 'KabC' facilitates conversion of prekainic acid (PKA) to the isomeric neurotransmitters kainic acid (KA) and kainic acid lactone (KAL). Our workflow allows for ionization of these isomeric products directly from E. coli colonies grown on agar containing the precursor substrate (PKA) and resolving them using tims, rapidly providing insight into product conversion. For KabC library generation, site directed, site saturation, and random mutagenesis (via PCR) were used. For sample analysis we utilized a Bruker timsTOF fleX and 1,5-diaminonaphthalene (1,5-DAN) matrix in negative mode. For increased robustness, we applied ten randomized bursts of 300 laser shots per spot at a laser frequency of 5000 Hz. All data were analyzed using MALDI-PharmaPulse and DataAnalysis (Bruker Daltonics).

Novel Aspect

A MALDI-tims-MS DE assay offers high-throughput isomeric separation and identification, drastically increasing the scope and efficiency of DE enzyme engineering.

Preliminary Data or Plenary Speaker Abstract

The goal of our DE screening campaign is to generate a library of engineered KabC enzymes with variable kainoid biosynthetic capabilities. We aim to mutate the KabC homolog dsKabC, isolated from the red macroalgae species Diginea simplex, to invert its biological activity from primarily synthesizing KA to primarily synthesizing the KAL product. This will facilitate direct and efficient access to KAL as an understudied ionotropic glutamate receptor antagonist, thus further expanding access to neuroactive tools while also providing a proof of concept for our analysis. Using synthetic standards of PKA, KA, and KAL, an ESI-tims method was generated so tims parameters could be altered and resolution of KA and KAL could be observed in real-time. Once resolution of KA and KAL was achieved, the resulting tims parameters were incorporated into a MALDI-tims-QTOF MS method optimized for small molecule analysis in negative mode. Despite showing that our method could detect differences in the production of KAL between wild-type (WT) and mutant dsKabC, our preliminary screening results using a small library of 176 mutant colonies were not consistent with the enzyme activity that we observed in vitro. To further improve signal intensity of the products, 10 µM of isopropyl β-D-1-thiogalactopyranoside (IPTG) was incorporated into the LB agar growth plates alongside PKA to induce expression of dsKabC inside the E. coli cells and increase product formation. Upon addition of IPTG, a nearly 27-fold improvement to the average signal-to-noise ratio for m/z 212.09 (N = 8) was observed. Incorporation of heavy-labeled PKA (PKA-d5) into the matrix solution also improved consistency of product detection and ratio calculation, as observed by our analysis using Bruker’s MALDI-PharmaPulse MALDI-tims-MS acquisition and visualization software.

Pharmapulse allows for visualization based on the tims peak area ratio between KA and KAL, allowing for streamlined interpretation of high-throughput MALDI-tims data.

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Tin Cham Mak[1], Professor Emeritus Ronald Quinn[1], Dr Miaomiao Liu[1]
[1]Griffith Institute for Drug Discovery

Discovery of inhibitor against Mycobacterium tuberculosis Leucyl-tRNA synthetase (LeuRS) via Mass Spectrometry-based screening

Tin Cham Mak 197 - Discovery of inhibitor against Mycobacterium tuberculosis Leucyl-tRNA synthetase (LeuRS) via Mass Spectrometry-based screening, Meeting Room 110, August 21, 2024, 10:44 - 11:03

Tuberculosis (TB) remains a major global health threat, causing significant morbidity and mortality. The emergence of drug-resistant strains of M. tuberculosis has further highlighted the urgent need for novel therapeutic targets and treatments. Targeting aminoacyl-tRNA synthetases, essential enzymes in protein synthesis, has emerged as a promising strategy for developing new anti-TB drugs. This project aims to investigate the feasibility of using mass spectrometry for screening of tRNA synthetases as drug targets.

Methods

LeuRS was mixed with 100 compounds and injected into a Q-TOF-MS after incubation. The quadrupole was setup to capture the protein–ligand complex and filter off unbound molecules. Ligand was dissociated from the protein-ligand complex in the collision cell for detection. Identified ligands were then tested for inhibition in an enzyme assay and for antimycobacterial activity using M. smegmatis as a surrogate to evaluate inhibition against M. tuberculosis.

Novel Aspect

The identification of inhibitors targeting M. tuberculosis LeuRS which show antimycobacterial activity on M. smegmatis.

Preliminary Data or Plenary Speaker Abstract

Screening resulted for the identification of 24 compounds from a pool of 2000 candidates. Subsequent investigations into 20 of these compounds revealed 10 exhibiting inhibition on the M. smegmatis assay and categorised into 6 distinct series. Notably, one series demonstrated enzyme inhibition under low ATP concentrations and screening of analogues lead to a marine natural product with IC50 19 µM. The remaining series are under investigation via amino acid or tRNA competition assays.

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Rachel Smith[1], Tabea Gerlach[1], Joseph Sharratt[1], Reynard Spiess[1], Emrys Jones[3], Catherine Brookes[2], Professor Perdita Barran[1]
[1]Manchester Institute of Biotechnology, [2]Bristol Myers Squibb, [3]Waters Corporation

Development of automated high-throughput mass spectrometry methods for biotechnology and biomedical targets using desorption electrospray ionisation

Rachel Smith 229 - Development of automated high-throughput mass spectrometry methods for biotechnology and biomedical targets using desorption electrospray ionisation, Meeting Room 110, August 21, 2024, 11:03 - 11:22

Current validation of potential targets from biotransformation's or biofluids are often reliant on extensive purification which involves relatively high volumes of sample and solvent as well as plastic/glassware. Desorption electrospray ionisation mass spectrometry (DESI-MS) has been demonstrated to facilitate higher throughput for target ID, along with simplified sample preparation workflows that use less solvent and sample. Features of minimal sample preparation, spatial mapping capabilities and ease of automation indicate that DESI-MS is well-suited technique for HT-MS. The work presented here demonstrates a DESI-MS platform that is appropriate for HT screening applications, providing automated sample preparation and loading with analogous data processing.

Methods

Contactless acoustic-based liquid transfer performed with a Labcyte Echo 650 was used to prepare the Teflon coated glass slides prior to DESI analysis. A Waters prototype automated slide loader DESI source, termed AutoDESI, coupled to a Waters SYNAPT XS was used for all work presented here. Directed evolution screening reactions performed with this platform include biotransformations of Paroxetine and enzyme mutagenesis screening for the homocoupling reaction of aminopropanols. Sample preparation, sample support and spot density are also explored and optimised.

Novel Aspect

First presented HT screening applications for biotransformations using an AutoDESI source and Echo spotting.

Preliminary Data or Plenary Speaker Abstract

Presented here is a complete highly automated HT-MS screening workflow, from sample preparation to analysis, with an achievable throughput of 0.5 s/sample. With a lower density spot deposition, the AutoDESI is capable of screening <3500 samples a run in approximately 2.5 hours. Data based on increasing spot density will be presented which suggests a maximum achievable throughput in a single run as x10 higher. Both screening reactions explored with this platform demonstrate the suitability of DESI-MS as a HT screening method.

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Mr. Xiaobo Tian[1], Mr Gérard Hopfgartner[1]
[1]University Of Geneva

Differentiating specific and non-specific protein-metabolite interactions using gradient open port probe electrospray ionization mass spectrometry

Xiaobo Tian 45 - Differentiating specific and non-specific proteinmetabolite interactions using gradient open port probe electrospray ionization mass spectrometry, Meeting Room 110, August 21, 2024, 11:22 - 11:41

Native electrospray ionization mass spectrometry (ESI-MS) using nano ESI or desorption electrospray ionization (DESI) has been used to study interactions between macromolecules and ligands, usually protein-metabolite interactions. In ESI the charge state distributions (CSD) of proteins differ between native and non-native conditions, and we report a method that can differentiate specific protein-metabolite interactions from non-specific binding based on protein unfolding by methanol. We found that forming high charges of protein-metabolite complexes requires higher proportions of methanol than free protein while, for non-specific complexes, there is no obvious difference in the CSD. By comparing the changes in the CSD of free protein and protein-metabolite complex versus the increase of methanol, we can distinguish metabolites that specifically interact with the target protein.

Methods

Our approach is based on a 3D-printed open port probe electrospray system (gOPP-ESI) using mobile phase gradients (aqueous to methanol) for the ionization of protein/protein-metabolite complex.

Novel Aspect

Recognizing metabolites that specifically interact with the target protein based on protein unfolding by methanol on the gOPP-ESI-MS system

Preliminary Data or Plenary Speaker Abstract

Notably, we found that a true protein-metabolite complex is more resistant to the denaturing effect of methanol compared to the free protein. This is corroborated by the observation that forming high charge states of protein-metabolite complexes requires higher proportions of methanol than free protein while, for non-specific complexes, there is no obvious difference in the CSD. Therefore, by comparing the changes in the CSD of free protein and protein-metabolite complex versus the increase of methanol, we can distinguish metabolites that specifically interact with the target protein. The approach is evaluated with well-characterized protein-ligand pairs, and we confirmed that cytidine phosphates, N, N', N"-triacetylchitotriose, and fluvastatin are specific ligands for ribonuclease A, lysozyme, and beta-lactoglobulin respectively. However, cytidine-5'-triphosphate (CTP) interacts non-specifically with lysozyme and beta-lactoglobulin. We believe that after first-round native-MS assays to identify which metabolites cause mass shifts to the free protein, the gOPP-ESI-MS could be used as a quick second-round check to exclude non-specific binding and discover metabolites truly interacting with the protein of interest, reducing the number of candidates for subsequent validation experiments.

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Cellular-level resolution DESI-MS imaging

Chengyi Xie 429 - Cellular-level resolution DESI-MS imaging, Meeting Room 110, August 21, 2024, 11:41 - 12:00

Desorption electrospray ionization (DESI), introduced by Cooks and his team in 2004, stands out as an innovative ambient imaging technique. It is renowned for its minimal sample preparation, user-friendliness, and greater stability compared to other surface-based liquid droplet extraction and desorption imaging methods. Nevertheless, its spatial resolution, typically ranging from 50 to 150 µm, falls short of other soft ionization-based molecular mass spectrometry imaging (MSI) techniques, such as matrix-assisted laser desorption ionization (MALDI) and nanoDESI. This limitation stems from the inherent spray geometry of the ESI process, which leads to a larger projection diameter of the solvent plume on the tissue section. Although optimizing the spray head can enhance resolution, the potential for improvement remains constrained.

Methods

Expansion DESI mass spectrometry imaging was developed to improve the resolution of DESI MSI. The samples were first embedded in a swellable hydrogel. During gelation, both proteins and lipids were anchored to the gel network under well-controlled conditions. After gelation, the sample is fully expanded in water. The tissue is then dried and desalted before DESI imaging. Finally, imaging was conducted using our cost-effective homebuilt DESI source, coupled with an orbitrap mass spectrometer.

Novel Aspect

Cellular-level DESI imaging at a resolution of about 5 µm was achieved for the first time.

Preliminary Data or Plenary Speaker Abstract

In this study, we introduced a cellular-level mass spectrometry imaging method termed expansion DESI MSI. A low-cost homebuilt DESI ion source was employed with a measured resolution of about 50 µm for MSI. After implementing a nearly ten-fold expansion technique, DESI mass spectrometry imaging has achieved a lateral spatial resolution of approximately 5 µm, making it suitable for cellular-level analysis. This improved resolution allowed for more detailed structural imaging of mouse brain tissue, surpassing the capabilities of standard DESI imaging. Furthermore, the expanded DESI MSI technique demonstrated improved detection capabilities for specific lipids, such as ceramide and cerebroside. Importantly, the total lipid classes identified were not compromised compared to the control DESI MSI method. In summary, this study introduces a notable advancement in mass spectrometry imaging with the development of the expansion DESI MSI technique. This method achieves a nearly tenfold enhancement in spatial resolution, facilitating detailed cellular-level analysis. This advancement provides a more accessible method for analyzing complex biological samples at high spatial resolution, representing a significant improvement compared to traditional DESI MSI techniques.

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Dr. Makoto Arita[1,2]
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Lipidome signatures associated with aging and the host-microbiome interaction

Keynote: Professor Makoto Arita Keio University 673 - Lipidome signatures associated with aging and the host-microbiome interaction, Plenary 3, August 21, 2024, 15:00 - 15:25

Abnormal lipid metabolism is often a background factor of diseases, which may lead to the discovery of new seeds for drug discovery and medical applications such as early diagnosis and treatment. Recent advances in mass spectrometry have provided a major impact on lipid biology, suggesting that the lipid molecules analyzed in the past are only the tip of the iceberg.

Methods

We established a LC-QTOF/MS-based untargeted lipidomics platform equipped with MS-DIAL4 that enables us to identify the structural diversity of lipids in human and mouse tissues, cells, and commensal bacteria. Also, the Lipidome Atlas Project is ongoing by utilizing the timsTOF fleX MALDI-2-based mass spectrometry imaging (MSI) to visualize the local environment created by specific lipids on the dynamics and functions of multicellular systems.

Novel Aspect

LC-MS-based untargeted lipidomics and MSI-based spatial lipidomics revealed the lipidome signatures associated with aging and the host-microbiome interactions.

Preliminary Data or Plenary Speaker Abstract

By combining LC-MS-based untargeted lipidomics with feature-based molecular spectrum networking and MSI-based spatial lipidomics, we revealed that lipids with complex structures are produced by gut microbiota, and the local distribution of bacterial lipids were visualized that would help for the understanding of host-microbiome interactions. Also, we conducted untargeted lipidomics across different tissues from mice at various life stages to explore the potential link between aging and lipid metabolism, considering microbiome dependencies.

Professor Kohta Nakatani[1], Professor Yoshihiro Izumi[1], Professor Hironobu Umakoshi[2], Dr. Maki Yokomoto-Umakoshi[2], Ms. Tomoko Nakaji[1], Mr. Hiroki Kaneko[2], Mr. Hiroshi Nakao[2], Professor Yoshihiro Ogawa[2], Dr. Kazutaka Ikeda[3], **Professor Takeshi Bamba**[1]
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Solid phase extraction and LC/MS/MS methods for comprehensive targeted profiling of bioactive lipids

Takeshi Bamba 126 - Solid phase extraction and LC/MS/MS methods for comprehensive targeted profiling of bioactive lipids, Plenary 3, August 21, 2024, 15:25 - 15:44

The objective assessment of health status using information from blood metabolites can be applied to medical strategies such as early detection and prevention of disease. However, the number of clinical applications is stagnating despite the increase in biomarker research. Bioactive lipids such as sterols and eicosanoids bind to a nuclear and membrane receptor, and exert physiological and pathophysiological activity in biological systems. Therefore, comprehensive and quantitative information on bioactive lipids in the blood is expected to provide useful indicators for a better understanding of health status. Here, we have developed a new analytical method that combines solid-phase extraction (SPE) and a liquid chromatography tandem mass spectrometry (LC/MS/MS) to monitor a wide range of bioactive lipids.

Methods

The LC/MS/MS analyses were performed using a Nexera X2 UHPLC system coupled to an LCMS-8060 QqQMS (Shimadzu Co, Kyoto, Japan) equipped with a heated electrospray ionization source. The optimized final LC conditions were as follows: column, InertSustain C18 (GL Sciences Inc, Tokyo, Japan); injection volume, 10 µL; flow rate, 0.3 ml/min; column temperature, 50°C; mobile phase (A), 5 mM ammonium acetate in water/acetonitrile 75/25 (vol/vol); and mobile phase (B), 5 mM ammonium acetate in 2-propanol. The optimized gradient conditions were as follows: 1% B, 0 min; 1–38% B, 0–17 min; 38–99% B, 17–25 min; 99% B, 25–35 min; 99–1% B, 35–36 min; and 1% B, 36–46 min.

Novel Aspect

Breakthrough LC/MS/MS and sample preparation optimization for accurate and quantitative analysis of bioactive lipids, revealing new physiological insights

Preliminary Data or Plenary Speaker Abstract

The MS/MS conditions, including precursor ion, collision energy, product ion, and prequadrupole focusing voltage, for the 144 bioactive lipids and 28 stable isotope-labeled compounds were optimized using flow injection analysis of each authentic standard, with up to two transitions for each metabolite. LC conditions were evaluated under two different additive conditions using four LC columns: Inertsil ODS-4, InertSustain C18, Inertsil ODS-HL, and Inertsil ODS-P, which exhibit different hydrophobicities and stereoselectivities. The optimal condition was InertSustain C18 with ammonium acetate as an additive. We investigated a sample preparation method for human plasma, assuming that proteins, hydrophilic metabolites, and highly hydrophobic metabolites, such as phospholipids and neutral lipids, interfere with the LC/MS/MS measurements of bioactive lipids. As a result, the optimal SPE method successfully removed most of the interfering components, with the exception of lysophospholipids. The recovery rate of the bioactive lipids to be measured was more than 70%. The SPE method and LC/MS/MS methods were used to analyze human plasma samples. Samples were collected from eight healthy volunteers, including five males and three females. A total of 46 bioactive lipids (19 steroids, 20 bile acids, and 7 polyunsaturated fatty acid metabolites) were successfully quantified in the human plasma. The results indicated the presence of bioactive lipids with sex differences and circadian rhythms beyond those previously reported. Taken together, these

results demonstrate that the analytical system combining SPE and LC/MS/MS methods is useful for the simultaneous quantitative analysis of comprehensive bioactive lipids.

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Seung Hee Shin[1], Professor Myeong Hee Moon[1]
[1]Yonsei University

Optimization of skin sampling method for lipidomic analysis by nanoflow nUHPLC-ESI-MS/MS

Seung Hee Shin 72 - Optimization of skin sampling method for lipidomic analysis by nanoflow nUHPLC-ESI-MS/MS, Plenary 3, August 21, 2024, 15:44 - 16:03

The skin's outer layer, the stratum corneum, primarily comprises lipids such as ceramides and glycerolipids, serving as a crucial barrier against environmental factors. To efficiently collect the skin sample, tape stripping is commonly used, where an adhesive tape is used to collect the stratum corneum layers. However, variations in lipid removal per each attempt necessitate a standardized approach. This study aims to optimize skin sampling techniques by exploring ideal sampling locations and determining the optimal number of tape strips for repeated trials. Such improvement is crucial for enhancing the reliability and accuracy of skin sampling procedures, ensuring consistency in lipid analysis and aiding in the advancement of dermatological research and clinical practices.

Methods

Skin samples were obtained using the tape stripping procedure, where an adhesive disc was used. Samples were collected from seven consecutive tape strips at a single location and from four distinct areas: the forehead, forearm, cheek, and neck. Lipids were extracted from these samples and subjected to analysis using nanoflow ultrahigh performance liquid chromatography electrospray ionization tandem mass spectrometry (nUHPLC-ESI-MS/MS). Lipid profiles were examined by comparing the normalized peak area of each lipid species to the peak area of an internal standard specific to each lipid class. This comprehensive approach allowed for a detailed assessment of lipid composition and distribution across various skin regions, providing insights into skin lipid dynamics.

Novel Aspect

A proper sample pooling method was suggested as a representative tape stripping method to avoid loss of skin lipids.

Preliminary Data or Plenary Speaker Abstract

Repeatedly obtaining skin samples from the same spot revealed a trend where the number of identified lipid species tended to decrease, particularly becoming significant after the 6th layer. To overcome this variation in lipid composition between layers, a pooling method of different tape strips was adopted. Rather than analyzing each tape strip individually, extracts from the initial 3 or 5 consecutive layers were pooled, respectively, before analysis. This approach led to the identification of over 50 additional lipid species compared to individual tape strip extracts. Prior to optimizing the number of tape strips for sample pooling, an assessment of lipid composition and quantities among adjacent spots was conducted. Statistical comparison of the quantified results revealed that the normalized peak area of each lipid species from three adjacent spots did not differ significantly from each other. Therefore, sample pooling was adopted to establish a representative tape stripping method. By combining samples from multiple layers, this approach ensures a more accurate reflection of skin lipid composition while minimizing the risk of losing quantifiable lipids.

Mr. Patrick Mueller[1], Dr. Laura Gisela González Iglesias[1], Mr Romain Giraud[1], **Prof. Dr. Gérard Hopfgartner**[1]
[1]University of Geneva, Life Sciences Mass Spectrometry

Enhanced Mass Spectrometry Workflows using ESI and APPI with Multi Ion Activation Methods for Characterization of Lipids in Plasma Samples.

Gérard Hopfgartner 367 - Enhanced Mass Spectrometry Workflows using ESI and APPI with Multi Ion Activation Methods for Characterization of Lipids in Plasma Samples, Plenary 3, August 21, 2024, 16:03 - 16:22

The physiological functions of lipids are highly impacted by small structural differences in isomeric lipid molecules. However, the complete structural characterization and absolute quantitation of lipids in complex biological samples are still challenged by the high structural similarity and great structural diversity. In this study we apply complementary 2D liquid chromatography mass spectrometry and supercritical fluid chromatography–mass spectrometry with electrospray (ESI) or atmospheric pressure photoionization (APPI) workflows to achieve a more effective annotation of in human plasma lipids, in particular for isomeric double bond position. Informative spectra for ESI precursors and radical cation precursors generated by dopant assisted APPI can be obtained using multi-ion activation methods including collision (CID), electrons (EAD) or photons (UVPD) activated dissociation.

Methods

The human plasma lipids were extracted using the modified Matyash (MTBE) method using a PAL RTC (CTC Analytics, Zwingen, Switzerland). Off-line 2DxLC extracts analysis was performed using an Acquity UPLC BEH HILIC column and Xterra C18 both from Waters. SFC separation was achieved using a Nexera UC system (Shimadzu, Kyoto, Japan) using a Viridis HSS C18 SB column (Waters).
Mass spectrometric detection was performed on a QqTOF (6600 TripleTOF, Sciex)) equipped with a prototype ExD collision cell and a differential mobility cell, SCIEX, Concord, ON, Canada) or a ZenoTOF 7600 MS (Sciex) equipped with a UV laser operated at 213 nm. For APPI chlorobenzene was used as dopant. Data were collected in SWATH-DIA mode and scheduled MRMHR acquisition mode.

Novel Aspect

CID, EAD and UVPD fragmentation to improve the annotation and characterization of lipids ionized by ESI and dopant assisted APPI.

Preliminary Data or Plenary Speaker Abstract

The lipids extracted in the organic phase were analyzed using the HILIC method to achieve lipid class separation. Lipids were annotated as the first step using an in-house program, Compound Calculator, and the Skyline Lipid creator tool to create an HR-MS series of precursor and fragments-related features for rapid data processing. The implementation of a unit mass Q1 isolation SWATH/CID acquisition allowed the quantification and identification of specific lipids with minimized isotopic cross-talk. The fractions containing the target lipid classes were identified and collected automatically using the RTC robot. The eluant for each fraction was injected to separate the lipids according to their carbon number and unsaturations. The MS2 data using EAD-MRMHR and or UVPD-MRMHR yield additional structural information to assign the double bond position and regioisomeric positions of the acyl chains. Using this approach, it was possible to accurately annotate and quantify around 300 human plasma glycerophospholipids between SM, PC/PC-Os, LPC/LPC-Os, PE/PE-Os, LPE/LPE-Os, PI and PG.
The benefit of dopant-assisted APPI mass spectrometry is illustrated for the analysis of triglycerides and subsequent formation of triglyceride radical cations as well as deprotonated cations. The signal response of formed radical cations is higher than ammonia adducts formed by SFC-APCI and

comparable to ammonia adduct intensities formed using SFC-ESI. Moreover, collision induced dissociation of generated radical cations and deprotonated cations yield typical losses which allow to pinpoint the position of all double bond positions within acylglycerols. In addition, acylglycerols fragment ions can be used to differentiate between stereospecific numbering (Sn) positions of attached fatty acids.
Finally, the observed fragmentation patterns in CID of radical cations of lipids were rationalized and compared to the fragmentation pattern of lipids ionized by ESI and subjected to electron activated dissociation allowing in both cases to obtain information about double location.

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Comprehensive analysis of structural glycosphingolipids in clinical samples using trapped ion mobility spectrometry mass spectrometry

Huong Giang Vo 336 - Comprehensive analysis of structural glycosphingolipids in clinical samples using trapped ion mobility spectrometry mass spectrometry, Plenary 3, August 21, 2024, 16:22 - 16:41

To understand the impact of glycosphingolipids on disease mechanisms a comprehensive structural characterization and quantification of GSLs that extensively covers the GSLs pathways in samples is essential to pin point GSLs functions such cell-cell recognition, signaling, neuronal functions, immune responses, host-pathogen interaction, etc. in different diseases. We introduce here an optimized GSLs extraction method from human serum and brain tissue combined with a 4D-Glycospingolipidomics method using liquid chromatography (LC) and trapped ion mobility spectrometry (TIMS) with mass spectrometry (MS) that enables extends the qualitative and quantitative coverage of GSLs in clinical specimens. Furthermore, we demonstrate the potential of this methodology to identify Parkinson's Disease (PD) disease-associated GSLs in PD patients' serum and brain tissue.

Methods

Extraction of GSLs method was developed based on Kirsch et al. (2012)[1] to enhance extraction efficiency and detection yield in microflow LC-MS analysis. This method encompassing phospholipid depletion, reversed-phase purification and fractionation to enrich sialylated long chain GSLs aimed at enabling the structural resolution of the mixture using microflow tims-tof/PASEF. Additionally, parallel extraction of 24 samples increased workflow throughput. Sialylated GSLs were analyzed using Elute-UHPLC and timsToF Pro (Bruker Daltonics) in negative ion mode with PASEF-based fragmentation technique. A 4D- GSL database (m/z, RT, CCS, MS/MS spectra) was generated in-house from standards, porcine brain extract, human serum, and brain tissue. The method was applied on PD and control sample specimens to enable elucidation the relationship between GSLs and PD.

Novel Aspect

This method provides improved capability for analyzing GSLs, enabling more precise and in-depth investigation into complex biological samples.

Preliminary Data or Plenary Speaker Abstract

Normal-phase or HILIC chromatography has been widely used to separate and analyze GSLs in biological samples, distinguishing isomers arising from differences in the glycan chain. However, this study demonstrates that TIMS, a method within the capacity to separate molecules according to their structural conformation, acts as an efficient orthogonal separation technique to reversed-phase chromatography for GSLs analysis. Additionally, we showcased the effectiveness of 4D-Glycosphingolipidomics using microflow LC-TIMS-MS, demonstrating its ability of rapidly profile GSLs with increased sensitivity, depth and high resolution of structural heterogeneity of complex GSL mixtures.
As an intitial proof-of-concept for 4D-Glycosphinglipidomics method, we analyzed GSLs in reference serum lipidome (NIST serum 1951c) and sera from healthy volunteers. Overall, we detected 167 sialylated GSL species in human serum samples. Importantly, this approach surpasses previous nanoflow-based method[1] due to broader coverage of longer carbohydrate chain and an increased diversity of ceramides within GSLs.
The application and efficacy of the method were further evaluated by analyzing PD serum and clinical tissue samples compared to controls, which underpinned PD- specific GSL patterns.

1. Kirsch S, Souady J, Mormann M, Bindila L, Peter-Katalinić J (2012) Ceramide Profiles of Human Serum Gangliosides GM2 and GD1a exhibit Cancer-associated Alterations. J Glycomics Lipidomics S2: 005. doi:10.4172/2153-0637. S2-005

Ms Huaqi Su[1], Dr. Christopher Fowler[1], Prof. Colin Masters[1], Prof. Kevin Barnham[1], Dr. Laura Vella[1,2], Prof. Gavin Reid[3,4,5]
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Multi-omics characterization of highly enriched human plasma extracellular vesicles

Huaqi Su 54 - Multi-omics characterization of highly enriched human plasma extracellular vesicles, Plenary 3, August 21, 2024, 16:41 - 17:00

Small extracellular vesicles (sEVs) encapsulate and carry lipid cargo derived from various cellular processes and organelles such as mitochondria, endosomes and lysosomes. Small EVs circulating in the blood provide valuable insights into disease mechanisms and offer biomarkers for diseases that involve lipid dyshomeostasis, dysregulated cellular processes and dysfunctional organelles. However, co-isolation of plasma sEVs with highly abundant high-/low- density lipoprotein particles and proteins in blood plasma, complicates their molecular characterization, and potential utility. Here, to explore the potential of plasma sEVs, we describe the development of a plasma sEV isolation method based on density gradient ultracentrifugation (DGUC) and size exclusion chromatography (SEC) and utilized an integrated sample extraction and mass spectrometry based lipidomic and proteomic techniques to facilitate their characterization.

Methods

Human plasma sEVs were isolated by DGUC and four different SEC columns that have commonly been used in sEV isolation, featuring different resins and pore sizes, to test the efficiency in separating and enriching EVs from lipoprotein particles and blood proteins. The identity of sEVs was confirmed by western blotting (WB) and transmission electron microscopy. The highly enriched plasma sEVs were subjected to monophasic lipid extraction then analysed by semi-quantitative direct infusion ‘shotgun’ lipidomic analysis. From the pellet remaining after lipid extraction, proteins were digested, and peptides were analysed via label free quantitative data independent acquisition LC-MS/MS based proteomics.

Novel Aspect

Via lipidomics and proteomics, we demonstrate that reducing contaminants enables detection of potential protein and lipids biomarkers in plasma sEVs.

Preliminary Data or Plenary Speaker Abstract

According to WB, DGUC successfully separated the high density band (HD, composed of sEVs and high density lipoprotein particles) from low- and very low- density lipoprotein particles (represented by ApoB100). Then, the HD band was subjected to further sEVs separation and enrichment using SEC. The IZON qEVoriginal Legacy 70nm SEC was found to provide the greatest enrichment of plasma sEVs containing known EV markers, flotillin-1 and syntenin, and the depletion of a marker for high density lipoprotein particles, ApoA-I. A total of 620, 866 and 1167 proteins were identified from the platelet free plasma, HD fraction and sEVs, respectively. The reduction of plasma and HD protein contaminants enabled the detection of numerous sEV associated proteins that have not previously been detected in plasma or plasma EVs, including proteins known to be involved in networks associated with mitochondria, endosomal autophagic and lysosomal pathways, and the central nervous system. Lipidomic analysis of the highly enriched sEVs resulted in the identification and quantitation of 358 lipids. Consistent with previous reports, the sEVs were found to be enriched in sphingomyelin (33.7 mol% total lipids), glycerophosphoethanoamine and glycerophosphoserine, which have been implicated in diseases associated with lipid dyshomeostasis. Notably, however, we

report here for the first time a 16-fold decrease in cholesteryl ester (CE) lipids in plasma sEVs compared to platelet free plasma, suggesting that CE lipid depletion could be used to assess the effectiveness of current and future methodologies for enriching plasma sEVs.

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Professor Jesper Olsen[1]
[1]University Of Copenhagen

High-throughput and scalable single-cell proteomics with narrow-window data-independent acquisition mass spectrometry

Keynote: Professor Jesper Velgaard Olsen University of Copenhagen 700 - High-throughput and scalable single-cell proteomics with narrow-window data independent acquisition mass spectrometry, Meeting Room 105, August 21, 2024, 15:00 - 15:25

Mass spectrometry (MS)- based single-cell proteomics (SCP) is emerging as the next frontier in proteomics and has already enhanced our understanding of cellular differentiation and diseases by allowing for the direct measurement of single-cell proteomes and their post-translational modifications (PTMs). This capability is instrumental in delineating the functional phenotypes within cell populations, elucidating cellular and embryonic development, forecasting disease trajectories, and pinpointing specific surface markers and potential therapeutic targets unique to each cell type. However, SCP is nascent and faces challenges including limited sequence depth, throughput, and reproducibility, constraining its broader utility. This presentation introduces key methodological and technological advances, which considerably improve the sensitivity, coverage and dependability of protein identification from single cells.

Methods

Single cells of HeLa and differentiated iPSCs were sorted using the cellenONE. Sample lysis and digestion is performed within the proteoCHIP EVO 96 inside the CellenONE with a master mix consisting of 0.2% DDM, 100mM TEAB, 20 ng/µL trypsin, and 10 ng/µL Lys-C. The LC-MS/MS analysis was conducted using an Orbitrap Astral Mass Spectrometer operated in narrow-window DIA mode coupled with an Evosep One LC using Whisper flow rates. For peptide identification and quantitation, raw MS files were analyzed using DIA-NN 1.8 or Spectronaut v18 using a spectral library-free directDIA+ approach.

Novel Aspect

High-throughput single-cell proteomics workflow with over 5000 proteins identified per cell

Preliminary Data or Plenary Speaker Abstract

Single-cell proteomics (SCP) promises to revolutionize biomedicine by providing an unparalleled view of the proteome in individual cells. We introduce a streamlined SCP workflow encompassing sample preparation to MS analysis for LFQ-based quantitative analysis of over 5000 proteins and 40,000 peptides in single HeLa cells. Our workflow involves single cell dispensing and sample preparation using the cellenONE with a proteoCHIP EVO96 and direct transfer to Evotip disposal trap columns, and subsequent LC-MS/MS analysis using the Evosep One LC with Whisper flow gradients coupled to narrow-window data-independent acquisition (nDIA) on the Orbitrap Astral mass spectrometer. The methods have been optimized to deliver a throughput of processing and analyzing up to 120 label-free SCP samples per day. A comprehensive evaluation of analytical software tools, alongside strict false discovery rate (FDR) controls solidified the reliability of our results. These enhancements also facilitated the direct detection of post-translational modifications (PTMs) such a site-specific phosphorylation in single cells, negating the need for enrichment and thereby simplifying the analytical process. An optimized tissue dissociation buffer enables effective single cell disaggregation of drug-treated cancer cell spheroids, refining overall SCP analysis. Analyzing non-directed induced pluripotent stem cell (iPSC) differentiation, we can consistently quantify stem cell markers such as OCT4 and SOX2 in single hiPSCs and lineage markers like GATA4 (mesoderm), HAND1 (endoderm) and MAP2 (ectoderm) in different embryoid body cells. Our workflow sets a new benchmark in LFQ-

based SCP for sensitivity and throughput, with broad applications in basic biology and biomedicine for identification of cell type-specific markers and therapeutic targets.

Mr Nathan Burke[1,2,3], Dr David Skerrett-Byrne[1,2], Mrs Amanda L Anderson[1,2], Dr Shaun Roman[4], Dr John E Schjenken[1,2], Professor Brett Nixon[1,2,5], Dr Liz Bromfield[1,2,3,5]
[1]Priority Research Centre for Reproductive Science, School of Environmental and Life Sciences, Discipline of Biological Sciences, The University of Newcastle, [2]Infertility and Reproduction Research Program, Hunter Medical Research Institute, [3]School of BioSciences, Faculty of Science, Bio21 Institute, The University of Melbourne, [4]NSW Health Pathology, [5]These authors contributed equally to this work

Phosphoproteomic analysis of human sperm capacitation reveals novel, druggable kinases offering new non-hormonal male contraceptive targets

Nathan Burke 248 - Phosphoproteomic analysis of human sperm capacitation reveals novel, druggable kinases offering new non-hormonal male contraceptive targets, Meeting Room 105, August 21, 2024, 15:25 - 15:44

Extrinsic microenvironments provide crucial stimuli to transcriptionally and translationally silent human spermatozoa priming them for fertilisation. Residency in the female reproductive tract is one such microenvironment that establishes fertilisation competency by promoting capacitation associated phosphorylation signalling cascades. With some kinases considered synonymous with successful sperm capacitation (e.g. protein kinase A), protein phosphorylation forms a dynamic and essential component of sperm maturation. Despite the essential nature of phosphorylation to mammalian fertilisation, a comprehensive analysis of the phosphoproteomic landscape of capacitating human spermatozoa has yet to be reported.

Methods

To characterise the cellular signalling events underpinning sperm capacitation we performed phosphopeptide enrichment and high-resolution tandem mass spectrometry to quantify protein phosphorylation in populations of non-capacitated human spermatozoa as well as those subjected to capacitation stimuli in-vitro. Sample preparation followed the EasyPhos protocol with parallel digestion, alkylation and TiO2-bead phosphopeptide enrichment. Reverse phase nLC-MS/MS was performed using an Orbitrap Exploris 480 MS coupled to a Dionex UltiMate 3000RSLC nano high-performance liquid chromatography system and Proteome Discoverer 2.4 was used for protein identification and label-free quantitation. To provide further biological context to the phosphoproteomes, we loaded the normalized abundances of phosphorylated residues (1086 serine, 97 threonine, and 47 tyrosine residues) into Phosphomatics, an interactive substrate-kinase mapping tool for global phosphoproteomic data.

Novel Aspect

Mapping the phosphoproteomic landscape of capacitating human spermatozoa reveals kinase candidates as novel, highly coveted male contraceptive targets.

Preliminary Data or Plenary Speaker Abstract

This strategy successfully identified 2,350 site-specific phosphorylation events mapped across 902 unique sperm proteins. In congruence with previous findings indicating the importance of tyrosine phosphorylation to fertilisation, a 2-fold increase (representing a 104% gain) in tyrosine phosphorylated sites was observed following capacitation, compared to a modest 5% gain in the phosphorylation of serine residues under the same conditions. Capacitation significantly upregulated phosphorylation in 124 proteins (1.5-fold change, $p<0.05$) and stimulated phosphorylation of a further 40 proteins. Of this subset of capacitation-sensitive phosphoproteins, 44% had a previously characterised role in sperm function, including A-kinase anchoring protein 4 (AKAP4) and heat shock protein A2 (HSPA2), which are critical contributors to motility and sperm-egg binding.
Mapping of phospho-residues to upstream kinases revealed a suite of novel sperm kinases with previously unappreciated functions in sperm. Pharmacological inhibition of p21 activated kinase 1 (PAK1) and polo-like kinase 1 (PLK1) during capacitation hampered sperm progressive-motility, while

PLK1 inhibition significantly attenuated capacitation associated tyrosine phosphorylation to levels congruent with a loss of fertilisation competency. AKT serine/threonine kinase 1 (AKT1) inhibition during capacitation suppressed acrosomal exocytosis, an essential process penultimate to sperm-egg fusion and fertilisation. These results demonstrate the utility of kinase inhibition in arresting sperm activation following external stimuli. Further, these findings permit a new understanding of key kinases that act as functional regulators of human spermatozoa and shed light on new candidates for highly coveted male contraceptive targets.

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Miss Molly Talbot[1,3], Dr Alexander W. Rookyard[2,3,5], Dr Desmond K. Li[2,4], Dr Stuart J. Cordwell[2,3,5], Dr Melanie Y. White[1,3]

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Reversibly oxidised cysteine post-translational modifications in diabetic cardiomyopathy following antioxidant N-propionylglycine, identified using quantitative mass spectrometry.

Molly Talbot 617 - Reversibly oxidised cysteine posttranslational modifications in diabetic cardiomyopathy following antioxidant Npropionylglycine, identified using quantitative mass spectrometry, Meeting Room 105, August 21, 2024, 15:44 - 16:03

Overproduction of reactive oxygen species (ROS) is a key determinant in obesity, hyperglycaemia, and insulin resistance to promote Type 2 Diabetes (T2D) and cardiovascular disease (CVD). When cellular redox levels outweigh endogenous cellular antioxidant capacity reactive sulphur groups in cysteine resides are altered, manifesting as cardiac functional deficiencies. Current pre-clinical evidence reveals the potential for antioxidant therapies to attenuate ROS and limit the extent of damage and dysfunction by restoring redox balance. To date, the translation of antioxidant treatments to clinical settings has been unsuccessful, driving further investigation. Using mass spectrometry, we aimed to characterise modified cysteine-containing peptides in response to pathological levels of ROS and potential sites protected by acute antioxidant treatment.

Methods

Using a rat model that combines 8 weeks of a high-fat diet with a low-dose treatment using streptozotocin (STZ) at week 4, we have modelled the clinical development of T2D and diabetic cardiomyopathy. Treatment controls (diet alone and STZ alone) and physiological conditions provide a delineation of the discrete contributions of obesity, hyperglycaemia and insulin resistance. Hearts were excised to undergo Langendorff ex vivo perfusion, which permitted acute and targeted cardiac delivery of antioxidant N-propionylglycine (MPG; 1mM). For this study, we created three subsets, 40-minute perfusion (+/- MPG) and 5-minute perfusion to define the native redox balance (-MPG). Redox-modified cysteine residues enriched by thiol-disulfide exchange enrichment and identified using liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Novel Aspect

Characterising reversible redox modifications upon acute MPG treatment, identifying key proteins modified in diabetic hearts contributing to decreased contractility.

Preliminary Data or Plenary Speaker Abstract

Ex vivo perfusion of T2D hearts in the presence of MPG preserved myocardial contractility (97% maintenance RPP) by comparison to time-matched T2D hearts without MPG which showed a gradual decline in contractility (84% maintenance RPP). Contractility was measured by considering the maintenance of the rate pressure product (left ventricular developed pressure X heart rate). To identify cysteine sites “at risk” of being redox modified during ex vivo perfusion, we generated a third group of T2D hearts to capture the native redox balance. Using differential alkylation, we distinguished free-cysteines from those reversibly modified (e.g. disulfides, S-nitrosylated and S-gluthathionylated). The current study observed that MPG treatment protected cardiac antioxidants (e.g. Peroxidasin) and mitochondrial and contractile proteins. In contrast, proteins with known oxidoreductase activity were less captured by TDE upon MPG treatment. Finally, MPG showed little capacity to protect against redox changes to proteins regulating fatty acid metabolism, suggesting that the impact of caloric excess provided by long-term high-fat diet feeding promoted redox changes in metabolic pathways that could not be protected by acute MPG treatment. This study

shows contractile dysregulation observed during ex vivo perfusion reflects the susceptibility of cysteine-containing proteins in the mitochondria and contractile apparatus to redox alterations, which occur along a separate time course to those redox modifications targeting fatty acid metabolism.

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Ms. Atreyee Sengupta[1], Dr. Kanika Narula[1], Dr. Arunima Sinha[1], **Professor Subhra Chakraborty**[1]
[1]National Institute of Plant Genome Research

Climate Change & Food Security:

Organellar Crosstalk and Post-translational Control Shaping Plant Immunity

Subra Chakraborty 712 - Climate Change & Food Security: Organellar Crosstalk and Post-translational Control Shaping Plant Immunity, Meeting Room 105, August 21, 2024, 16:03 - 16:22

Impending changes in the global climate coupled with mortality associated with fungal infections have resulted in challenges related to food and nutrition. Cell compartmentalization into different subcellular organelles is an attribute conserved in eukaryotes, including plants. Evidences suggest role of these organelles in defense response. However, the dynamic role of their intrinsic crosstalk in cellular signalling and PTM regulation of organellar proteins during pathogen infection remains largely unknown. Blast is one of the most destructive diseases of rice, causing considerable productivity loss.

Methods

To substantiate the role of Extracellular matrix (ECM) and nucleus in plant immunity, we carried out proteomics and phosphoproteomic analyses using TMT and iTRAQ coupled with liquid chromatography-tandem mass spectrometry (LC-MS/MS) in resistant rice variety challenged by Magnaporthe. Temporal lysine acetylome was developed using LC-MS/MS. GC-MS based metabolome was developed to explore molecular interactions that occur between organelles. To functionally characterize an immune regulator (OsIRF6), in-silico analysis and RNA expression profile were performed followed by an assessment of biophysical characterization. Interacting partners were identified using bacterial pull-down and protoplast-based co-IP followed by MS/MS analyses. The interactors were validated using BIFC assay. To further characterize the effect of OsIRF6, CRISPR/Cas9-mediated genome editing was carried out to generate OsIRF6 edited lines.

Novel Aspect

High-resolution cellular map and functional characterization depicted that OsIRF6 is a key regulator of immunity.

Preliminary Data or Plenary Speaker Abstract

Integrative multiomics data of two different organelles, extracellular matrix (ECM) and nucleus, revealed unique signatures characterizing each stage of infection besides uncovering the convergence and divergence of defense signalling. Altogether, our data highlighted five major signalling cascades operating between ECM and nucleus. The multilevel regulatory network generated in this study sets the foundation for in-depth mechanistic dissection of the inter-organellar crosstalk in immunity. Furthermore, we identified and functionally characterized a nucleotide-binding multi-functional protein with differential acetylation status upon infection, OsIRF6, as a key regulator of immunity that functions downstream of the oxylipin signalling pathway. This study provides valuable insight into understanding the intricate molecular mechanism that governs immunity against blast pathogens and biotechnological strategies for fungal disease resistance in crops.

Scott Peterman[1], Romain Huguet[2], Dr. Cristina Jacob[2], Alan Atkins[2], Jimmy Zeng[3], Sangeet Adhikari[3], Hao Qian[3], Megan Mora[3], Bruce Wilcox[3], Claudia Martins[2]
[1]Thermo Fisher Scientific, [2]Thermo Fisher Scientific, [3]PrognomIQ

Novel Hybrid Nominal Mass Instrument Enables Rapid Development of Large-Scale Targeted Plasma Proteomics Assays

Scott Peterman 518 - Novel Hybrid Nominal Mass Instrument Enables Rapid Development of Large-Scale Targeted Plasma Proteomics Assays, Meeting Room 105, August 21, 2024, 16:22 - 16:41

Mass spectrometry (MS) based targeted proteomics provides quantitative measurements of specific proteins or peptides in subject samples, but development of large-scale targeted quantitative assays to answer biological and clinical questions has seen limited success. Many of the inherent challenges of developing these assays (i.e. expense, transition selection, development time) with a triple quadrupole MS are addressed with a recently developed novel hybrid nominal mass instrument developed by ThermoFisher. This new instrument captures all transitions for every peptide and is simultaneously >3x faster than an Exploris 480 in PRM mode (75Hz vs 22Hz). To demonstrate the capabilities of this novel instrument, we developed a 24-min quantitative PRM assay of 804 peptides (1608 precursors) to evaluate the analytical figures of merit.

Methods

A pooled male plasma sample (BioIVT) was digested with in-house developed automated protocol and quantified with a Quantitative Fluorometric Peptide Assay kit (Pierce). The same pooled plasma standard was also processed on the Proteograph™ Product Suite (Proteograph) and all nanoparticle (NP) fractions were pooled to create a pooled NP (PNP) standard. PQ500 standard peptides (Biognosys) were prepared according to manufacturer's guidance and were spiked-into a digested plasma sample or Proteograph plasma for sample preparation. All prepared samples were subjected to a 60SPD PRM collection on a ThermoFisher Vanquish NEO Novel Hybrid Nominal Mass Instrument. Proteome Discoverer (v3.1), Skyline (v23.1) and PRM conductor(beta, skyline plug-in) were used to accelerate PRM methods construction and to evaluate data quality.

Novel Aspect

Systematic evaluation of a novel hybrid nominal mass instrument for large-scale quantitative plasma proteomics biomarker studies.

Preliminary Data or Plenary Speaker Abstract

The current gold standard for quantitative LCMS targeted proteomics assays utilizes a QQQ instrument, which offers superior precision, quantitative accuracy, and robustness. The development cycle for a QQQ quantitative proteomics assay is significant due to the need to carefully select transitions that are specific to each peptide, provide optimal S/N and minimal CV's. These issues are further exacerbated when creating assays with 100's to 1000's of endogenous and SIL peptides. Utilizing the new hybrid nominal mass instrument, we created a quantitative assay of 804 peptides (1608 targets) in <2 days utilizing the Gas Phase Fractionation DIA with HCD fragmentation mode of this instrument to quickly map retention times, select charge state and optimal transitions. The laborious task of selecting transitions when using a digested plasma sample with the complexity of Proteograph plasma was addressed through PRM data collection and subsequent filtering of transitions. This new instrument collects PRM data at >3x the speed of an Orbitrap instrument (75 Hz vs 22 Hz), which provided the opportunity to create a high throughput (60 SPD) quantitative targeted assay. To evaluate the quantitative capabilities of the instrument we performed a serial dilution of PQ500 SIL peptides into a fixed concentration Proteograph plasma matrix and demonstrated a 4-5x linear dynamic range. Early evaluation of 804 peptide assay in a pooled plasma digest sample (n=4 injections) suggests promising quantitative performance. A total 800/804 SIL peptides were

observed; with a median CV of 4.3%, and 91.3% of detected peptides have a CV below 20% with median of 11 Datapoints Per Peak (DPP). On the endogenous peptides, total 578/804 peptides were observed with median of 17.6% CV and 11 DPP. The robustness, reproducibility, sensitivity and overall utility of the instrument for biomarker detection is under evaluation through the analysis of a large cohort of cancer samples.

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Mr. Li Zhong[1], Prof. Zongwei Cai[1]
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Mass Spectrometry Proteomics Reveals PLEK as a Biomarker for the Early Phase of Severe COVID-19

Li Zhong 11 - Mass Spectrometry Proteomics Reveals PLEK as a Biomarker for the Early Phase of Severe COVID-19, Meeting Room 105, August 21, 2024, 16:41 - 17:00

SARS-CoV-2 infection, which is responsible for the ongoing COVID-19 pandemic, presents a wide range of clinical manifestations, from mild respiratory symptoms to severe respiratory distress and multi-organ failure. Understanding the factors that contribute to disease severity is critical to the development of effective therapeutic strategies. Identification of biomarkers associated with disease severity may aid in early detection and risk stratification.

Methods

In this study, mass spectrometry-based proteomics was applied as the main analytical method to the sera of mild and severe COVID-19 patients infected with wild-type and omicron variants to find the potential protein biomarkers. A well-established golden hamster model was used to verify the proteomic profiling with both the analytical method of ELISA and prm-PASEF, as well as to study the pathology. To illustrate the mechanism behind the biomarkers, a pull-down assay was performed in the Calu-3 cell line to screen the potential interaction proteins of the biomarkers. Then, a knock-down experiment was performed to verify the proposed mechanism.

Novel Aspect

This mechanism provides insight into the potential role of PLEK in promoting the inflammatory response associated with severe COVID-19.

Preliminary Data or Plenary Speaker Abstract

Proteomics analysis in serum of COVID-19 patients reveals that pleckstrin (PLEK) in sera of severe COVID-19 patients was significantly upregulated during the first ten days of infection, which is likely to be an early indicator of disease progression. Experimental studies in golden hamsters confirmed that PLEK was significantly upregulated in early severe infections. Using intranasal administration of exogenous PLEK in hamsters, increased PLEK expression was shown to exacerbate COVID-19 lung injury. PLEK expression is significantly correlated with levels of interleukin-6 (IL-6), interleukin-8 (IL-8) and monocyte chemoattractant protein-1 (MCP-1) in both patient sera and Calu-3. The observed correlation suggests a potential regulatory role for PLEK in modulating the inflammatory response during SARS-CoV-2 infection. Using pull-down and knock-down experiments, the mechanistic studies show that PLEK upregulation enhances virus-mediated inflammation through activation of the ERK1/2 signaling pathway.

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Dr. Xiaopeng Li[1]
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Multidimensional Mass Spectrometry Assisted Supramolecular Chemistry

Keynote: Professor Xiaopeng Li Shenzhen University 226 - Multidimensional Mass Spectrometry Assisted Supramolecular Chemistry, Meeting Room 106, August 21, 2024, 15:00 - 15:25

Supramolecular self-assembly, inspired by the precise organization of bio-macromolecules in living systems, allows for the spontaneous formation of ordered complexes from a chaos state. Among various noncovalent driving forces, metal–ligand coordination plays a vital role in the self-assembly of structurally and functionally sophisticated metallo-supramolecules, benefitting from the high directionality and predictable feature of coordination bonds. As a result, metallo-supramolecular chemistry has witnessed an explosion in constructing a myriad of artificial supramolecular architectures. Compared to the flourish of self-assembly strategies, however, the lack of efficient characterization methods always limits the investigation of assembled complexes at equilibrium and the kinetic details of self-assembly processes in solution because of the weak interaction and dynamic nature of metallo-supramolecules.

Methods

During the past 10 years, our lab focused on the characterization of synthetic macromolecules using multidimensional mass spectrometry, which enabled the construction and characterization of large metallo-supramolecules with high precision.

Novel Aspect

Multidimensional mass spectrometry is emerging as a powerful tool to assist the development of new synthetic chemistry.

Preliminary Data or Plenary Speaker Abstract

We first modified commercial mass spectrometer to minimize the fragmentation of metallo-supramolecules and provide high resolution mass spectra. Then, ion mobility mass spectrometry was applied to obtain the size and shape information of metallo-supramolecules. Moreover, we elucidated the stability of metallo-supramolecules using tandem mass spectrometry. Finally, we investigated the kinetic and thermodynamic features of coordination self-assembly processes.

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Mr. Nicolas Pizzala[1], **Professor Scott Mcluckey**[1]
[1]Purdue University

Ion/Ion Reactions for Complex Mixture Analysis:

Precursor Resolution via Ion Z-state Manipulation (PRIZM)

Scott McLuckey 629 - Ion/Ion Reactions for Complex Mixture Analysis: Precursor Resolution via Ion Z-state Manipulation (PRIZM), Meeting Room 106, August 21, 2024, 15:25 - 15:44

Electrospray ionization (ESI) is often the ionization method of choice, particularly for high mass polar molecules and complexes. When mixtures of analyte species are present, however, charge state ambiguities can arise along with overlap in mass-to-charge (m/z) from species with different mass and charge. Solution conditions can sometimes be used to generate relatively low charge states, which tends to reduce charge-state ambiguity and m/z overlaps. However, for complex mixtures, charge state reduction alone may be insufficient to resolve mixture components. A mass-selection step can simplify the mixture subjected to charge state manipulation, thereby allowing for improved separation of mixture components. The general tandem mass spectrometry approach is referred to here as precursor resolution via ion z-state manipulation (PRIZM).

Methods

Complex mixtures of proteins, protein complexes, or synthetic polymers were subjected nano-ESI, thereby generating a complex mixture of ions over a range of masses and charge states. A sub-set of the ions was mass-selected, in some cases following an ion parking step. The selected ions were then subjected to reaction with a mass-selected population of oppositely charged ions generated either via nano-ESI or atmospheric pressure chemical ionization. Upon mutual storage in an electrodynamic ion trap, the analyte ion charge states are reduced, thereby facilitating the resolution of the mixture components. The preceding procedure constitutes a PRIZM experiment. Experiments of this type were performed either on Q/TOF or 3-D ion trap platforms enabled for ion/ion reaction studies.

Novel Aspect

The PRIZM experiment constitutes an approach for the study of complex mixtures comprised of high mass and hetergeneous species.

Preliminary Data or Plenary Speaker Abstract

Examples in which a mass-selection step is followed by a charge manipulation step for the purpose of mass determination go back several decades. Examples of charge manipulation strategies include, ion/molecule proton transfer reactions, electron capture/transfer reactions, ion/ion proton reactions, and multiply-charged ion attachment (MIA) reactions. Here, we review briefly a few early examples of experiments that fall into the category of a PRIZM experiment. We then illustrate PRIZM for the characterization of the molecular weight distribution of synthetic polymer distributions using single charge transfer experiments as well as for mass determination of components in large biomolecule complexes using either proton transfer or MIA. Because PRIZM involvles a mass-selection step, it is desirable to be able to concentrate the signals from multiple charge states into a narrow m/z window for mass-selection and subsequent charge manipulation. This can be done using the ion parking technique, which involves the selective inhibition of ion/ion reaction rates. Ion parking at high m/z (e.g., m/z > 10,000), however, can be challenging due to the shallow well-depths associated with high m/z ions in electrodynamic ion traps. We have developed a forced-damped harmonic oscillator model to predict conditions for optimal ion parking at high m/z and demonstrate efficient ion parking out to roughly m/z 20,000. We demonstrate parking of ions of interest in native mass spectrometry and demonstrate a PRIZM experiment on species as large as GroEL (0.8 MDa).

Takaya Satoh[1], Azusa Kubota[1], Masaaki Ubukata[1]
[1]JEOL Ltd.

High Mass Resolution Mass Spectrometry for Assessing Polyethylene terephthalate Degradation: A Comprehensive Study using MALDI-TOFMS and GC-TOFMS

Takaya Satoh 128 - High Mass Resolution Mass Spectrometry for Assessing Polyethylene terephthalate Degradation: A Comprehensive Study using MALDI-TOFMS and GC-TOFMS, Meeting Room 106, August 21, 2024, 15:44 - 16:03

Conducting degradation analysis on PET products is important in ensuring quality control and maintaining long-term performance. PET is susceptible to degradation due to environmental factors and chemical attacks, which can alter its physical and chemical properties over time. Reactive-pyrolysis GC-MS is the most popular method for analyzing variations in the main chain of polyethylene terephthalate (PET), and MALDI-TOFMS is one of the major methods to analyze the variations in polymer end groups. MALDI-TOFMS can also analyze the sample surface of polymers. Combining different types of methods for a detailed characterization of UV-degraded PET is essential. This report describes the results from investigating structural variation at the early stage of UV irradiation using reactive-pyrolysis GC-TOFMS and MALDI-TOFMS.

Methods

For reactive-pyrolysis GC-TOFMS, the 0-8 hour UV irradiated PET films were mixed with 10 µL of tetramethylammonium hydroxide 25% w/w methanol solution (TMAH). The reactive-pyrolysis GC-TOFMS data were acquired in both EI and FI modes, and structure analysis was performed using an EI mass spectral database made by an AI model. For MALDI-TOFMS analysis, 2,4,6-trihydroxyacetophenone monohydrate (THAP) was used as the matrix. Two types of measurements were performed to investigate the UV degradation on the sample surface: first, the sample surfaces of the 0-1 hour UV irradiated PET films were directly measured, and second, PET film slices of different depths (0-1 and 2-4µm) after 0-4 hour UV irradiation were dissolved in HFIP and measured.

Novel Aspect

Detailed characterization of UV-degraded polyethylene terephthalate was performed by combining reactive pyrolysis GC-TOFMS and MALD-TOFMS results.

Preliminary Data or Plenary Speaker Abstract

Variance analysis was performed on the results of reactive-pyrolysis GC-TOFMS data before and after UV irradiation. Twelve characteristic pyrolysis products generated after UV irradiation were confirmed around RT 9-13 minutes. Three of the twelve pyrolysis products were identified by NIST library search. Automatic structure analysis was performed on pyrolysis products not registered in the NIST library database using the AI structure analysis function of msFineAnalysis AI. Molecular formulae were determined by integrated data analysis using electron ionization (EI) and field ionization (FI) data. Compound structures were estimated using library search results from the AI-predicted EI library created using structgures listed on the PubChem compound database. Furthermore, ion intensities of UV-degradation compounds increased with UV irradiation time. This variance was observed even in the early stages of UV degradation, such as one-hour UV irradiation. Compared to the bulk analysis of GC-TOFMS, MALDI-TOFMS is more suitable for sample surface analysis. As a result of direct measurement of the sample surface, the cyclic PET oligomers (series A) were observed in the mass spectra before UV irradiation. The polymer series changed to PET oligomers with COOH/COOH end-groups (series B) due to the photooxidation of the sample surface. The series B can be observed from the sample surface after 0.5 hour UV irradiation. This indicates the importance of analyzing the sample surface to confirm early-stage UV degradation. The degradation to depth direction from the sample surface was also investigated. The series A and B were both

observed in the mass spectra of 0-1μm and 2-4 μm slices from the sample surface after 4 hour UV irradiation. Comparing the ratio of series A to B, it increased from 0-1μm to 2-4 μm slices from the sample surface. The results suggested that the degradation degree will change in a few micrometer depth directions.

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Mr Callan Littlejohn[1,2], Dr Meng Li[2], Mr Sam Weekes[2], Miss Anna Cordiner[2], Dr Mark P. Barrow[2], Prof Peter O'Connor[2]
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Tools for Polymer Identification using Ultra-high Resolution Mass Spectrometry

Callan Littlejohn 554 - Tools for Polymer Identification using Ultrahigh Resolution Mass Spectrometry, Meeting Room 106, August 21, 2024, 16:03 - 16:22

Polymer contamination is now ubiquitous in nearly every ecosystem and there is a growing concern for the impact of these plastics on the wildlife within these environments. Accurate identification of plastics can be challenging using traditional methods, especially when the degradation of the plastic begins. The lack of accurate identification post degradation can be problematic as some polymers have higher impacts on life once degraded due to modifications making its uptake by cells more efficient or through shortening of chain lengths increasing its cytotoxicity.
Using specialised tools with ultra-high resolution mass spectrometry, it is possible to accurately identify polymers even after degradation has begun. The ultra-high resolution of FTICR coupled with tandem MS allows for enhanced analysis of these species.

Methods

Analytes were dissolved in toluene or chloroform, followed by ionisation using either APPI or n-ESI and analysis was performed using a Bruker 12 T solariX which had been externally calibrated using ESI-tuning mix to aid untargeted analysis of the polymer species. This reduced the permissible uncertainty in the assignment algorithm.
After acquisition data was analysed within the Polymer Suite of Touchstone MS, an in-house software designed specifically for polymer analysis. The modified Kendrick mass defect was used to group analyte peaks without the use of clustering algorithms, and mean values of mKMD for each series was generated with standard deviations allowing for assignment of analytes within a series with reasonable accuracy dependent on the quality of the initial calibration.

Novel Aspect

Projected modified Kendrick Mass Defect plots to assign polymers throughout degradation.

Preliminary Data or Plenary Speaker Abstract

Polystyrene was studied at various stages of degradation by exposure to ultraviolet light and heat. It was found that the initial main method of degradation was chain scission and using mKMD it was possible to confirm the appearance of more degradation products as the polymer become more degraded. This led to MS/MS works which gave further information on the structure of these degradation products. It has been previously shown that the mKMD can be used to accurately group fragmentation types in MS/MS and this technique was combined with a new projected mKMD plot to assign the fragmentation plot. An exact mass calculator designed for Polymer analysis was then used to determine the heteromonomer content of the fragment which aids in assigning fragmentation and determining structure. It was also shown that it was possible for the algorithm to propose polystyrene against a number of other common polymers without any metadata.
Poly Methyl-Methacrylate co-Butyl methacrylate has been studied for its degradation in different conditions and the analysis was performed using this new workflow. It was found that it was possible to observe the effect that the butyl group had on the degradation of these products by observing the change as a product of the content of the two monomers. It was possible to assign the monomer content in each series using the mKMD plot and track the degradation using the projected mKMD plot.

It is possible that some degradation may be beneficial to assignment of a polymer as having more peaks increase the number of samples and therefore increase the intensity of the peaks within the projected mKMD plot, therefore, where other techniques such as GPC or IR may struggle with a complex degradation cycle, this algorithm should benefit from it.

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Dr Elvis Okoffo[1], Dr Jake O'Brien[1], **Dr Cassandra Rauert**[1], Dr Francisca Ribeiro[1], Stacey O'Brien[1], Tania Toapanta[1], Emma Yenney[1], Gabriel De la Torre[1], Professor Kevin Thomas[1]
[1]Queensland Alliance For Environmental Health Sciences (QAEHS), The University of Queensland

Quantitative analysis of micro- and nano-plastics in environmental samples by pyrolysis gas chromatography mass spectrometry.

Cassandra Rauert 548 - Quantitative analysis of micro- and nanoplastics in environmental samples by pyrolysis gas chromatography mass spectrometry, Meeting Room 106, August 21, 2024, 16:22 - 16:41

Micro-plastics contamination within environmental samples is now widely described and through recent analytical developments nano-plastics contamination has begun to be reported. However, little is currently known about the size distribution of nano- and micro-plastics contamination of environmental samples. Pyrolysis-gas chromatography-mass spectrometry (Pyr-GC/MS) has recently emerged as a quantitative technique for the analysis of micro and nano-plastics contamination of environmental samples. This includes seafood and potable water destined for human consumption through to biosolids destined for agricultural applications and plastics in marine sediments, road dust, and wastewater. The technique offers several advantages, including being independent of particle sizes, the ability to analyse a wide range of plastic polymers, exhibiting high specificity and the potential for quantification based on mass concentration.

Methods

Our aim was to go beyond visual identification and spectroscopy techniques for microplastics analysis and develop and apply an analytical workflow for concentrating and analysing micro and nano-plastics in complex environmental and potable water samples. Quantitative analysis were performed by (1) using a pressurized liquid extraction to dissolve plastic types at high temperatures and pressures followed by pyrolysis coupled to gas chromatography mass spectrometry and (2) concentrating large volumes of water samples using Amicon Stirred Cell ultrafiltration (at 100 kDa, approximately 10 nm) with subsequent detection by pyrolysis gas chromatography–mass spectrometry. The combined methods allows for the identification and quantification of nine selected plastic types, including poly(ethylene terephthalate), polyethylene, polycarbonate, polypropylene, poly(methyl methacrylate), polystyrene, polyvinylchloride, nylon 6, and nylon 66.

Novel Aspect

The application of Pyr-GC/MS for quantitative analysis of plastic contaminants, including both micro and nano-sized particles, across different environmental matrices.

Preliminary Data or Plenary Speaker Abstract

In this presentation we will provide an overview on the use of pressurised liquid extraction and/or Amicon® Stirred Cell ultrafiltration followed by Pyr-GC/MS for the analysis of micro- and nano-plastics in complex environmental samples including biosolids (concentration range between 0.4 and 23.5 mg/g dry weight), seafood (0.04 and 2.4 mg/g of tissue), environmental and portable water (from 0.01 to 0.44 µg/L), marine sediments (from 3.3 to 2194.2 µg/g), wastewater (from 0.04 and 7.3 µg/L across samples), bioplastics in environmental samples and antifouling paint particles in marine environments. Interestingly, the method was applied to quantify plastics concentrations in archived biosolids samples from Australia and the United Kingdom from between 1950 and 2016, with increasing concentrations observed over time for each plastic. Prior to the 1990s, the leakage of plastics into biosolids was found to be limited except for polystyrene. Increased leakage was observed from the 1990s onwards; potentially driven by increased consumption of polyethylene, polyethylene terephthalate and polyvinyl chloride. In a similar work, we have investigated for the first time the historical deposition of plastic in Moreton Bay Australia by use of sediment cores. The results showed a decrease in total plastic abundance with core depth and sediment age. Evidence of plastic emerged in the sediment cores around 1975. Polyethylene was found in the highest

concentration overall, and most prevalent on the surface of the cores (8,520 µg/g dry weight sediment). From the current findings, plastic concentrations in the Central Bay are predicted to reach 2%-4% g/g sediment by 2050. Additionally, we will discuss how sequential size fractionation prior to analysis can be used as a quantitative method to determine size distribution of micro- and nano-plastics. Limitations of this methodology will be discussed, in conjunction with recommendations on how this workflow can advance future research on nano- and micro-plastics contamination.

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Miss Laura Puente-De La Cruz[1,2], Professor Kevin Thomas[1,2], Dr Cassandra Rauert[1,2]
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Micro and nanoplastic migration from plastic breast milk storage bags and storage bottles

Laura Puente-De La Cruz 379 - Micro and nanoplastic migration from plastic breast milk storage bags and storage bottles, Meeting Room 106, August 21, 2024, 16:41 - 17:00

Recently, human exposure to micro (< 5mm) and nanoplastics (< 1µm) has become one of the most important areas of research regarding plastics, however infant exposure is much less studied. Ingestion is thought to be one of the main sources of exposure for infants, some studies have reported micro and nanoplastics in plastic feeding bottles and storage containers for breast milk as well as in breast milk and infant formula powder. Taking this knowledge gap into account, the aim of the present study is to evaluate the micro and nanoplastic migration from breast milk storage bags and storage bottles in order to provide an accurate baseline of oral exposure for infants originating from these sources.

Methods

Five brands of commercial breast milk storage bags and two brands of storage bottles were selected. The experimental procedure consisted of filling the bags and bottles with 150 mL of milk simulant (50% v/v Ethanol/water) or 150 mL of filtered ultrapure water (all by triplicate) and storing them for 3 days in the fridge. Afterwards the contents from each bag were filtered through 1 µm and 0.3 µm glass fibre filters in a purpose-built plastics clean lab to minimise background contamination. Plastic analysis of the filters was performed with Pyrolysis gas chromatography-mass spectrometry (Py-GC/MS). Strict QA/QC procedures both during the sample processing and data analysis were implemented during this study.

Novel Aspect

The novelty of this study resides in providing accurate results regarding infant exposure to micro and nanoplastics following strict QA/QC.

Preliminary Data or Plenary Speaker Abstract

The results of the breast milk storage bags, made of polyethylene or polyethylene and polyethylene terephthalate, showed that there was an interference in the detected polyethylene signal. This has been previously reported, due to the chemical structure of polyethylene, long chained hydrocarbon compounds cause an increase in the pyrolysis product signals (i.e., C10, C12 and C14 alkenes and C21 diene) that are monitored to identify and quantify it. Thus, the quantities measured from the storage bags indicate false positives while the actual polyethylene concentration is below the method limit of detection (1.14µg). It is possible that this interference is originating from slip agents or other additives used during the manufacturing process of the plastic. Regarding polyethylene terephthalate none was detected in the storage bags. For the storage bottles, made of polypropylene, a polypropylene signal was detected in the sterilised bottles with concentrations ranging between <MDL to 1.96µg/bottle (particles > 300 nm), where the MDL was 0.37µg. Further experiments will be conducted in order to determine if repeated sterilisation of the bottles contribute to micro and nanoplastic release

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Dr. Jin-Ming Lin[1]
[1]Tsinghua University

Microfluidic Chip Combined with Mass Spectrometer for Single Cell Analysis

Keynote: Professor Jin-Ming Lin Tsinghua University 750 - Microfluidic Chip Combined with Mass Spectrometer for Single Cell Analysis, Meeting Room 109, August 21, 2024, 15:00 - 15:25

The micron-scale space of the microfluidic chips can be well matched to the cell size, which can easily simulate and precisely manipulate the cell growth microenvironment, and is an ideal platform for studying cell-cell interactions, signal transduction and communication, and cellular drug metabolism and delivery. Our research group has carried out research related to the development and application of microfluidic chip and mass spectrometry [1-3], successfully developed the world's first microfluidic chip mass spectrometry analysis device, and industrialized the technology transfer enterprises. This report mainly introduces the development of microfluidic chip combined with mass spectrometry for single cell analysis [4-6].

Methods

The single cell extraction - on-line pretreating - mass spectrometry, a combined microfluidic method with single cell extractor, extremely efficient mixer and mass spectrometry (MS) for online derivatization and analysis was applied. The cell extraction mixture was injected by an auto sampler into the LC-MS/MS with an injection time of 2 s. The injection volume was 10 μL, a little less than 16 μL, to avoid air being injected to HPLC. In the LC part, hexamethylenetetramine(N4(CH2)6), the pretreated product of ammonia, was isolated from the mixture and then went into the ESI-QqQ MS. Multistage Response Monitoring(MRM) mode was applied to analyze the quantity of hexamethylenetetramine. Data were acquired in positive mode.

Preliminary Data or Plenary Speaker Abstract

As a demonstration of concept, the ammonia content in a single cell was analyzed: An extraction probe was applied onto a single cell, where the extraction fluid dissolved the target cell. The cellular ammonia was transported to the redesigned micromixer and react with the pretreatment agent to achieve the derivatization for signal-enhancement. The micromixer chip had a high mixing efficiency with a very low volume (0.192 μL), showing a broad potential for online pretreatment of single-cell mass spectrometry. After validating the mixing efficiency and optimizing the HPLC-ESI-QqQ MS parameters, a series of single-cell extraction and MS analysis of ammonia were performed. Four types of cell samples, including HUVEC cells, HepG2 cells, A549 cells and MCF-7 cells was analyzed, as representatives of traditional non-tumor cells and tumor cells. Additionally, the results showed a significant difference in the ammonia content between these cells. Furthermore, ammonia content changes in A549 cells after hypoxia stimulation was also analyzed. With its resolution on a single-cell level, this method could have the potential of rapid detection of a single tumor cell in a tissue slice and other biologic samples, or delve into the drug mechanisms at metabolite level. This presents a great prospect in the clinical preliminary diagnosis of cancer, and shows a greater potential in further more elaborate single-cell material analysis.

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Prof. Dr. Hartmut Schlüter[1]
[1]University of Hamburg - Section Mass Spectrometric Proteomics

Towards original compositions of proteoforms in tissues via soft laser ablation

Hartmut Schlüter 232 - Towards original compositions of proteoforms in tissues via soft laser ablation, Meeting Room 109, August 21, 2024, 15:25 - 15:44

Analysis of proteoforms require careful sample treatment because these molecules are quickly converted into different proteoforms by enzymatic and chemical reactions in vitro. As soon as cells are homogenized, enzymes are released from their compartments, which can e.g. proteolytically degrade proteoforms (proteases) or remove phosphate groups (phosphatases) or other posttranslational modifications (PTMs). Consequently, identification and quantification of proteoforms by mass spectrometry, which currently is the only technique enabling an exact analysis of proteoforms, will yield false results. In this study we compared samples obtained from liver by classical mechanical homogenization with samples obtained by soft and cold vaporization of the tissue with a nano-second infrared laser (NIRL) and analyzed both samples for the first time with top-down proteomics.

Methods

Liver tissue was cut into two comparable pieces. One piece was irradiated with NIRL resulting in a conversion of the tissue into a tissue aerosol. The tissue aerosol obtained by NIRL was condensed. The second piece was homogenized using the bead-mill homogenization procedure at 4°C. Both samples were applied to a top-down mass spectrometric proteomics protocol developed by the group of Andreas Tholey (published in Angew Chem Intl Ed, 2023; 62: e202301969). This group also performed the top-down proteomics procedure. The LC-MS data were processed, and the resulting features used for identification and quantification of the proteoforms. In addition, aliquots of both samples were applied to SDS-PAGE, comparable bands were cut, proteins digested and analyzed with bottom-up proteomics.

Novel Aspect

For the first time tissue samples obtained by laser ablation were analyzed with a top-down approach using LC-MS.

Preliminary Data or Plenary Speaker Abstract

In the sample and homogenate of the liver tissue obtained by irradiation with NIRL a significant higher quantity of proteins as well as higher number of large proteoforms was yielded. In the tissue homogenate obtained by classical homogenization a significantly higher number of truncated proteoforms were identified. Furthermore, NIRL is giving the opportunity to get closer to the original composition of the proteoforms in the tissue. The higher yields in the amount and number of proteoforms in NIRL homogenates can be explained by the complete conversion of the tissue into a liquid. After centrifugation of the NIRL tissue homogenate no pellet was observed in contrast to the classical homogenization. For the minimized proteolytical degradation of proteoforms in the NIRL homogenate the very fast homogenization process is responsible. In conclusion NIRL-based sampling and homogenization of tissues is optimal for proteoform analysis.

Andrei Fedorov[1], Gianna Slusher[1], Mason Chilmonczyk[2], Austin Culberson[2]
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MicroTAS (Total Analysis System) for ESI-MS Dynamic Monitoring of Extracellular Proteome and Intracellular Metabolome in Cell and Gene Therapy Biomanufacturing

Andrei Fedorov 67 - MicroTAS (Total Analysis System) for ESI-MS Dynamic Monitoring of Extracellular Proteome and Intracellular Metabolome in Cell and Gene Therapy Biomanufacturing, Meeting Room 109, August 21, 2024, 15:44 - 16:03

New analytical technologies must be developed to identify and control the critical process parameters that govern cell growth and differentiation for safe, effective, and potent cell and gene therapies biomanufacturing. Existing measures of bulk properties should be complemented by in-process measurement of endogenous factors that more directly correlate to cell state and function throughout cell culture processes. This work demonstrates a new microfluidic lab-on-a-chip platform for at-line/inline dynamic ESI-MS monitoring of extracellular proteome and intracellular metabolome with sampling directly from the cell culture. By decreasing the sample size (down to a single/few cells) and increasing the speed of label-free metabolic and proteomic measurements, spectral markers and metabolic pathways can be correlated with dynamic cell processes at early time points assessment.

Methods

The platform integrates local extracellular secretome and intracellular metabolome measurements, which can be applied in sequence or in parallel depending on the application needs. The device is equipped with a minimally invasive sample probe which uptakes small volumes of cell media (~1 uL) directly from the bioreactor microenvironment. For extracellular secretome, sample treatment relies on a micro/nano-fabricated tangential flow mass exchanger with an ultra-thin nanoporous membrane for size-selective mass exchange, enabling ESI-MS with a temporal response of ~1 minute from uptake to analysis. For intracellular metabolome, custom-integrated microfluidics allows for pre-concentrating the cells, isolating the cells from the media, and extracting the intracellular metabolites via electrical lysis. This rapid, hands-free sample preparation enables ESI-MS dynamic mesurements on minutes time scale.

Novel Aspect

Inline/at-line in-situ cell culture analysis, multi-mode dynamic ESI-MS of secretome and metabolome, microfabricated platform for total analysis, real-time biomanufacturing monitoring.

Preliminary Data or Plenary Speaker Abstract

For secretome measurements, mouse preosteoblast cells (MC3T3) were intermittently sampled (every 3 days) throughout the entire cell cycle, totaling over 2 weeks, without affecting culture sterility or cell viability. The platform was used for inline sample cleanup and ESI-MS analysis of samples from both the bulk media (i.e., far from cell membrane) and the cell microenvironment (i.e., ~50 μm from cell membrane). Principal component analysis (PCA) reveals that only sampling from the cell microenvironment, enriched with cell secretomes, made it possible to detect differences in cells at key physiological states including proliferation, confluence, and differentiation. Conventional ESI-MS analysis of the bulk samples lacks the sensitivity due to substantial dilution of secreted biomolecules. Offline HPLC analysis was carried out on aliquoted media from each DSP-analysis time point to tentatively identify biomarkers correlating with cells in known states. These biomarkers were compared to spectral loading plots resulting from the PCA that demonstrates the potential of the platform to serve as both a process-monitoring tool and a discovery tool.

Early detection and metabolic pathway identification of T-Cell activation was demonstrated using an intacellular metabolome analysis on the platform. The number of cells required for analysis is reduced by orders of magnitude compared to conventional workflows. Multivariate spectral fingerprint analysis of the cell lysates over time was able to distinguish between activated and unactivated T-cells without a priori classification within 6 hrs of activation. Up/downregulation of individual metabolic pathways indicative of successful activation were also robustly detected at early time points.

By sampling secretome locally from the immediate vicinity of cells and rapidly analyzing the metabolome from only a few cells, our approach provides insight into unknown mechanisms of cellular behavior and identify candidate critical quality attributes (CQAs) and critical process parameters (CPPs) for real-time monitoring in complex biomanufacturing workflows of advanced cell-based immunotherapies.

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Magnus Rydberg, **Associate Professor Nick Manicke**[1]
[1]Indiana University

On-Paper Electrophoretic Stacking and Separations Coupled to Paper Spray Mass Spectrometry

Nick Manicke 365 - On-Paper Electrophoretic Stacking and Separations Coupled to Paper Spray Mass Spectrometry, Meeting Room 109, August 21, 2024, 16:03 - 16:22

Paper spray and related methods enable rapid analysis by mass spectrometry (MS). Two causes of higher detection limits are 1) analyte band broadening during extraction/wicking through the paper and 2) ion suppression. We are working to improve detection limits by adding rapid (<5 minute) electrophoretic manipulations on the paper. We have demonstrated on-paper analyte stacking for small molecule drugs, pollutants, and proteins using field amplified sample stacking (FASS) and faradaic ion concentration polarization (f-ICP) coupled online with paper spray MS detection. Stacking improves detection limits by over 100X with modest increases in time (a few minutes) and device complexity. Crude separations on the paper are achievable as well.

Methods

Platinum wires at opposing ends of a paper fluid channel imposed a DC field across the stacking device for simultaneous electrokinetic manipulation and MS ionization. In the case of f-ICP, the driving mechanism is rapid neutralization of background electrolyte (BGE) anions by protons generated by electrolysis at the anode. BGE titration produces an ion depletion zone (IDZ) with a steep electric field gradient at its boundary. Electroosmotic counterflow (EOF) moved analyte cations towards the IDZ, while the elevated electric field inside the IDZ pushed them away. Analytes concentrate at the IDZ boundary and migratedto the sharp paper tip as the IDZ expanded. Ionization occurred at the tip as the device was floated at 4kV relative to the MS inlet.

Novel Aspect

First demonstration of electrophoretic stacking coupled with paper spray MS for biofluid analysis

Preliminary Data or Plenary Speaker Abstract

The presentation will present device designs to carry out electrophoretic manipulations with online paper spray MS detection, discuss modification of paper surface charge using derivatization agents to control electrophoresis, and show results for stacking and separations on paper. Devices were prepared by 3D printing using inexpensive hobby-grade printers using polypropylene filaments to achieve acceptable solvent compatibility. Papers were cellulose or glass fiber papers. The surface charge of the papers needed to be controlled in order to control both the magnitude and direction of the EOF. This was achieved using dipodal silane coupling reagents with amine functional groups to adjust the surface zeta potential of the papers. Applications explored thus far include detection of Per- and Polyfluorinated Substances (PFAS) at sub-part-per-trillion (ppt) concentrations and detection of illicit drugs in urine at low ppt levels. For PFAS, 500 microliters of tap water was stacked using FASS and yielded detection limits below 1 ppt, an approximately 600 fold improvement over paper spray without stacking. For illicit drug detection in urine, f-ICP stacking proved to be more robust when stacking salty samples. Drugs were stacked directly from urine specimens in minutes, yielding detection limits for fentanyl and cocaine of around 10 ppt, about 50 fold lower than achievable by unstacked paper spray. The approach for stacking samples by f-ICP could not use as large a sample volume as our FASS-based device, hence the more modest improvement. We have also achieved crude electrophoretic separations on-paper within a few minutes, including baseline separation of methamphetamine and fentanyl. Such separations can improve specificity and decrease ion suppression by separating matrix components and co-eluting analytes.

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Professor John Langley[1]
[1]University of Southampton

Sustainable fuels and the need for different hyphenated solutions

John Langley 26 - Sustainable fuels and the need for different hyphenated solutions, Meeting Room 109, August 21, 2024, 16:22 - 16:41

Renewable, sustainable fuels continue to present analytical challenges, related to stability, complexity and their combination with already complex fuel matrices, e.g., diesel. The latest generation of hydrotreated materials, SAF (Sustainable Aviation Fuel) and HVOs (Hydrotreated Vegetable Oils) add to this complexity. Each requiring different hyphenated approaches to determine quality and follow their inclusion into the different fuel chains.

First generation biodiesels, e.g., from vegetable oils, fatbergs, UCO (Used Cooking Oil) demanded good analytics and hyphenation. Recently HVOs have been introduced, presenting different, new challenges that require different hyphenated solutions, i.e., GC-MS, 2D GC-MS SFC-MS and SFC-FID to determine fuel quality of the newly processed material, a challenge made much more difficult once they are mixed with a fuel matrix.

Methods

GC-MS, 2D GC-MS, SFC-MS and SFC-FID methods have been developed to fully characterise these new, sustainable materials. Orthogonal chromatographic approaches are required due to the complexity of the materials, particularly related to their different feed stocks. Similarly, the specificity of the different ionisation techniques has been used to probe and identify species present that are not revealed by one technique alone

Novel Aspect

New hyphenated methods for new sustainable fuels.

Preliminary Data or Plenary Speaker Abstract

Impurity profiling of these sustainable fuels is now possible, together with determination of the quality and make up of these new fuels. Specific features and materials can be identified across the different techniques, sometimes confirming the presence of components, other times identifying unique components related to the hyphenated technique employed. A major break-through is the ability to quantify the degree of hydrocarbon branching produced through the processing as well as the efficiency of the hydrotreatment method. Further, the long-term stability of these materials can also be tracked, this linked to the original source of the feedstock, e.g., UCO, fatbergs, vegetable oils.

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Dr Russ Grant, Dr J. Will Thompson, Dr Erin Redman, Dr Scott Mellors, Dr Christopher Shuford
[1]Labcorp

High Throughput Quantitative Amino Acid Analysis for The Masses

Russell Grant 562 - High Throughput Quantitative Amino Acid Analysis for The Masses, Meeting Room 109, August 21, 2024, 16:41 - 17:00

Analysis of amino acids plays a key role in diagnosing a multitude of inborn errors of metabolism, particularly in pediatric populations. Mass spectrometry has been globally deployed for newborn screening from DBS and subsequent 2nd-tier screening of blood, plasma, CSF and urine for diagnostic differentiation prior to confirmatory genetic analysis. Since 2012, Labcorp has deployed a fully clinically validated quantitative LC-MS/MS assay for direct diagnostic support in patients samples, introduced into a single mass spectrometer. This presentation will describe our efforts to reduce the technical complexity of the workflow through evaluation and evolution of the ZipChip microchip electrophoresis system coupled to an Exploris 240 MS to enable accurate and precise quantification of amino acids for patient management.

Methods

Sample preparation: 50uL samples/cals/QC's + 250 uL internal standard solution (50:50 MeOH:MeCN). Mixed for 10 mins and centrifuged prior to LC-MS/MS analysis. Samples were dried, frozen at -80C prior to resuspension for CE analysis (500 uL 70/30 v/v MeOH/water with 0.1M ammonium acetate).
LC-MS/MS Analysis: 5uL of supernatant is injected across 3 different LC separations (staggered parallel multiplexing, ARIA Transcend TX4, Thermo) into a single ABSciex API5500 system (+ve ion ESI, scheduled MRM.
ZipChip HR analysis: 5.5 nL injection (20 uL loaded to chip). Peptides BGE (MeCN/water/formic acid) coupled to an Exploris 240 MS (500 V/cm for 10 minutes for the CE separation). Quantification occurring from MS1 scans (m/z 70-800) at 60k resolution, 300% AGC, max accumulation of 50 ms.

Novel Aspect

Accurate and precise quantitative analysis of amino acids for diagnostic application via ZipChip CE-MS balancing throughput, resolution and sensitivity.

Preliminary Data or Plenary Speaker Abstract

The currently validated LC-MS/MS assay incorporates 3 discreet LC separations (RPLC+ion-pairing, HILIC and RPLC) to resolve 106 of 107 analytes and internal standards that contribute to each other following sample preparation. All quantitative measures demonstrate bias/imprecision <15%, accuracy within 2 SD for proficiency analysis and has been used for clinical diagnostic support for > 10 years.
Of the 36 analytes quantitatively measured routinely by multiplexing LC-MS/MS across a nearly 5-log dynamic range, 31 were successfully quantified by CE-MS across the same dynamic range – demonstrated by acceptable calibration curve fit (R2>0.99; ±15%bias) and quality control results (two levels within ±2SD of target; with exception of ornithine +2.3 SD at one level). However, in plasma specimens, argininosuccinic acid, cystathionine, and homocitrulline had a majority of patient results below the LLOQ by both CE-MS and LC-MS/MS, which precluded quantitative comparison. Of the 5 unsuccessful CE-MS measures, Taurine was not detectable given it is negatively charged in solution, homocystine and cystine degraded prior to CE-MS studies, while the isomers GABA and B-Aib were unresolved by CE.
For the 28 analytes quantifiable by CE-MS, comparison of LC-MS/MS and CE-MS results in 77 plasma specimens demonstrated good correlation (R>0.887) and quantitative agreement (Deming Slope: 0.851-1.096; Mean Bias <±11.4%) for all but 7 analytes. Quantitative discordance for four of these analytes (glutamic acid, hydroxylysine, glycine, methionine) utilized analogue internal standards, suggesting an unresolved matrix effect in the CE-MS analysis that could be alleviated by use of

alternate internal standards. However, three analytes (proline, histidine, threonine) showing quantitative discordance used matched isotope-labeled internal standards, suggesting unresolved selectivity and/or non-linearity issues of the CE-MS measurement requiring further investigation. Ongoing studies will be presented which include optimal CE separation time and MS resolution settings (isomeric/isobaric interferences and quantitative accuracy (points-per-peak)), together with enhancement in sensitivity using on-chip SPE enrichment.

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Prof. Dr Kevin Pagel[1]
[1]Freie Universität Berlin

Mass Spectrometry-Based Techniques to Elucidate the Sugar Code

Keynote: Professor Dr Kevin Pagel Freie Universität Berlin 751 - Mass Spectrometry-Based Techniques to Elucidate the Sugar Code, Meeting Room 110, August 21, 2024, 15:00 - 15:25

Cells encode information in the sequence of biopolymers, such as nucleic acids, proteins, and glycans. Although glycans are essential to all living organisms, surprisingly little is known about their biological role. The reason glycobiology lags behind its counterparts dealing with nucleic acids and proteins lies in the complexity of carbohydrate structures, which renders their analysis extremely challenging. Building blocks that may differ only in the configuration of a single stereocenter, combined with the vast possibilities to connect monosaccharide units, lead to an immense variety of isomers, which poses a formidable challenge to conventional mass spectrometry.

Methods

In recent years, a combination of innovative ion activation methods, commercialization of ion mobility–mass spectrometry, progress in gas-phase ion spectroscopy, and advances in computational chemistry have led to a revolution in mass spectrometry-based glycan analysis.1

Novel Aspect

Combination of ion mobility spectrometry and gas-phase spectroscopy for omics applications

Preliminary Data or Plenary Speaker Abstract

Here we showcase for a couple of examples how complex oligosaccharide structures can be unambiguously identified using ion mobility mass spectrometry2 and cryogenic ion spectroscopy.3 Particular focus will be put on particularly challenging glycoconjugates such as mucin-type O-glycans and glycosaminoglycans. Further, we discuss the potential of mass spectrometry-based techniques combined with theory to elucidate the structure of reactive intermediates from glycosylation reactions such as glycosyl cations.4-6

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Ms Felicia Hansen[1], Mr Lachlan Jekimovs[2], Dr Berwyck Poad[1], Professor Stephen Blanksby[1]
[1]Queensland University Of Technology, [2]University of Queensland

Direct observation of phospholipase A activity via isomer-resolved mass spectrometry.

Felicia Hansen 497 - Direct observation of phospholipase A activity via isomer-resolved mass spectrometry, Meeting Room 110, August 21, 2024, 15:25 - 15:44

Phospholipase-A1 (PLA1) and -A2 (PLA2) enzymes selectively catalyse ester hydrolysis of the sn-1 or sn-2 position of membrane glycerophospholipids, respectively, releasing a free fatty acid signaller and lysophospholipid. As PLA2 has been implicated as the rate limiting step in the inflammatory pathways known as the arachidonic cascade, understanding its activity, substrate specificity and selectivity is paramount. Monitoring PLA activity requires rapid separation and the unambiguous identification of the lysophospholipids, including resolution of regioisomers differing at only the sn-1 or -2 position on the glycerol backbone. The high-resolution cyclic ion-mobility enabled quadrupole time-of-flight mass spectrometer (Waters Select Series Cyclic IM-MS) is demonstrated herein as a suitable platform for real-time monitoring of enzymatic reactions.

Methods

Methanolic solutions of lysophosphatidylcholine (LPC) standards were spiked with sodium acetate and infused via electrospray ionisation in the positive ion mode, with mass selection of the [M+Na]+ precursor within the quadrupole mass filter. Following multi-pass separation around the cyclic IM device of the Waters Select Series Cyclic IM-MS, ions were subjected to collision-induced dissociation (CID), post ion mobility separation. Enzymatic reactions of PLA1 or PLA2 with a small range of diacylphosphatidylcholines (PC) were undertaken, requiring 2 hours incubation at 37 °C to produce the lysophospholipid products. The respective samples were infused both individually and as mixtures. NIST plasma underwent a 100-fold dilution and spiked with sodium, whereas the sodium citrated plasma was diluted 10-fold and not spiked.

Novel Aspect

Cyclic ion-mobility method for the rapid separation and unambiguous identification of sn-positional lysophosphatidylcholine isomers.

Preliminary Data or Plenary Speaker Abstract

This novel method successfully and efficiently separated LPC lipid isomers over 20-40 passes (300-600 ms) of the cIMS device, displaying two distinct arrival features. Integration of individual features presented diagnostic fragments m/z 104 and 147, representing acyl attachment to the sn-1 or sn-2 position, respectively.
Isopure standard separation indicated fragment exclusivity, allowing for unambiguous isomer identification. LPC 16:0 showed complete separation over 20 passes (314 ms) with LPC 0:0/16:0 arriving first, reversed to the elution order observed in HILIC separation. Alternatively, LPC 18:1 required 40 passes for complete separation (622 ms). Successful separation was observed for both the n-9 and n-12 (cis) variants. Interestingly, the arrival order swapped such that LPC 18:1/0:0 exited first. This reiterates the necessity of unambiguous identification.
As NIST human blood plasma is extensively researched and characterized, it was trialed to both validate the separation method and attempt its viability on biological matrices. Isomeric LPC 18:1 relative abundancies were consistent with literature, thus verifying the method. Whilst results for LPC 16:0 did not match analytical abundancies previously reported, agreement with the usual structural motif of PCs (shorter unsaturate on the sn-1 position) was considered validating.
Observation of precursor signal decreasing and diagnostic fragment signal increasing indicated PLA activity. Symmetrical diacyl PC 18:1/18:1 (standard) was digested with one of PLA1 or PLA2 to observe enzyme selectivity (sn preference). Both phospholipases were found to produce their isomer abundance ratio of 5:2 in favour of the expected product. Future work should be conducted on

asymmetric diacyl PCs to determine if PLA catalysis is more specific to the FA or selective of the sn attachment position.

Msc. Bram van de Put[1], Prof. Henk Schols[1], Dr. Wouter de Bruijn[1]
[1]Wageningen University And Research

Towards De Novo Sequencing of Oligosaccharides Using Cyclic Ion Mobility Spectrometry

Bram van de Put 325 - Towards De Novo Sequencing of Oligosaccharides Using Cyclic Ion Mobility Spectrometry, Meeting Room 110, August 21, 2024, 15:44 - 16:03

The bioactivity of oligosaccharides is dictated by their molecular structure. However, the effects of the individual structural features of oligosaccharides on their functionality remain poorly understood. Therefore, the complete structural characterization of oligosaccharides may kickstart the development of targeted nutraceutical oligosaccharide formulations.
The structural characterization of oligosaccharide formulations, such as galacto-oligosaccharides (GOS) present in infant formula, is challenging due to their inherent complexity. GOS, although only composed of galactose and glucose, usually follows a complex pattern of assembly resulting in hundreds of isomers with different sequences and linkage types. Current techniques fail to fully characterize such complex mixtures of isomeric oligosaccharides in a reasonable timespan. We introduce several approaches for the fast sequencing of GOS using cyclic ion mobility spectrometry.

Methods

The separation of galactose and glucose monomers and di- and trimers thereof with different linkage types and anomeric configurations is demonstrated using a combination of high resolution multi-pass cyclic ion mobility spectrometry (cIMS), and fragmentation-based deconvolution. Reducing-end anomers of dimers and trimers are identified using cIMS2, where the reducing-end anomers are partly separated using cIMS, isolated, fragmented, and their monosaccharide fragments again separated using cIMS. The monosaccharide fragments generated from dimers and trimers can then be compared with those of monosaccharide standards to identify the sugar type and anomeric configuration of the reducing end. These methods were used to establish rules for the recognition of structural features of oligosaccharides by their ion mobility and fragmentation behavior.

Novel Aspect

Novel strategies using cIMS-MS and MS-fragmentation for the full structural characterization of oligosaccharides.

Preliminary Data or Plenary Speaker Abstract

To understand the effects of the different structural features on the drift time behavior of oligosaccharides, we studied twelve GOS dimers representing different linkage types and monosaccharide sequences using cyclic ion mobility spectrometry (cIMS).
The performance of several metal adduct ions (Li+, Na+, K+, Rb+, Cs+) for the cIMS separation of oligosaccharides with various structural features were compared. The separation of reducing-end anomeric configuration was found to improve with increasing adduct-ion size. However, with increasing adduct-ion size the separation of the isomers with different sequences and/or linkage types was found to decline. None of the tested adduct ions allowed complete separation of all disaccharide standards. Multivariate curve resolution (MCR-ALS) was successfully applied to deconvolute mixtures of the disaccharide standards by spectral differentiation after post-cIMS fragmentation. It was found that fragment yields sharply diminished with increasing adduct ion size, limiting the use of fragmentation strategies. Despite its lesser separation power, the smallest adduction ion, lithium, was selected for its far superior fragmentation yields.
A database of drift times for the individual reducing-end anomers of GOS dimers was created. The anomeric configurations were identified using cIMS2 by comparison of the drift times of monosaccharide fragments of the disaccharides with monosaccharide standards. It was found that drift times were predictably related to composition in the order Gal-Gal < Glc-Gal < Glc-Glc, whereas linkage types were straightforwardly differentiated by fragmentation patterns. The obtained

disaccharide drift time database was successfully used to fully characterize trisaccharides by their disaccharide fragments.

The cIMS-based sequencing strategies, here demonstrated for di- and trisaccharides of galactose and glucose, are powerful tools for the structural characterization of oligosaccharides. The incorporation of these approaches in LC-MS methods is a promising high-throughput characterization approach that can be further exploited for the analysis of complex food oligosaccharide formulations.

Dr David Marshall[1], Michael Pfrunder[1], Jason Hong[1], Dr Berwyck Poad[1], John McMurtrie[1], Professor Stephen Blanksby[1], Kathleen Mullen[1]
[1]Queensland University of Technology

Separation and characterisation of isomeric coordination complexes with high-resolution ion mobility mass spectrometry

David Marshall 281 - Separation and characterisation of isomeric coordination complexes with high-resolution ion mobility mass spectrometry, Meeting Room 110, August 21, 2024, 16:03 - 16:22

Self-assembled metallosupramolecular cages are discrete 3-dimensional assemblies with well-defined internal cavities that have myriad applications including catalysis, molecular sensing, and drug delivery. Unambiguous characterisation of these coordination cages is challenging due to their dynamic behaviour in solution, such as reversible guest binding, isomer exchange and structure interconversion. Moreover, NMR spectroscopy is limited to diamagnetic species and X-ray crystallography relies on the challenging preparation of single crystals. While the population of diastereomers can be selectively tuned through ligand design, guest binding or crystallisation, the efficient separation of all isomers is necessary to investigate their individual properties. Here we demonstrate the utility of high-resolution ion mobility mass spectrometry to resolve and characterise individual coordination cages within a dynamic mixture.

Methods

Tetrahedral M4L6.X8 (M = metal, L = ligand, X = anion) coordination cages were prepared by self-assembly of the appropriate metal salt (MX2) and a neutral bis bidentate ligand, formed in situ from 4,4’-diaminobiphenyl and 2-formylpyridine. Acetonitrile solutions of the resulting mixture of stereoisomeric cages were infused into a Waters Select Series Cyclic ion mobility mass spectrometer via electrospray ionisation. From the mixture of charge states generated by ESI, [M4L6.X]7+ ions were mass-selected in the quadrupole and separated over a variable number of passes around the cyclic ion mobility device prior to mass analysis by time-of-flight.

Novel Aspect

First application of high-resolution ion mobility to separation of coordination cage diastereomers

Preliminary Data or Plenary Speaker Abstract

Tetrahedral coordination cages were formed as a mixture of stereoisomers (T, S4, C3 point groups), arising from different ligand arrangements around each metal vertex. Stereoisomers of [Fe4L6.X]7+ ions were partially resolved after 30 passes of the cyclic ion mobility cell. Complete resolution required the selective ejection of the larger feature (longer drift time) from the cyclic device in order to avoid ‘wrap-around’ and re-injection of the smaller feature for a further 120 passes (resolving power > 700). The observed ratio of diastereomers varied depending on both the metal cation and the templating counter ion (X = BF4-, Br-, I-, ClO4-, CF3SO3-). By comparison to the isomer population in solution and calculated collision cross sections, the identity of each resolved peak could be assigned. Post-mobility collision-induced dissociation in the transfer region enabled the relative stability of each isomer within the mixture to be probed, with T-symmetric isomers more resistant to dissociation than the C3 and S4 analogues.
Finally, this method was successfully applied to high-spin paramagnetic complexes (M = Co2+, Ni2+), which are particularly problematic for NMR characterisation. Moreover, individual stereoisomers of low-symmetry, bimetallic cages containing mixtures of Fe2+, Co2+ and/or Ni2+ could be resolved and characterised by the same approach.
It is envisioned that ion mobility-based methods will encourage chemists to embrace coordination cages containing a broader range of metal ions and ligands, significantly expanding the scope of accessible supramolecular architectures.

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Dr Madelien Wooding[1], Dr Yvette Naudé[1]
[1]University Of Pretoria

The Story of a Sorptive Sampler: From Fairy Circles to the Detection of Tuberculosis-Associated Compounds using GC×GC-TOFMS and UPLC-IMS-HRMS

Madelien Wooding 74 - The Story of a Sorptive Sampler: From Fairy Circles to the Detection of Tuberculosis-Associated Compounds using GC×GC-TOFMS and UPLCIMS-HRMS, Meeting Room 110, August 21, 2024, 16:22 - 16:41

The variation and complexity of matrices, and as well as time and monetary constraints often call for novel sampling approaches. The development of a sorptive polydimethylsiloxane (PDMS) sampler has enabled the sampling of various matrices such as soil, water, and the human skin surface. As its efficacy has been validated across these matrices, attention now turns towards diagnostic applications. Tuberculosis (TB), a predominant global infectious disease, has prompted exploration into less invasive diagnostic methods, moving away from traditional procedures like blood-draws or sputum collection. However, these substitute samples remain infectious, requiring clinical expertise for sampling. Since there is a correlation between chemicals detected in skin and exhaled breath, sampling of human skin emanations presents an attractive substitute.

Methods

Addressing the limitations of existing skin chemicals sampling methods, we employed our in-house developed and -made wearable sorptive sampler. To enhance the polarity of PDMS, a nonpolar material, the sampler was modified with ethanol or isopropanol which also increased the sorption volume. The solvent modification step simultaneously infuses the band with an internal standard. The small band was placed on human skin and shielded with Mylar® to minimise background exposure during sampling. After sampling, the band was desorbed directly in the inlet of a GC or in a commercial thermal desorption system, for VOCs analyses by GCxGC-TOFMS. For non-volatile compounds the silicone rubber band was extracted with microlitres amount of solvent and the extract analysed by UPLC-IMS-HRMS.

Novel Aspect

We present a novel versatile non-invasive wearable sorptive sampler for the safe and simple detection of infectious disease.

Preliminary Data or Plenary Speaker Abstract

To investigate the feasibility of using human skin emanations for infectious disease diagnosis, the PDMS samplers were worn by TB-positive and TB-negative individuals. Results confirmed a correlation between compounds found in this study, and those reported for TB from other biofluids. In a comparison to known TB-associated compounds from other biofluids analysis established the presence of 27 of these compounds emanating from human skin using GCxGC-TOFMS. Additionally, 16 previously unreported compounds were found as potential biomarkers. Two compounds that have previously been identified as potential TB biomarkers in serum were tentatively identified from human skin excretions using UPLC-IMS-HRMS. The wearable sorptive sampler and human skin emanations show excellent potential as a non-invasive, point-of-care, health monitoring tool.

Patricia Skowronek[1], Georg Wallmann[1], Maria Wahle[1], Sander Willems[2], Matthias Mann[1]
[1]Department Proteomics and Signal Transduction, Max Planck Institute of Biochemistry, [2]Research and Development, Bruker Belgium nv.Optimal trapped ion mobility workflows coupled with dia-PASEF and synchro-PASEF for high throughput and sensitivity

Patricia Skowronek 312 - Optimal trapped ion mobility workflows coupled with dia-PASEF and synchro-PASEF for high throughput and sensitivity, Meeting Room 110, August 21, 2024, 16:41 - 17:00

Recent developments in mass spectrometry have rekindled the interest in Time-of-Flight (TOF) mass analyzers and established them as most sensitive mass analyzers to date. Coupled with Trapped Ion Mobility Spectrometry (TIMS) and a quadrupole, they enable Parallel Accumulation - Serial Fragmentation (PASEF), a principle that allows to decouple ion accumulation and mass detection. Building on this principle, we recently introduced synchro-PASEF, a data-independent acquisition (DIA) scan mode and successor to dia-PASEF. This new mode combines efficiency and sensitivity with specificity, offering improved identification and quantification. Here, we explore the potential of synchro-PASEF in the context of high throughput and high sensitivity applications, such as the analysis of single cells with short gradients.

Methods

We extended our Python package, py_diAID, to generate optimally designed dia-PASEF and synchro-PASEF methods. With these methods, we acquired raw data using a TIMS quadrupole time-of-flight mass spectrometer (timsTof Pro and timsTof Ultra, Bruker Daltonics) coupled with the EvoSep One liquid chromatography system. Evosep gradients separated 5 ng to 200 ng tryptic K562 digest (Promega) using a PepSep C18 reversed-phase column, 10 µm electrospray emitter (Bruker Daltonics) or Aurora IonOpticks columns at throughput of 200 samples per day (SPD), 60 SPD or 40 SPD (nano-flow rates). Lastly, we analyzed the raw data using our novel search software AlphaDIA that efficiently uses the ion mobility dimension.

Novel Aspect

An optimal synchro-PASEF workflow unites efficiency and sensitivity with selectivity as required for high throughput and high sensitivity workflows.

Preliminary Data or Plenary Speaker Abstract

The synchro-PASEF scan mode utilizes a rapidly sliding quadrupole window for constant and fast-moving mass isolation, synchronized with the TIMS release. This two-dimensional isolation has two primary advantages. First, it enables coverage of the precursor space with only four scans leading to a cycle time of only half a second – a two-fold improvement over dia-PASEF. Secondly, by scanning only a portion of the fragment ion mobility peak, it partitions the signals across several fragment spectra, thereby encoding the precursor mass into each spectrum. To employ this additional criterion for identification efficiently, we developed a feature in AlphaDIA. Moreover, we extended py_diAID to generate optimal dia-PASEF and synchro-PASEF methods. For dia-PASEF, we previously showed that variable windows comprehensively cover the precursor space, leading to higher precursor identifications. Applying this concept to synchro-PASEF, we confirmed that synchro-PASEF achieves a similar coverage as dia-PASEF, but with higher quantitative reproducibility. Specifically, using the standard K562 cell line digest, both acquisition modes identify around 6,000 proteins with 21-minute gradients, but synchro-PASEF quantified 30% more proteins with a CV below 10%. This improvement persists across measurements with short gradients or for low sample inputs coupled with nano-flow gradients.
In conclusion, we demonstrate that dia-PASEF and notably synchro-PASEF acquisition workflows enable deep and accurate quantification of proteomes, even at high throughput and high sensitivity applications, a prerequisite for extensive systems biology and clinical studies including those focusing on the single cell level.

Plenary – Advancing Personalized Proteomics by DIA-MS: From Large-Scale Profile to Single-Cell Resolution Professor Yu-ju Chen, Academia Sinica Taiwan

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Advancing Personalized Proteomics by DIA-MS: From Large-Scale Profile to Single-Cell Resolution

Personalized proteomics starts to inspire new strategies for precision medicine by facilitating discovery of disease biomarkers, molecular signature of disease subtypes and druggable pathways of treatment of individual patients. Due to the high complexity of human proteome and disease, however, clinical proteomic still faces challenges to achieve high sensitivity for limited specimens in different stage of a patient's journey and sufficient profiling depth to "see" the disease biology. To advance the personalized proteomics, we explored the data-independent acquisition mass spectrometry (DIA MS) for rapid and deep profiling from large-scale profiling down to single cell landscape. For low-input nanoscale sample (i.e., nanogram cells, ~5−50 cells), a sample size-comparable spectral library strategy greatly enhances the proteome coverage over a 5-order dynamic range. Spectral similarity analysis revealed that the fragmentation ion pattern in the DIA-MS/MS spectra of the dataset and spectral library play crucial roles for mapping low abundant proteins. Combining with microfluidic chips for highly streamlined all-in-one proteomic sample preparation, the chip-DIA strategy achieves sensitivity of thousands of proteins across tens of single mammalian cells, allowing good mapping coverage and reproducibility (<20% CV) on the non-small cell lung cancer pathway. To implement DIA into the more challenging PTMomics, using phosphoproteomics that has lower LC-MS/MS detectability, extremely low abundance and stoichiometry as a model, we developed a simple and rapid one-pot phosphoproteomics workflow (SOP-Phos) for microscale phosphoproteomic samples (1000 – 10 cells). With an ultra-streamlined workflow combining integrated proteomics chip (iProChip), we ultimately demonstrated nanoscale-to-single cell phosphoproteomic profiling to reveal heterogeneity of patient with acquired resistance to third-generation EGFR therapy. Notably, the sensitivity and coverage enable illumination of heterogeneous cytoskeleton remodeling and cytokeratin signatures among patient-derived cells, stratifying mixed-lineage adenocarcinoma-squamous cell carcinoma subtypes and identifying alternative next-line therapy for late-stage patients.

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Pushing the Limits of Quantitative Proteomics to Advance Alzheimer's Research

Keynote: Professor Rena A. S. Robinson Vanderbilt University 685 - Pushing the Limits of Quantitative Proshibteomics to Advance Alzheimer's Research

Large-scale proteomics studies of clinical samples and animal models are readily increasing across disease applications, including in Alzheimer's disease (AD). These studies require diversity in cohorts, accessible sample types, multiple disease timepoints, robust analytical workflows, and high-throughput capabilities in order to have the greatest impact towards disease understanding and discovery efforts. Our laboratory has been working in the last few years to address these requirements primarily to advance health disparities research in Alzheimer's disease. Bottom-up proteomic workflows leveraging in-house and commercial isotopic and isobaric labeling strategies, sample preparation automation, and high-resolution mass spectrometry have been developed. Importantly, to handle sample sizes (>1000), standard operating procedures and quality control measures have been implemented in our proteomics workflows. This presentation will highlight our efforts in establishing quality control metrics and implementation of automation to advance our understanding of disparities and peripheral contributions in AD.

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Marco Jochem[1,2], Anthony Cerra[1], Dr Simon Cobbold[1,2], David Komander[1,2]
[1]WEHI, [2]Uni Melbourne

Analysing Non-Proteinaceous Ubiquitination by Mass Spectrometry

Marco Jochem 444 - Analysing Non-Proteinaceous Ubiquitination by Mass Spectrometry, Plenary 3, August 22, 2024, 10:25 - 10:44

Ubiquitin is an essential eukaryotic protein that can be covalently attached to other molecules in complex combinatory ways, with key functions in signalling the degradation of proteins via proteasomal or lysosomal pathways. Ubiquitin is involved in the regulation of almost every cellular process and therefore plays a role in many human diseases, including various cancers, infections or neurodegenerative and autoimmune diseases. Recent studies have described the ubiquitination of lipopolysaccharides (LPS), sugars, lipids, and nucleic acids, expanding the role of ubiquitin modifications beyond proteins. However, many aspects of non-proteinaceous ubiquitination remain elusive, and investigations have so far been hampered by a lack of suitable methodologies and reagents. Here we present a novel MS-based workflow for the analysis of non-proteinaceous ubiquitination.

Methods

We explore non-proteinaceous ubiquitination through the development and use of a new, unbiased, mass spectrometry-based workflow called NoPro-clipping. A key component of our method is the use of viral and bacterial proteases, referred to as ‘clippases’, which cleave ubiquitin at its C-terminus and leave characteristic GlyGly marks on ubiquitinated substrates. Subsequently, we use molecular weight filtration to enrich GlyGly-modified small molecules, including non-proteins, and analyse them by ammonium acetate/acetonitrile-based liquid chromatography (LC) mass spectrometry (MS). We further increase the sensitivity of NoPro-clipping via sortagging, a strategy where we use the bacterial transpeptidase enzyme Sortase A to attach a short peptide onto GlyGly-modified small molecules. This transformative step renders non-proteinaceous metabolite samples peptidic to characterize them through conventional proteomics workflows.

Novel Aspect

We established a novel MS-based method for the detection of ubiquitinated non-proteins and demonstrate its utility by analysing ubiquitinated sugars.

Preliminary Data or Plenary Speaker Abstract

Ubiquitin is an essential eukaryotic protein that can be covalently attached to other proteins, regulating their function and fate through diverse mechanisms. Recent studies have described the ubiquitination of lipopolysaccharides (LPS), sugars, lipids and nucleic acids, expanding the role of ubiquitin modifications beyond proteins. However, many aspects of non-proteinaceous ubiquitination remain elusive, hindered by the absence of suitable methodologies and reagents.

In this study we explore non-proteinaceous ubiquitination through the development and use of a new, unbiased, MS-based workflow called NoPro-clipping. A key component of NoPro-clipping is the use of viral and bacterial proteases, referred to as ‘clippases’, which cleave ubiquitin at its C-terminus and leave characteristic GlyGly marks on ubiquitinated substrates. Subsequently, we enrich GlyGly-modified small molecules, including non-proteins, and analyse them by LC-MS.

We established and optimized NoPro-clipping by using a variety of different sugars that were in-vitro ubiquitinated by the E3 ligase HOIL-1 (Glucose, Maltose, GlcNAc, LacNAc etc.). Our method robustly detects those ubiquitinated species in both the purified form and when spiked into lysates from human cell lines.

To further increase the sensitivity of NoPro-clipping, we utilize sortagging, a strategy employing the bacterial transpeptidase enzyme SortaseA to attach a short peptide onto GlyGly-modified small molecules. This step transforms non-proteinaceous metabolite samples into peptidic samples that can be analysed through proteomics workflows.

Aiming to unravel the potential implications of glycogen ubiquitination in glycogen storage diseases, we established a human liver cell system where the E3 ligase HOIL-1 can be actively recruited to glycogen via induced proximity. Using fluorescent microscopy, we demonstrate that this recruitment leads to the formation of granular glycogen bodies that stain positive for ubiquitin. Following α-Amylase digestion, NoPro-clipping and sortagging, we successfully detect multiple ubiquitinated maltose species (GG-maltose, GG-maltotriose etc.), showing that NoPro-clipping is sensitive enough to detect ubiquitinated glycogen in cells.

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Dr. Guangcan Shao[1], Zhen-Lin Chen[2], Shan Lu[1], Si-Min He[2], Li-Lin Du[1], Dr. Meng-qiu Dong[1]
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Mapping the Modification Sites of Ubiquitin-like Proteins (UBLs)

Guangcan Shao 503 - Mapping the Modification Sites of Ubiquitin-like Proteins (UBLs), Plenary 3, August 22, 2024, 10:44 - 11:03

Ubiquitin like proteins (UBLs) are a family of evolutionarily conserved proteins that share a similar structure and a modification mechanism with ubiquitin. More than 10 UBLs have been identified, of which the best-known example is small ubiquitin-related modifier (SUMO). UBLs play important roles in many biological processes including signal transduction, DNA repair, inflammation, and autophagy. However, identification of UBL modifications remains a challenge because most of them cannot be identified directly using the ubiquitination mapping method. This study aims to develop a high-throughput method for identifying modification sites of UBLs on substrate proteins.

Methods

We developed a software tool called pLink-SUMO to identify SUMOylated peptides, pLink-SUMO was trained using several thousands of Higher-energy Collisional Dissociation (HCD) spectra generated from synthetic peptides in vitro and tested using independent datasets. Fission yeast cells were lysed in the presence of guanidine hydrochloride to minimize loss of UBL modification. Samples were digested using different proteases or their combinations. For complex samples, the peptide mixtures were separated into up to six strong cation exchange (SCX) fractions. We developed an antibody against the C-terminal peptide of Pmt3, the fission yeast SUMO, to enrich SUMOylated peptides. All samples were analyzed on a Q-Exactive HF mass spectrometer coupled with an Easy-nLC HPLC. UBL modified peptides were identified from HCD spectra using pLink-SUMO.

Novel Aspect

A MS-based method for mapping UBL modification sites without introducing mutations to UBL proteins.

Preliminary Data or Plenary Speaker Abstract

Compared to other software tools such as pLink1, pLink2 and MaxQuant, pLink-SUMO demonstrated superior precision, sensitivity, and speed in both simple and complex samples. For example, pLink-SUMO identified from fission yeast data ten times as many unique SUMOylation sites as MaxQuant, and was ten times faster.
We identified a total of 858 SUMOylation sites in 500 substrate proteins from fission yeast. A motif ΨKXE (Ψ represents hydrophobic amino acids, X represents any amino acids) was found enriched among these SUMOylation sites, which is in agreement with a published study (Køhler J B. et al., communications, 2015).
The antibody against the C-terminal peptide of SUMO displayed good specificity, increasing the ratio of identified SUMO or SUMOylated peptides over the total number of identified peptides from 0.9% to over 80% in complex samples. Moreover, it enriched not only SUMOylated peptides but also non-protein substrates of SUMO from complex samples.
The expression levels of three other UBLs in fission yeast (Urm1, Ned8, and Atg12) are much lower than that of Pmt3. Little is known about their attachment sites on substrate proteins. So far, we identified 12 Urm1 modification sites in 7 substrate proteins and 6 Ned8 modification sites in 3 substrate proteins.

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Miss Siqi Li[1], Professor Tara Pukala[1], Wayne Leifert[2,3]
[1]University Of Adelaide, School of Physics, Chemistry, and Earth Sciences, [2]University of Adelaide, School of Biological Sciences, [3]CSIRO Health and Biosecurity

Development of highly sensitive mass spectrometry methods for phosphorylated protein analysis

Siqi Li 189 - Development of highly sensitive mass spectrometry methods for phosphorylated protein analysis, Plenary 3, August 22, 2024, 11:03 - 11:22

Alzheimer's Disease (AD) is by far the most common cause of dementia, which makes developing an effective diagnosis method for AD essential. The overarching aim for this project is to develop an efficient mass spectrometry-based analysis workflow for salivary phosphorylated tau protein as a biomarker for the early diagnostic stage of AD. Such investment is important to overcome current limits in detecting low abundance salivary AD biomarkers and contribute to the development of AD screening in clinical trials with higher resolution and sensitivity.

Methods

The approach to build up the mass spectrometry (MS)-based clinical diagnosis method focusses on improving sensitivity of phosphorylated Tau detection using mass-tag enrichment. A biotinylated phosphate affinity dinuclear zinc(II) metal complex was designed and synthesised as the mass-tag. Trypsin digested phosphorylated Tau peptides were identified through bottom-up phosphoproteomics analysis from AD patients' saliva samples. Enrichment of phosphorylated Tau was carried out by cross-linking biotinylated mass-tag with streptavidin on a solid-phase support. Standard LC-MS and timsTOF were employed to develop a robust workflow. A shift in drift time in ion-mobility (IM) would be observed for tagged phosphopeptides.

Novel Aspect

Utilizing mass-tag enrichment and drift time differences by ion-mobility MS to improve sensitivity for phosphorylated peptide analysis.

Preliminary Data or Plenary Speaker Abstract

Our approach exploits development of a novel ion mobility MS-based shift reagent. Here we incorporate features for both enrichment and enhanced analytical identification. The mass-tag we chose 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. The tagged phosphorylated peptides are readily cleaved from biotin through reduction of disulfide bond by adding TCEP or DTT as reducing agent. Improvement of MS sensitivity is observed when comparing phosphorylated peptides enrichment methods using TiO2 and our mass-tag enrichment methods. A distinguishing shift of drift time vs m/z from un-tagged phosphopeptide to tagged phosphopeptide was observed, assisting in rapid identification and characterisation.

Dr Lin Zhu[1], Dr Yuanyuan Song[1], Mr Zuowei Xu[1], Dr Hongzhi Zhao[2], Mr Zhangsheng Shi[1], Mr Zhitong Zhu[1], Mr. Xin Diao[1], Mr Xiao-Liang Liao[3], Dr. Zhi-Feng Chen[3], Prof Zongwei Cai[1]
[1]State Key Laboratory of Environmental and Biological Analysis, Hong Kong Baptist University, [2]Ministry of Education Key Laboratory of Pollution Processes and Environmental Criteria, College of Environmental Science and Engineering, Nankai University,Ministry of Education Key Laboratory of Pollution Processes and Environmental Criteria, College of Environmental Science and Engineering, Nankai University, [3]Guangdong Key Laboratory of Environmental Catalysis and Health Risk Control, Guangdong-Hong Kong-Macao Joint Laboratory for Contaminants Exposure and Health, School of Environmental Science and Engineering, Guangdong University of Technology

Real-world PM2.5 Exposure Causes Liver Metabolic Reprogramming via Oxidation of Specific Cysteine Residues on MDH2 and CPT2 in Mice

Lin Zhu 130 - Real-world PM2.5 Exposure Causes Liver Metabolic Reprogramming via Oxidation of Specific Cysteine Residues on MDH2 and CPT2 in Mice, Plenary 3, August 22, 2024, 11:22 - 11:41

Air pollution, particularly PM2.5, remains the top environmental death cause worldwide and causes millions of deaths annually. It is estimated that 2.5 billion urban population live in areas exceeding WHO's guideline on PM2.5, which continues to increase because of urbanization. The small particle size makes it possible for PM2.5 to enter blood circulation and affect distant organs. Epidemiological and experimental evidence demonstrated the association between PM2.5 exposure and fatty liver diseases featured by reactive oxidative species (ROS) mediated metabolic reprogramming, while exact targets remained vague. Utilizing redox proteomics, this study profiles PM2.5-oxidized cysteine residues through integrated in vivo, in vitro, and in silico techniques to identify novel key molecules that contributed to PM2.5-prompted hepatic metabolic reprogramming.

Methods

Adult male mice (6 per group) were exposed to filtered or ambient air with high PM2.5 in Taiyuan, Shanxi, China, during the coal-burning season from November 2018 to May 2019. Redox proteomics analysis was conducted for isolated liver mitochondria to obtain relative oxidation abundance and occupancy of each identified cysteine residue. IodoTMT labeled peptides were analyzed on Orbitrap Fusion mass spectrometer and searched against the Uniprot database using MaxQuant. Molecular dynamics (MD) simulation was conducted using AutoDock Vina. HepG2 and AML12 cell lines were used for targeted metabolomics and seahorse assays. MDH2/CPT2 knock-out cell lines were generated by CRISPR/Cas 9 system. Targeted metabolomics analysis was conducted on a QE mass spectrometer based on published methods.

Novel Aspect

PM2.5-induced cysteine oxidation on mitochondrial proteins shifts liver metabolism to lipogenesis, a novel mechanistic insight.

Preliminary Data or Plenary Speaker Abstract

A total of 2805 unique Cysteine-containing peptides from 704 proteins were identified and quantified from isolated hepatic mitochondria of exposed mice. Multiple cysteine sites on mitochondria enzymes regulating energy metabolism were found to be significantly oxidized, including Carnitine Palmitoyltransferase II (CPT2), Malate Dehydrogenase 2 (MDH2), Glycerol-3-Phosphate Dehydrogenase 2 (GPD2), Glutamic-Oxaloacetic Transaminase 2 (GOT2), and Acyl-CoA Dehydrogenase Long Chain (ACADL). In silico simulation suggested that PM-mediated oxidation identified at specific cysteine residues might inhibit energy metabolism regulators CPT2 and MDH2. Decreased MDH activity was confirmed by targeted metabolomics and activity assay in mouse liver tissues, which repressed glycolysis via malate shuttle malfunction. Lipid markers accumulation confirmed declined CPT2 activity and metabolic reprogramming towards lipogenesis was identified by elevated triglyceride abundance. The PM2.5-induced metabolic reprogramming was subsequently confirmed in PM2.5-exposed HepG2 and AML12 cell lines using the seahorse fuel flex assay. Genetic

manipulation by CRISPR/Cas9 on specific cysteine residues further validated that inhibitory cysteine oxidation events on CPT2 and MDH2 contributed to hepatic metabolic reprogramming, which repressed glycolysis and beta-oxidation to pivot lipogenesis. These PM-induced events were reported as novel effectors for hepatic triglyceride accumulation and potentially to the development of fatty liver disease. Our findings uncover a novel regulatory mechanism by which PM2.5 induces hepatic metabolic shifts, potentially contributing to fatty liver disease, and offer insights into the broader impact of environmental pollutants on mitochondrial function and metabolic disorders.

Dr. Maria Tanzer[1], Jenny Riedel[1], Kael Schoffer[1], Charlotte Williams[1], Dr. Lianju Shen[1]
[1]The Walter and Eliza Hall Institute of Medical Research

Proteome profiling of macrophage reprogramming upon dead cell clearance

Maria Tanzer 44 - Proteome profiling of macrophage reprogramming upon dead cell clearance,
Plenary 3, August 22, 2024, 11:41 - 12:00

Cell death drives inflammation. Macrophages mitigate inflammation by recognising and phagocytosing dead cells. Thereby they are reprogrammed towards a wound healing, anti-inflammatory state, which contributes to inflammation resolution. The mechanisms driving macrophage reprogramming during dead cell clearance, as well as the proteins these macrophages release, remain poorly understood.

Methods

Here we applied a systems-level secretome, phosphoproteome and ubiquitinome analysis of macrophages engulfing apoptotic T-cells to comprehensively detect and dissect regulators of macrophage reprogramming.

Novel Aspect

This knowledge will facilitate the development of therapeutic approaches that target macrophage programming to control inflammation and improve wound healing.

Preliminary Data or Plenary Speaker Abstract

By employing high sensitivity phosphoproteomics and ubiquitinome analyses, we identified dynamic modifications on both novel and established receptors like Mertk and Csfr1, along with other signaling components, implicating the signaling events that drive reprogramming. Subsequently, we investigated the downstream effects of dead cell engulfment and uncovered the release of hundreds of proteins involved in wound healing, fibrosis, and ECM remodeling. Increased detection of inflammatory receptors such as TNFR1 and 2 in the supernatants was inhibited by protease treatment, pointing towards extensive receptor shedding upon dead cell clearance. These findings highlight the profound impact of reprogramming on the macrophage proteome and secretome, identifying regulated and regulatory proteins.

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Light-mediated discovery of surfaceome nanoscale organization

Keynote: Professor Bernd Wollscheid Swiss Federal Institute of Technology 756 - Light-mediated discovery of surfaceome nanoscale organization

The molecular nanoscale organization of the surfaceome is a fundamental regulator of cellular signaling in health and disease. Technologies for mapping the spatial relationships of cell surface receptors and their extracellular signaling synapses would unlock theranostic opportunities to target protein communities and the possibility of engineering extracellular signaling. At the IMSC 2024, I will describe the development and application of an optoproteomic technology termed LUX-MS that enables the targeted elucidation of acute protein interactions on and in between living cells using light-controlled singlet oxygen generators (SOGs). By using SOG-coupled antibodies, small molecule drugs, biologics, and intact viral particles, I will demonstrate the ability of LUX-MS to decode ligand-receptor interactions across organisms and to discover surfaceome receptor nanoscale organization with direct implications for drug action. LUX-MS-based decoding of surfaceome signaling architectures thereby provides a molecular framework for the rational development of theranostic strategies. In the context of the TumorProfiler project under development, I will further highlight new Swiss/ETH projects integrating proteotyping with other technologies for the digitization of patient samples, such as pharmacoscopy, which are geared towards supporting clinical decision-making.

Mrs. Lan Anna Ye, Mr. Michael Woolman, Dr. Francis Talbot, Ms. Alexa Fiorante, Dr. Yuki Sata, Dr. Hiroyuki Ogawa, Dr. Fumi Yokote, Dr. Nicholas Bernards, Dr. Michael Cabanero, Dr. Nhu-An Pham, Mrs. Nikolina Radulovich, Prof.Dr. Benjamin Lok, Prof. Dr. Kazuhiro Yasufuku, Prof. Dr. Ming-Sound Tsao, Prof. Dr. Howard Ginsberg, **Prof. Dr. Arash Zarrine-afsar**[1]
[1]University Of Toronto

10-second Classification of Lung Cancer Subtypes by Picosecond Infrared Laser Mass Spectrometry: Evaluation of Diagnostic Power Across Various Tissue Models

Arash Zarrine-Afsar 14 - 10-second Classification of Lung Cancer Subtypes by Picosecond Infrared Laser Mass Spectrometry: Evaluation of Diagnostic Power Across Various Tissue Models, Meeting Room 105, August 22, 2024, 10:25 - 10:44

The diversity in prognosis, treatment and survival across lung cancer subtypes necessitates precise diagnosis. Current approaches involve histologic evaluations, immunohistochemistry but their turnaround times span tens of minutes to hours. Here, the differentiation between small cell and non-small cell cancers via endobronchial ultrasound biopsies using intraoperative cytopathology is important. Non-small cell cancers are candidates for surgery with complete resection being desirable. Small cell lung cancers on the other hand are treated with systemic therapy and in certain situations with radiotherapy. Picosecond InfraRed Laser (PIRL) ablation soft ionization mass spectrometry (MS) is a potential tool for rapid lung cancer subtype identification. With just ~10-second sampling and analysis duration, we hypothesize that PIRL-MS may offer rapid precise classification of lung cancer subtypes.

Methods

To evaluate the utility of PIRL-MS in rapid classification of different types of lung cancer we used a variety of tissue models including immortalized cell lines and xenografts thereof, patient derived xenografts, organoids and primary patient tissue, subjecting them to 10-second PIRL-MS analysis. Each specimen was sampled multiple times to capture within specimen heterogeneity with technical independent replicates (n>=3). Tissue molecular content in the form of a gas plume was captured and profiled by a Waters Xevo-G2-XS quadrupole Time of Flight (qToF) mass spectrometer. PIRL-MS profiles (m/z 100-1,000 Da) were processed with multivariate Principal Component Analysis Linear Discriminate Analysis (PCA-LDA). The accuracy was determined using 20% leave out test (PCA-LDA model) with Mahalanobis distance calculations (standard deviation of 4).

Novel Aspect

Rapid 10-second PIRL-MS can potentially guide surgical decisions in real time and serve as an intraoperative tool enhancing diagnosis speed.

Preliminary Data or Plenary Speaker Abstract

Immortalized cell lines (in n=3 technical replicates with multiple sampling): A549 for adenocarcinoma (40 spectra), H460 for large cell carcinoma (40 spectra), H526 for small cell carcinoma (43 spectra), H2170 for squamous cell carcinoma (31 spectra) resulted in 100% accurate classification from 20% cross-validation of the 4-component PCA-LDA model (10-second spectra). Ten independent subcutaneous murine xenografts of same cell lines resulting in 190 adenocarcinoma, 194 large cell carcinoma, 193 small cell carcinoma and 190 squamous cell carcinoma spectra also performed with 100% accuracy using 20% cross-validation. Likewise, patient derived xenografts (n=47 for adenocarcinoma with 703 spectra, n=3 large cell carcinoma with 63 spectra, n=14 small cell carcinoma with 191 spectra, n=17 squamous cell carcinoma with 297 spectra) exhibited a 98% accuracy. Primary patient tissue (n=50 for adenocarcinoma with 715 spectra, n=50 large cell carcinoma with 742 spectra, n=5 small cell carcinoma with 68 spectra, n=50 squamous cell carcinoma with 807 spectra) showed a 94% accuracy. Endobronchial ultrasound (EBUS) guided biopsies (n=42,

219 spectra) were classifiable with a balanced primary patient tissue (5 specimen / class) PCA-LDA model (n=5 small cell carcinoma with 68 spectra and n=15 non-small cell with 174 spectra) with a diagnostic accuracy of 89%.
PIRL-MS models revealed distinct m/z markers specific to each lung cancer type whose identification with chromatography and high-resolution mass spectrometry is currently on going. However, attempts were made to cross-classify primary patient tissue using various tumour tissue models utilizing unique m/z profiles to each tissue model. Immortalized cell lines and xenografts thereof failed to classify primary human tissue (accuracies of 0% for both). Patient derived xenografts, however, resembled primary human tissue most closely (with cross-classification accuracy of 85%) with organoids coming a close second judging by PCA-LDA model overlays. Immortalized cell lines and xenografts thereof have limited utility in classifying patient tissue.

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Assoc Prof Michelle Hill[1,2,3]
[1]Proseek Bio, [2]QIMR Berghofer Medical Research Institute, [3]The University of Queensland

Ovarian cancer serum glycoform biomarker panel discovery to lectin magnetic array-mass spec (LeMBA-MS) clinical assay

Michelle Hill 611 - Ovarian cancer serum glycoform biomarker panel discovery to lectin magnetic array-mass spec (LeMBA-MS) clinical assay, Meeting Room 105, August 22, 2024, 10:44 - 11:03

Ovarian cancer diagnosis currently relies on two non-specific tests for risk stratification prior to surgery, during which tissue is taken for pathology diagnosis. This procedure has resulted in diagnostic delays that causes psychological stress, and late stage diagnosis which is associated with higher mortality. Two unmet diagnostic needs were identified for ovarian cancer: differential diagnosis for pelvic mass/symptoms, and population screening in asymptomatic women. While previous efforts in ovarian cancer blood biomarkers have targeted proteins, circulating/cell-free nucleic acids and extracellular vesicles, our novel approach focuses on glycoform biomarkers. The scientific rationale includes the well-studied role of altered glycosylation in cancer pathogenesis, and the improved diagnostic performance of existing tumour markers when specific glycoforms are measured.

Methods

We conducted a two-phase age-matched case-control study for ovarian cancer serum glycoform biomarker discovery using our previously established lectin magnetic bead array-mass spectrometry (LeMBA-MS) platform [1], which was validated through oesophageal adenocarcinoma biomarker discovery and licensing [2]. This study aimed to discover biomarkers for differential diagnosis of high grade serous ovarian cancer from benign pelvic mass and healthy women. A secondary objective was to evaluate the potential of the identified biomarkers for screening. The discovery phase applied 7 lectin LeMBA-DDA proteomics on 60 serum samples from 2 UK cohorts for differential diagnosis and screening (~12 months before diagnosis). Candidate biomarkers were qualified in 95 Australia serum samples using LeMBA-MRM and 3 lectins. Multimarker signatures were developed using generalized regression.

Novel Aspect

LeMBA-MS is the first unified platform for glycoform biomarker discovery to clinical MS assay.

Preliminary Data or Plenary Speaker Abstract

For ovarian cancer diagnosis from benign and healthy groups, a 13-marker signature had 94.8% area under the receiver operating curve, 93% specificity and 81.5% sensitivity using leave-one-out cross validation. Excitingly, discovery results in the preclinical set showed C9, ITIH3 and A2MG glycoforms were already altered in sera collected 11.1 ± 5.1 months prior to ovarian cancer diagnosis, and significantly different from the benign and healthy samples [3].

To progress towards the goal of a clinical LeMBA-MS test, the next step is to further validate the biomarker candidates in additional samples including other ovarian cancer subtypes. In addition, we will determine biomarker specificity by measuring serum samples of other women's cancers. A diagnostic panel will be formulated in a shorter LeMBA-MS assay and technically validated for clinical diagnostics study.

For the screening test, additional longitudinal time-series samples from the UK Collaborative Trial of Ovarian Cancer Screening [4] will be analysed to determine the earliest onset glycoform biomarkers, before formulating a final panel for clinical study.

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Machine Learning-Driven Identification of sex and KRAS specific features for Ferroptosis-Targeted Drug Repurposing in Colorectal Cancer

Hong Yan 80 - Machine Learning-Driven Identification of sex and KRAS specific features for Ferroptosis-Targeted Drug Repurposing in Colorectal Cancer, Meeting Room 105, August 22, 2024, 11:03 - 11:22

The landscape of sex differences in Colorectal Cancer (CRC) has not been well characterized. In particular with respect to the mechanisms of action for oncogenes such as KRAS. However, our recent study showed that tumors from male patients with KRAS mutations have decreased iron-dependent cell death called ferroptosis. Building on these findings, we further examined ferroptosis metabolism in CRC, considering both sex of the patient and KRAS mutations, using public databases and our in-house CRC tumor cohort.

Methods

A semi-targeted metabolomics analysis was previously performed on 161 tumor tissues which identified 106 metabolites. We integrated multi-omics (metabolomic, transcriptomic) data to comprehensively characterize the ferroptosis signaling network and assess biological heterogeneity, and to identify the ferroptosis landscape according to sex and KRAS status in CRC. Bioinformatic machine learning methods (Variable Importance (VIMP) with subsampling inference) and Gaussian mixture models were used to identify genes and metabolites predictive of KRAS status. Random survival forest combined with a backward elimination (RSF-BE) algorithm using1000 bootstraps was utilized to obtain predictive genes and metabolites related to ferroptosis by sex and KRAS mutational status. Furthermore, the associations between ferroptosis gene expression levels, metabolite abundances, and drug responses were investigated.

Novel Aspect

This study takes into account several factors making it the most exhaustive study of the ferroptosis landscape in CRC

Preliminary Data or Plenary Speaker Abstract

Through subsampling inference and variable importance analysis (VIMP), 20 metabolites from the MSKCC cohort were found to be significantly different between KRAS mutant and wild type tumors in male patients, including lactate, lipoxin B4, prostaglandin F2α, stearic acid, histidine, xanthine, methionine, vitamin E and glutamine, while in female patients, only stearic acid was altered and identified to be related to ferroptosis. Additionally, we also found that differential gene expression related to KRAS mutations are significant only in male patients. These differentially expressed genes are known to suppress (e.g., SLC7A11) or drive (e.g., SLC1A5) ferroptosis. A new statistical method, discriminant analysis on high-dimensional Gaussian mixtures, is employed to validate the mentioned findings. Furthermore, we explored the prognostic value of ferroptosis regulating genes and discovered sex- and KRAS-specific differences at both the transcriptional and metabolic levels by random survival forest with backward elimination algorithm (RSF-BE). Of note, genes and metabolites involved in arginine synthesis and glutathione metabolism were uniquely associated with prognosis in tumors from males with KRAS mutations.
Further, we investigated the correlation between gene expression, metabolite levels, and drug sensitivity across all CRC primary tumor cell lines using data from the Genomics of Drug Sensitivity in Cancer (GDSC) resource. We observed that ferroptosis suppressor genes such as DHODH, GCH1, and AIFM2 in KRAS mutant CRC cell lines were resistant to classical chemotherapies for CRC such as cisplatin and paclitaxel, indicating that these drugs may not be as effective for these patients. The comprehensive map generated here provides valuable biological insights for future investigations, and the findings are supported by rigorous analysis of large-scale publicly available data and our in-

house cohort. In conclusion, this comprehensive approach opens doors for leveraging precision molecular feature analysis in KRAS mutant CRC.

Paula Nissen[1], Dr. Nadezhda V. Popova[2], Antonia Gocke[1,3], Dr. med. Dr. hum. biol. Daniel J. Smit[2,4], Geoffrey Yuet Mun Wong[5,9], Dr Matthew Mckay[9], Thomas J. Hugh[5], Kerstin David[6], Hartmut Juhl[6], Dr. Hannah Voß[1], Dr. Jens U. Marquardt[7], Dr. Björn Nashan[8], Prof. Dr. Hartmut Schlüter[1], Professor Mark Molloy[9], Dr. Manfred Jücker[2]

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Quantitative mass spectrometric proteome analysis of colorectal carcinoma liver metastasis reveals distinct phenotypes associated with specific signalling pathways and survival

Paula Nissen 120 - Quantitative mass spectrometric proteome analysis of colorectal carcinoma liver metastasis reveals distinct phenotypes associated with specific signalling pathways and survival, Meeting Room 105, August 22, 2024, 11:22 - 11:41

Colorectal carcinoma (CRC) is one of the most common carcinomas with 1.9 million new annual diagnoses and has the second highest mortality rate of all carcinomas. Liver metastases (LM) frequently occur and are associated with impaired overall survival. Particularly recurrence, even years after initial diagnosis, contributes to the high mortality. Clinical as well as molecular markers are lacking to predict recurrence in CRC-LM patients. Proteomic analyses of CRC LM can lead to the identification of novel biomarkers and druggable targets in order to understand the mechanism of recurrence. Thus, molecular characterization of CRC-LM is highly relevant to improve therapy and diagnosis for CRC LM patients.

Methods

In an international multi-center study we analyzed 144 fresh frozen samples of synchronous and metachronous CRC LM from three different medical centres in Germany and Australia by mass spectrometry based differential quantitative proteomics using LC-MS/MS with Orbitrap mass spectrometers in both countries (Germany, Orbitrap FusionTM and Australia, Orbitrap HF-X) in the data dependent and data-independent-acquisition modes. The proteomics data were harmonized through batch-effect correction algorithms to build a large international multi-centre cohort. Consensus Clustering with k-means identified three molecular subpopulations of CRC LM, that were correlated with distinct clinical features and differential proteomic profiles.

Novel Aspect

An international multi-cohort study of CRC-LM identifies proteomic signatures to stratify distinct subgroups associated with progression-free survival.

Preliminary Data or Plenary Speaker Abstract

In total we quantified 9,506 proteins of which 3,044 were identified in 70% of all samples. The CRC-LM could be separated into three distinct proteomic phenotypes (referred to as Gr1, Gr2 and Gr3), which correlated with the progression-free survival of those patients. Through Ingenuity Pathway Analysis (IPA), specific signalling cascades with key regulator proteins higher abundant in the individual CRC-LM subgroup were identified. Phenotype Gr1 showed a higher abundance of alternative splicing key regulator proteins as well as extracellular matrix proteins, whereas CRC-LM assigned to Gr2 were characterized by higher abundant proteins involved in the classical complement pathway part of the complement system. Phenotype Gr3 showed higher abundance of proteins

involved in various metabolism pathways. Based on these groups, we established a distinct set of biomarkers for each phenotype diagnosis. The identified biomarkers were orthogonal validated by immunohistochemical staining.
Our results demonstrated three main subgroups of CRC-LM with different functions according to IPA analysis that may be used as a novel proteomic signature for risk stratification, prognosis, and potentially novel therapeutic targets development for CRC-LM patients.

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Dr Nicole Brace[1], Associate Professor Antonia Pritchard[1]
[1]The University of the Highlands and Islands

A Study of Altered B Cell Responses to PAMP-Activation in Schizophrenia

Nicole Brace 680 - A Study of Altered B Cell Responses to PAMP-Activation in Schizophrenia, Meeting Room 105, August 22, 2024, 11:41 - 12:00

Schizophrenia, a severe mental health condition which affects about 1% of the population. Dysregulation of the immune system is observed in schizophrenia. B cells, produce antibodies and facilitate antibody presentation to T cells by presenting peptides on their major histocompatibility complexes (MHC). While antibodies typically neutralize foreign peptides, autoantibodies recognise self-proteins and can induce autoimmune disorders.
This study plans to measure MHC II peptides from B cell lines, following stimulation, derived from schizophrenia patients and healthy controls. Changes to B cell function may contribute to the causes of schizophrenia and be a biomarker of a subset of schizophrenia. Ultimately, this research aims to gain an insight into the MHC II immunopeptidome in schizophrenia and potentially uncover immune response abnormalities.

Methods

Four B cell lines (two from schizophrenia patients, two from healthy controls) will be cultured and stimulated with the TLR7 agonist imiquimod or a vector control for 24 hours. MHC-II peptides will be enriched using immunopeptidomics technology, initially capturing MHC-II bound peptide complexes with the monoclonal antibodies L243 and Tü39. The immunopeptidome comprises peptides presented on MHC (also known as HLA). Peptides will be further enriched via size exclusion chromatography, followed by identification and analysis using liquid chromatography coupled to high resolution mass spectrometry on an Orbitrap. Bioinformatics, including HLA-type binding prediction, will be employed to investigate properties of the identified peptides.

Novel Aspect

The analysis of MHC II peptides in these B cell lines and application of immunopeptidomics to schizophrenia is novel.

Preliminary Data or Plenary Speaker Abstract

Preliminary immunopeptidomics analysis has been carried out prior to PAMP (TLR7)-stimulation of the B cells. We are now conducting immunopeptide analysis following protocol optimisation from B cells with and without TLR7 stimulation.
In the preliminary dataset, 5572 unique peptides were identified from 4424 proteins. 1889 peptides from 1606 proteins were in at least 2 replicates and made up the final dataset. Analysis of the source proteins identified the membrane to be the most prominent source protein location. This was supported by gene ontology pathway analysis which identified significantly enriched pathways including plasma membrane cell-cell adhesion. Neuron differentiation cell morphogenesis was among other significantly enriched pathways which is supported by previous research in the causes of schizophrenia. Binding prediction software successfully mapped 1772 peptides to an allele. Of these, 934 had a strong affinity (<1500 nm). The allele HLA-DRB1*01:01 mapped to the greatest number of peptides (n = 582). 531 of these HLA-DRB1*01:01 mapped peptides were grouped into a single cluster (KLD: 8.331). This preliminary data has been used to guide method development and the bioinformatic workflow for the analysis of subsequent PAMP treated and untreated samples.

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Dr Jean Armengaud[1]
[1]CEA

Monitoring the environment by mass spectrometry of sentinel animals and microbiomes

Keynote: Dr Jean Armengaud CEA 753 - Monitoring the environment by mass spectrometry of sentinel animals and microbiomes, Meeting Room 106, August 22, 2024, 10:00 - 10:25

The Earth's global ecosystem is confronted with numerous interconnected challenges, largely due to human-induced pollution, significant loss of natural habitats, accelerated pathogen spread, and global climate warming. Ecotoxicology, which merges toxicology and ecology, seeks to elucidate the impact of toxic substances on organisms across various levels, including populations, communities, ecosystems, and the biosphere. To evaluate these threats, sentinel animals, plants, and microbiomes provide early warnings of potential dangers. Their analysis by advanced mass spectrometry of their protein content is challenging due to the high diversity of organisms within each species and the paucity of knowledge about protein functions for most of the relevant sentinels.

Methods

Since proteins are the workhorses of biological systems, their global study by proteomics provides a wealth of information. The identification and quantification of proteins from any sentinel organism, or even complex samples such as microbiota, is achieved by efficient extraction of proteins from samples, shotgun proteomics, and relies on high-throughput tandem mass spectrometry. Current metaproteomics performance represents a quantum leap in the functional analysis of environmental samples, with, for example, coverage of over 122,000 unique peptides and 38,000 protein groups in a 30-minute independent data acquisition run for a microbiome for which the 48 most abundant species can be described in depth.

Novel Aspect

Significant advances have been achieved in environmental monitoring through mass spectrometry, enhancing risk assessment with more precise measurements.

Preliminary Data or Plenary Speaker Abstract

The potential of proteomics in ecotoxicology will be exemplified by our research on the amphipod Gammarus fossarum, an aquatic sentinel species frequently found in rivers and with an important function of detritivore. We developed a comprehensive database of coding gene sequences and mass spectrometry (MS)-validated proteins. Specific protein biomarkers for toxicant exposure were identified. An MS-based, precise multi-biomarker quantification strategy was proposed to verify these biomarkers. Up to 40 protein biomarkers could be simultaneously quantified in a single animal sample through selected reaction monitoring (SRM). This was later expanded to 157 proteins using scheduled multiple reaction monitoring (MRM). The methodology was further refined to accurately quantify 470 protein markers, resulting in a robust index for assessing the health status of organisms. Additionally, the potential of metaproteomics in ecotoxicology will be commented using results we obtained from soil samples and amphipod gut microbiota. We will discuss the challenges of metaproteomics, including issues related to mass spectrometry, data interpretation, and the exploitation of results in their biological context.

Miss Simran Kaur[1], Dr Cassandra Rauert[1], Professor Kevin Thomas[1]
[1]Queensland Alliance For Environmental Health Sciences (QAEHS)

From Tyres to Tides: Investigating Tyre Additive Chemicals in the Moreton Bay Catchment, Queensland

Simran Kaur 265 - From Tyres to Tides: Investigating Tyre Additive Chemicals in the Moreton Bay Catchment, Queensland, Meeting Room 106, August 22, 2024, 10:25 - 10:44

Tyre wear particles, one of the largest sources of microplastic pollution, raise additional concerns due to their role as environmental carriers of a wide range of incorporated additive chemicals. These chemical compounds from tyres can leach into the surrounding environment, particularly waterways, posing a significant risk to aquatic species. Additives, such as the antioxidant derivative 6PPD-quinone, have been reported to cause aquatic toxicity at environmentally relevant concentrations. 6PPD-quinone has been identified as a contributor to acute toxicity in coho salmon (Oncorhynchus kisutch), resulting in their mass mortality in US streams. While there is still very limited environmental monitoring or toxicological data on tyre additive chemicals and their impact on the Australian environment is still largely unknown.

Methods

To assess environmental concentrations in Australia, freeze-dried sediment samples (1 gram each) were placed in 10 mL ASE cells filled with hydromatrix and spiked with 20 µL x 1 ppm d6-Diuron/d5-Atrazine and 20 µL x 1 ppm d6-5MBTR/d5-6PPD Quinone. Hexane: DCM (1:1) extraction was conducted using an ASE 350 system at 180°C and 1500 psi for 5 minutes(two cycles). Extracts were evaporated to ~1 mL with N2 blowdown, washed with methanol, and re-evaporated. Samples were then transferred to GC vials, adjusted to 200 µL in methanol with 800 µL of MilliQ, and spiked with 20 µL of 1 ppm 13C-Caffeine. The prepared samples underwent analysis for 15 common tyre additive chemicals using LC-MS/MS following established methods (Rauert et al., 2022).

Novel Aspect

The discovery of 6PPD-quinone in Australian sediment underscores the need for further research on storm impact and pollutant accumulation.

Preliminary Data or Plenary Speaker Abstract

Tyre additives were detected in sediments from 24 of the 30 sites at total concentrations (sum of 13 additives) ranging <MDL (0.04) to 311 ng/g dry weight (d.w.) and 13 of the 15 monitored additives were detected. 6PPD-quinone was detected at 22 of the 30 sites (<0.07 – 3.2 ng/g, d.w.) and this is the first reporting of 6PPD-quinone in sediment in the Australian environment. 6PPD-quinone has only previously been reported in sediment in two Chinese studies, including freshwater and marine sediments (0.43-18.2 ng/g d.w.) (Zeng et al., 2023) and river sediment (<MDL – 46 ng/g) (Zhu et al., 2024). Similar to the concentrations detected in the Australian environment. This study demonstrates the ubiquity of tyre additive chemicals in the Australian (Queensland) environment and that while complex dynamics are at play, these pollutants can be transported through key catchments into Moreton Bay.

Miss Tsz-ki Victoria Tsui[1], Dr Kenrick, Kai-yuen Chan[2], Dr Sirius, Pui-kam Tse[1], **Dr Hang-kin KONG**[1]
[1]The Hong Kong Polytechnic University, [2]Centre for Eye and Vision Research Ltd.

Identifying Morphologically Similar Toxic Microalgal Strains by Proteomic Approaches

Hang-kin Kong 96 - Identifying Morphologically Similar Toxic Microalgal Strains by Proteomic Approaches, Meeting Room 106, August 22, 2024, 10:44 - 11:03

Toxic microalgae often cause harmful algal blooms that could negatively impact aquacultures and human health. As microalgal species in the same genus were morphologically similar but had different growth rates and toxicities, thus it is challenging to correctly identify the blooming species for proper managements of the blooms. Traditionally, the blooming microalgae could be identified by their morphologies under microscopic examinations. However, this method fails to identify microalgae at the species level. Microalgal identifications could also be achieved by real-time polymerase chain reaction assay. However, environmental samples always contain DNA from multiple microorganisms which makes primer design difficult to minimize non-specific amplifications. Therefore, the current study aimed to develop various proteomic methods for identifying morphologically similar toxic microalgal species.

Methods

Three morphologically similar Alexandrium strains: CCMP113, CCMP1888, and ATCI03 were selected for the study. Two proteomic methods have been attempted to identify unique proteins in each strain for identification purposes. The first method was to label proteins on the surface of the microalgal strains with cell membrane impermeable tags, like sulfo-NHS-SS-biotin. The labeled proteins were then extracted for shotgun proteomic analysis to identify unique proteins on the surfaces of each Alexandrium strain. Subsequently, antibodies against these unique proteins have been raised for developing an antibody-based assay. The second method was to extract small proteins (> 30 kDa) for top-down proteomic analysis which aimed to develop a microalgal protein fingerprint database for their identifications.

Novel Aspect

Novel proteomic approaches have been developed for identifying morphologically similar toxic microalgae

Preliminary Data or Plenary Speaker Abstract

In the protein labeling approach, the bottom-up proteomic analysis revealed that around 20 to 50 proteins were successfully labeled by sulfo-NHS-SS-biotin for each microalgal strain. The results indicated that the labeled proteins were located on the surface of the Alexandrium strain facing the extracellular space. NAD(P) transhydrogenases were found to be labeled in all of the Alexandrium strains. The unique amino acid sequence of the NAD(P) transhydrogenases could be used as a surface protein biomarker for identifying these 3 Alexandrium strains. Isocitrate dehydrogenase homolog was solely identified on the surface of CCMP113 which could act as a species-specific protein biomarker for CCMP113. As a limited amount of proteins could be labeled, so the top-down proteomic approach was employed to build up a microalgal protein fingerprint database for identifying microalgal species. More than 100 proteins (> 30 kDa) were identified in each Alexandrium strain by top-down proteomic analysis. Moreover, around 10 unique small proteins were identified for each strain which could potentially act as unique protein biomarkers. Furthermore, a few small proteins were only identified in certain growth phases of the Alexandrium strains which could potentially act as indicators for their growth. Thus, the top-down proteomic approach was found to be a more reliable methodology for finding protein biomarkers for identifying microalgal species.

Dr Yuanyuan Song[1], Dr Yanhao Zhang[1], Dr Lin Zhu[1], Ms Yanyan Chen[1], Ms Yi-Jie Chen[2], Mr Zhitong Zhu[1], Ms Jieqing Feng[1], Dr. Zenghua Qi[2], Prof. Jian Zhen Yu[3], **Dr Zhu Yang**[1,4], Prof Zongwei Cai[1]
[1]State Key Laboratory of Environmental and Biological Analysis, Department of Chemistry, [2]School of Environmental Science and Engineering, Guangdong University of Technology, [3]Department of Chemistry, Hong Kong University of Science & Technology, [4]Department of Biology, Hong Kong Baptist University

Inducible energy source shift alleviates geo-specific PM2.5 components causing imbalances in energy metabolism

Zhu Yang 468 - Inducible energy source shift alleviates geospecific PM2.5 components causing imbalances in energy metabolism, Meeting Room 106, August 22, 2024, 11:03 - 11:22

Air pollution, specifically fine particulate matter ($PM_{2.5}$), poses the greatest environmental risk, contributing to roughly seven million annual deaths worldwide as per WHO. The composition of $PM_{2.5}$ varies significantly, and its toxicity is not uniform across all components. Limited data on the toxicity of specific $PM_{2.5}$ constituents hampers our understanding and prevents the development of effective prevention strategies for associated health effects. Through correlational analysis of year-round $PM_{2.5}$ samples from north and south cities of China with changes in cellular metabolomes, we identified geo-specific metabolic markers. Notably, phosphocholine emerged as a functional metabolite capable of counteracting the energy imbalances induced by $PM_{2.5}$ and its most harmful components.

Methods

We carried out a year-long particulate matter (PM) sampling campaign in Taiyuan and Guangzhou, cities in northern and southern China, respectively, aiming to identify the key components of PM in these areas. Building on existing literature regarding $PM_{2.5}$ compositions, our study encompassed a detailed analysis of 82 $PM_{2.5}$ components found in both cities. Furthermore, we utilized untargeted metabolomics to investigate how exposure to these pollutants affects lung cell metabolites, enabling a comparison of the metabolic shifts triggered by different $PM_{2.5}$ constituents. Our correlation analysis successfully highlighted the components with a significant impact on $PM_{2.5}$ cytotoxicity and identified specific metabolites capable of counteracting $PM_{2.5}$-induced cellular dysfunction.

Novel Aspect

We uncovered a metabolite, phosphocholine, exhibiting a capacity to mitigate energy deficiencies caused by PM2.5 and its major toxic components.

Preliminary Data or Plenary Speaker Abstract

Fine particulate matter ($PM_{2.5}$) is widely acknowledged for its adverse implications on human health. However, the specific contributions of individual $PM_{2.5}$ components to its overall toxicity, particularly in light of its high variations, remain underexplored. Furthermore, effective strategies to mitigate $PM_{2.5}$-related health issues are notably lacking. This study aimed to thoroughly assess and compare the key $PM_{2.5}$ constituents and their impact on metabolite alterations in two distinct cities in northern and southern China: Taiyuan and Guangzhou. Our year-long collection and analysis of $PM_{2.5}$ samples identified 82 primary constituents, including ions, metals, and organic compounds, alongside organic carbon (OC) and elemental carbon (EC). $PM_{2.5}$ from Taiyuan showed higher levels of contamination, associated health risks, dithiothreitol (DTT) activity, and cytotoxic effects compared to samples from Guangzhou. Through metabolomic analysis of BEAS-2B lung cells exposed to all $PM_{2.5}$ samples from both areas, significant metabolic changes were observed. Correlation analysis pinpointed metabolites affected by $PM_{2.5}$ exposure and identified key toxic components specific to each region. Among them, phosphocholine stood out as a viable option to counteract $PM_{2.5}$ cytotoxic effects via inducing a shift in energy sources. Its supplementation effectively mitigated $PM_{2.5}$-induced disruptions in energy metabolism and cell viability by promoting ATP production and reducing Phospho1 expression. The identified toxic chemicals exhibited synergistic toxic effects, which could

be alleviated by phosphocholine supplementation. Our findings introduce a promising functional metabolite for remedying $PM_{2.5}$-induced cellular dysfunction and offer new insights into the regional differences in toxic $PM_{2.5}$ components.

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Ms Siobhan Peters[1], Associate Professor David Bishop[1]
[1]University Of Technology Sydney

Bioaccumulation of the environmental neurotoxin BMAA in mussels exposed to cyanobacteria

Siobhan Peters 460 - Bioaccumulation of the environmental neurotoxin BMAA in mussels exposed to cyanobacteria, Meeting Room 106, August 22, 2024, 11:22 - 11:41

Cyanobacteria, commonly known as blue-green algae, are prevalent in aquatic environments. Anthropogenic causes such as climate change and water eutrophication have led to an increase in harmful cyanobacterial blooms. These blooms pose risks to public health from the production of cyanotoxins, including β-Methylamino-L-alanine (BMAA), a neurotoxin implicated in sporadic MND. BMAA exposure is of particular concern for populations near water bodies and is exacerbated by bioaccumulation into human food sources. BMAA analysis is challenging due to low concentrations and complex sample matrices. Mussels are regularly used as bioindicators for other water pollutants and offer a solution for long-term monitoring. This study examines BMAA uptake and elimination in Australian mussels exposed to cyanobacteria, aiming to establish their suitability as environmental bioindicators.

Methods

70 native Australian mussels (Velesunio ambiguus) were distributed across 5 tanks, each containing 14 L of freshwater and 14 mussels, and were exposed to bloom levels of cyanobacteria (2x107 cells/ mussel /day of Microcystis aeruginosa) for a period of 56 days. Following the exposure period, mussels were left to depurate for a further 56 days. Mussels were sampled a total of 14 times (n=5) across the duration of the experiment, with one mussel per tank being sacrificed for analysis at each sampling time point. Samples then underwent an optimised extraction protocol, including protein precipitation, hydrolysis, solid-phase extraction and derivatisation before analysis by LC-MS/MS.

Novel Aspect

This research shows the uptake of an environmental neurotoxin BMAA from cyanobacteria in mussels with a highly sensitive LC-MS/MS method.

Preliminary Data or Plenary Speaker Abstract

The newly developed sample extraction methods significantly enhanced sensitivity, with limits of detection more than 1000x lower than recent studies using similar samples. This enabled the detection of BMAA at concentrations as low as 0.4 ng BMAA/g dry mussel tissue. These concentrations are notably lower than previously reported levels of BMAA in mussels, which often range in the µg/g range. BMAA was detected in the majority of mussel samples, with BMAA concentration increasing over time throughout the exposure period. There was a strong correlation between BMAA concentration and length of exposure. Following the cessation of cyanobacteria feeding, BMAA concentrations quickly decreased to pre-exposure levels. However, BMAA was detected in the controls and time-point 0 samples, suggesting prior exposure, and slow or incomplete depuration. This is supported by the incomplete removal of BMAA from the mussels at the end of the 56-day depuration period.
This experiment provides insight into the accumulation dynamics of BMAA in mussels during and after a cyanobacterial bloom and suggests that V. ambiguus could act as a suitable solution for BMAA analysis in Australia.

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Dr Albert Lebedev[1,2]
[1]Shenzhen Msu-bit University, [2]Core Facility Center "Arktika", Northern (Arctic) Federal University

Formation of Specific Disinfection By-products in Water treatment by Aqueous Chlorination

Albert Lebedev 140 - Formation of Specific Disinfection By-products in Water treatment by Aqueous Chlorination, Meeting Room 106, August 22, 2024, 11:41 - 12:00

Although being successfully applied all over the world water disinfection brings to formation of hazardous disinfection by-products (DBP) from natural and anthropogenic compounds always present in water. Over 800 DBP are known so far, being formed from natural humic matter. Every novel anthropogenic compound shows its unique scheme of transformation with formation of dozens and even hundreds DBP. Reactions of electrophilic substitution, electrophilic addition, nucleophilic substitution, oxidation, single electron transfer, and even radical substitution result in a consecutive array of DBPs, finishing with haloforms. Identification of novel DBPs and study of the mechanisms of their formation represents a challenging scientific task aiming improvement of human health and prevention of ecosystems pollution.

Methods

Aqueous chlorination and bromination of humic matter and numerous emerging contaminants (UV-filters, pharmaceuticals, cosmetic products) was studied with GC-HRMS and LC-HRMS. The reactions were conducted in laboratory in conditions close to that used at the water treatment stations. Besides, drinking water from several Russian cities and from swimming pools was analyzed to find DBPs. The studies involved various disinfecting agents, variation of ratios, pH values, reaction time, addition of inorganic salts. GC-MS experiments were performed using high resolution TOF mass-spectrometers Pegasus GCxGC-HRT (LECO Corporation) and Orbitrap Exactive (Thermo Scientific). LC-MS analyses were carried out with high-resolution TripleTOF 5600+ quadrupole time-of-flight (Q-TOF) (AB Sciex) and Orbitrap Tribrid (Thermo Scientific) mass spectrometers. Besides several low resolution GC-MS and LC-MS systems were used.

Novel Aspect

Numerous novel desinfection by-p-roducts were discovered and the mechanisms of their formation involving a number of reactions were elucidated.

Preliminary Data or Plenary Speaker Abstract

Numerous novel DBPs were identified in the drinking water in the current study. However, the most serious efforts were applied to elucidate the mechanisms of aqueous chlorination. Conjugated double bond appears to be more reactive in aqueous chlorination even than activated aromatic ring. Primary reactions of active chlorine with resveratrol and avobenzone involve double bond, rather than aromatic ring. The presence of two double bonds in limonene allowed studying consecutive reactions of nucleophilic addition and elimination with formation of various chlorinated and hydroxylated products. Radical reactions may also take place as aqueous halogenation of benzalkonium chloride resulted in formation of numerous products with halogens in the aliphatic chain. Pharmaceutical products (doxazosin, umifenovir) demonstrated rather complex transformation including substitution, elimination, and oxidation. Interesting reaction involves dealkylation of alkylamines, proceeding by single electron transfer with formation of the corresponding aldehyde as the second product. Bromides and iodides in natural water form more toxic organohalogens than the corresponding chlorinated ones. Long-term monitoring of DBPs in drinking water demonstrated fast penetration of bromine into the organic compounds at the initial stages of aqueous chlorination. Final DBPs contain more chlorine than bromine due to higher content of chlorine in the reaction mixture and substitution of bromine for chlorine. The latter reaction was studied on haloaromatic standards. The results show fast substitution of iodine for

chlorine in the aromatic substrates due to ipso-substitution. Bromine cation being substituted with chlorine may attack another substrate forming polybrominated species.
A wide range of DBPs containing up to five chlorine atoms, hydroxyl and carbonyl groups were revealed in aqueous chlorination experiments with cocamidopropyl betaine. The proposed mechanism of their formation involves nucleophilic substitution of the secondary amide hydrogen followed by subsequent free radical and electrophilic addition resulting in non-selective introduction of halogen atoms and hydroxyl groups into the alkyl chain.

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Professor Xinxing Zhang1
1Nankai University

Investigation of Microdroplet Chemistry with Mass Spectrometry

Keynote: Professor Xinxing Zhang Nankai University 758 - Investigation of Microdroplet Chemistry with Mass Spectrometry, Meeting Room 109, August 22, 2024, 10:00 - 10:25

Water microdroplet chemistry has drawn tremendous attention in the recent several years for the abilities to accelerate chemical reactions by several orders of magnitude compared to the same reactions in bulk water, and to trigger spontaneous reactions that cannot occur in bulk water. Among the unique properties of water microdroplets, the strong spontaneous redox power is especially intriguing. Several groups deduced that there is a high electric field (~109 V/m) at the air-water interface of microdroplets due to the formation of electric double layers, the alignment of the free O–H bonds of the interfacial water molecules, or the partial charge transfer that results in the interfacial H2O+--H2O- pairs. This high field is large enough to pull electrons out of the hydroxide ions (OH-) in water, yielding hydroxyl radicals (•OH) and electrons. The strong reducing power of water microdroplets is a result of the electron, and the strong oxidizing power of microdroplets is a result of •OH and the high electric field per se.

Methods

Mass spectrometry has been used as the major probe for the microdroplet reactions, not only for its advantages in tracing reactions by identifying the structures of the reactants and products, but also because sprayed microdroplet is a form that can be directly injected into a commercial mass spectrometer, facilitating fast and in-situ monitoring of the reactions, and sometimes helping to capture short-lived radical intermediates that bear key mechanistic information of the reactions. The water solution of a certain substrate is forced by a syringe pump through a fused silica capillary that sits inside a larger coaxial capillary through which high-pressure N2 sheath gas flows. The resulting plume of microdroplets is aimed toward the inlet of a mass spectrometer. The sizes of the droplets generated in this manner depend on the sheath gas pressure, and are in the range of several to several tens of microns.

Novel Aspect

Merely spraying water into small droplets can open an avenue rich in opportunities in synthetic chemistry.

Preliminary Data or Plenary Speaker Abstract

Water is considered as an inert environment for the dispersion of many chemical systems. However, by simply spraying bulk water into micro-sized droplets, the water microdroplets have been shown to possess a large plethora of unique properties, including the abilities to accelerate chemical reactions by several orders of magnitude compared to the same reactions in bulk water, and/or to trigger spontaneous reactions that cannot occur in bulk water. A high electric field (~109 V/m) at the air-water interface of microdroplets has been postulated to be the probable cause of the unique chemistries. This high field can even oxidize electrons out of hydroxide ions or other closed-shell molecules dissolved in water, forming radicals and electrons. Subsequently, the electrons can trigger further reduction processes. In our lab, by showing a large number of such electron-mediated redox reactions, and by studying the kinetics of these reactions, we opine that the redox reactions on sprayed water microdroplets are essentially processes using electrons as the charge carriers. The potential impacts of the redox capability of microdroplets are also discussed in a larger context of synthetic chemistry and atmospheric chemistry.

Prof. Jongcheol Seo[1]
[1]Pohang University Of Science And Technology (postech)

Investigating Protein Conformations Using Protein-Iodine Interactions

Jongcheol Seo 359 - Investigating Protein Conformations Using Protein-Iodine Interactions, Meeting Room 109, August 22, 2024, 10:25 - 10:44

Native ion mobility spectrometry-mass spectrometry (IMS-MS) sheds light on the quaternary structure and non-covalent interactions of protein complexes, facilitating the analysis of protein interactions in near-physiological environments. The collision cross-section (CCS) values of protein ions obtained from IMS-MS enable us to infer protein conformations indirectly. However, CCS value often does not provide enough structural/conformational details. In the present work, we demonstrate that observing protein-iodide adduct ions in IMS-MS can provide additional insights into the protein conformation and unfolding process in solution, which can extend the scope of MS-based study of protein conformations beyond current native IMS-MS and CCS.

Methods

Globular proteins with heme group (Myoglobin and cytochrome c), without heme group (ubiquitin) and intrinsically disordered proteins (β-casein and α-synuclein) were prepared in concentration of 50 μM in various solutions (5 mM ammonium acetate, 5 mM ammonium iodide or 5 mM HI). Experiments were carried out using a hybrid ion mobility quadrupole time-of-flight (IM-QTOF) mass spectrometer, Agilent 6560 equipped with home-built nano ESI source (4 μm emitter I.D.) in a soft tuning condition. Thermal denaturation of proteins was induced in real-time by applying heat from an external heat source to the nano emitter tip, and it was monitored in real-time using IMS-MS.

Novel Aspect

This study presents a new approach that provides additional insights into the protein conformation.

Preliminary Data or Plenary Speaker Abstract

Under native MS conditions in the ammonium acetate buffer, protein ions with low charge states were predominantly observed in the mass spectra. IMS revealed one or two arrival time distribution (ATD) peaks which could be assigned to the native conformation in solution. On the other hand, intrinsically disordered proteins (IDPs) exhibited broad charge distributions spanning from low to high charges. Interestingly, the proteins dissolved in an ammonium iodide solution generated protein-iodide adduct ions only for the low charge states which correspond to the native-like conformations. In contrast, the high-charge protein ions generated from the acidic solution containing HI, which are supposed to have highly unfolded, chain-like conformations, did not have iodide adducts. Furthermore, IDPs that do not have well-defined compact domains in solution did not show many iodide adducts.
Iodides, known as representative chaotropic ions, are typically associated with protein denaturation. However, our findings indicate that compact, globular protein ions contains numerous iodides, whereas denatured, chain-like protein ions do not bind iodides. Since iodides show favorable interactions with the interface, we speculate that the large protein-solvent interfaces in the globular domains of proteins attract iodides. In contrast, the chain-like domains without any large protein-solvent interface cannot hold iodides. Importantly, the number of bound iodides is correlated with the relative amount of loose chain-like domains in the protein ions, which provides additional insights into the protein unfolding process beyond what we can determine solely from CCS values.

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Microsolvation of charged residues prevents backbone collapse and aids in retention of native-like structural features after desolvation

Lukas Benzenberg 29 - Microsolvation of charged residues prevents backbone collapse and aids in retention of nativelike structural features after desolvation, Meeting Room 109, August 22, 2024, 10:44 - 11:03

Native mass spectrometry is frequently used to characterize proteins and their complexes, thus, seeking to find conditions under which native structural features are retained upon desolvation. However, charged residues are believed to compensate for their loss of solvation by engaging in non-covalent interactions with the backbone, which consequently leads to distortion of protein structure. In this work, we employ gas-phase FRET and ion mobility-mass spectrometry to investigate whether microsolvation of charged sites by crown ether variants can sufficiently preserve native features of α-helical structures after desolvation by prohibiting collapse with the backbone. Further, comparing distance constraints from gas- and solution-phase FRET with values of microsolvated species grants insights into structural dynamics in the field of native MS.

Methods

Gas-phase fluorescence spectroscopy experiments of a FRET pair-labeled α-helical peptide were carried out in a modified quadrupole-ion trap mass spectrometer, which enables excitation of mass-selected, trapped species by a femtosecond laser. Nano ESI was employed to generate ions under soft conditions. Fluorescence decay curves were acquired for charge states 6+, 7+, and 8+ and converted into donor-acceptor distances. The experiments were repeated for adducts with 21-Crown-7, 18-Crown-6, 15-Crown-5, and two 18-Crown-6 variants, which exhibit electron-rich substituents. Further, IM-MS experiments were performed on the same species to obtain complementary structural information. Lifetime values were also acquired in the solution phase and helicity of the polyalanine peptide was confirmed in circular dichroism spectroscopy experiments.

Novel Aspect

Microsolvation of charged residues prevents backbone collapse but lacks crucial charge shielding for preserving native-like structures after desolvation.

Preliminary Data or Plenary Speaker Abstract

Distance constraints from terminal fluorophore lifetimes revealed insights into the helical structure in the gas phase. For 6+, 7+, and 8+ species, distances were 73.5±1.27 Å, 92.5±3.04 Å, and 105.5±9.55 Å, respectively, indicating significant α-helix unfolding with higher charge states. Adding 100:1 equivalents of 18-Crown-6 resulted in the formation of adducts with lysine residues. Fluorescence spectroscopy experiments demonstrated helix compaction of 16.0 Å and 19.5 Å upon complexation with up to five and four crown ethers for 8+ and 7+ species, respectively, with minor shrinking of 2.9 Å for 6+ species. Collisional cross sections (CCS) from ion mobility experiments increased for higher charged species, suggesting Coulombic unfolding, consistent with fluorescence results. CCS values for 6+, 7+, and 8+ species were 1304±2 Å2, 1438±9 Å2, and 1499±3 Å2, respectively. CCS of 18-Crown-6 adducts increased slightly with roughly 25 Å2 per crown ether bound. With an expected CCS for 18-Crown-6 of 159±1 Å2, retention of more compact helical structures by microsolvation might be offset by increased CCS due to crown ether addition to the lysine sidechain. Experiments with 21-Crown-7, 15-Crown-5, and 18-Crown-6 variants with electron-rich substituents yielded similar results in fluorescence spectroscopy and ion mobility-mass spectrometry experiments. These findings suggest that although microsolvation retains native-like helical features after desolvation, its impact is weaker compared to absolute charge effects. Microsolvation effectively inhibits the collapse of charged sites, but lacks sufficient charge shielding against Coulomb repulsion, contributing mainly to unfolding mechanisms. Similar results across crown ether variants suggest negligible charge

shielding, regardless of ring size or substituent pattern, while however achieving sufficient non-covalent saturation of charged sites. In solution, donor-acceptor distances measure 48.0±0.92 Å, contrasting with microsolvated species at e.g. 73.0±2.27 Å. Circular dichroism confirmed helicity in solution, but desolvation leads to charge-dependent partial unfolding, distorting native conformations.

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To unfold, or not to unfold, that is the question. On the preservation of solution phase structures upon electrospray ionization

Valérie Gabelica 307 - To unfold, or not to unfold, that is the question. On the preservation of solution phasestructures upon electrospray ionization, Meeting Room 109, August 22, 2024, 11:03 - 11:22

Electrospray is reputedly the ionization method that best preserves non-covalent interactions from the solution to the gas phase. Many examples show that inter-molecular complexes are preserved intact down to the mass analyzer, or that folded protein structures are preserved intact down to the ion mobility analyzer. But should we always take the preservation of solution structures for granted? Here we will examine a few exceptions, which deepen our understanding of the scope and limitations of native mass spectrometry using electrospray. In contrast to most studies devoted to proteins, we examine here nucleic acid and organic foldamer structures specifically designed to test the influence of the strength of intramolecular forces and of the distribution of charge carriers on the ion structures.

Methods

We examined the bi-dimensional charge state distribution and collision cross section distributions date using an Agilent 6560 drift tube ion mobility Q-TOF. The instrument is tuned to minimize internal energy uptake before the drift tube [1]. We used the step-field method to obtain experimentally derived collision cross sections in helium drift gas, which are compared with trajectory model calculations on various molecular models. The models were generated with DFT calculations with PM7 semi-empirical calculations for larger systems, and with AMBER molecular dynamics when needed. The goal of the CCS comparison is to visualize what degree of folding/unfolding is compatible with the observations.

Novel Aspect

Better understanding of cases where solution non-covalent interactions may not be preserved upon electrospray ionization.

Preliminary Data or Plenary Speaker Abstract

For nucleic acids, we varied the strength of intramolecular forces by choosing the sequences so as to form predictable G-quadruplex subunits (stacked guanine quartets stabilized by inner NH_4+ or K+ cations), i-motif structures (stacked intercalated hemi-protonated cytosine base pairs), or non-folded single strands. For foldamers, we used 16-mer oligoquinolines (stabilized by a hydrogen bond network and by stacking interactions [2]) with peripheral phosphonate groups as charge carriers. We varied the number and position of phosphonate groups. For proteins, we studied multi-domain therapeutic proteins with disulfide-stabilized subunits separated by intrinsically disordered linkers [3]. We studied aqueous solutions and modulated the charging level by changing the ionic strength of the solution or by adding supercharging agents. We found that, when charge carriers are predominantly of one polarity (e.g., phosphate or phosphonate groups, deprotonated in solution), the ionic strength was the most important factor influencing the charge state. Higher ionic strength (e.g., higher [NH_4OAc]) increases the concentration of counter-ions that will eventually neutralize the solution deprotonated groups. However, solution folding also plays a role: sufficiently stable G-quadruplex and i-motif structures cannot be charged as much as non-folded strands, and as a result are observed only as compact ion structures [4]. We believe these are formed via the charged residue mechanism. When portions of the molecule can unfold (because they are already non-folded in solution, or because they can readily unfold in the solution-gas interface), bead-on-a-string structures can form. We believe these structures of intermediate collision cross sections are formed

via an intermediate scenario between charged residue and chain ejection, coined the bead-ejection mechanism (BEM). We argue that this ionization scenario is relatively common.

[1] Gabelica etal., JASMS 2018, 29, 2189
[2] Qi etal., Chem. Commun. 2012, 48, 6337
[3] Khristenko etal., JACS 2023, 145, 498
[4] Ghosh etal., Anal. Chem. 2022, 94, 15386

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Derivatization of Single Cell Saccharides and Analysis by Induced ESI MS

Sangwon Cha 637 - Derivatization of Single Cell Saccharides and Analysis by Induced ESI MS, Meeting Room 109, August 22, 2024, 11:22 - 11:41

Saccharide analogues like glycolipids and glycoproteins are essential for understanding vital biological processes. Detecting saccharides via mass spectrometry is challenging due to their neutral nature. One method involves derivatizing saccharides into detectable imine forms using the Schiff base reaction. Previous research used a thin-film reactor to speed up this reaction, yet it took minutes to complete. We explored induced electrospray ionization (inESI) to further accelerate the Schiff base reaction, potentially overcoming previous limitations.

Methods

The study utilized a custom-built induced electrospray ionization (inESI) source paired with an alternating current (AC) power supply, connected to a Thermo LTQ XL linear ion trap mass spectrometer. The acquisition of single-cell fluid was meticulously performed using a Nikon SMZ18 stereo microscope, a NARISHIGE MHW-10 micromanipulator, and a finely pulled borosilicate capillary with an outer diameter of 1.2 mm and an inner diameter of 1 µm.

Novel Aspect

Exploring the applicability of induced electrospray ionization to the study of chemical reaction acceleration

Preliminary Data or Plenary Speaker Abstract

The induced electrospray ionization (inESI) technique efficiently generates fine microdroplets from trace volumes, significantly accelerating reactions compared to traditional ESI. We studied various primary alkylamines under inESI to understand their reactivity in microdroplets, influenced by their pKb values and volatility. Through this, we developed an empirical formula that considers these properties to predict reactivity for unknown amines. We also refined parameters influencing microdroplet size and de-solvation. Our optimized inESI conditions achieved over 90% conversion efficiency in milliseconds, using a sample volume as minute as that within a single plant cell. This advancement in inESI demonstrates the technique's potential in rapid and efficient chemical analysis, with promising applications in biochemical research and diagnostics.

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Monitoring the Dynamic Fate of Interfacial Electrochemical Intermediates by Ambient Mass Spectrometry

Qianhao Min 652 - Monitoring the Dynamic Fate of Interfacial Electrochemical Intermediates by Ambient Mass Spectrometry, Meeting Room 109, August 22, 2024, 11:41 - 12:00

The in-situ dynamic monitoring of interface reactions within electrochemical systems promises to significantly enhance our grasp of key principles governing electrode interface charge transfer, chemical conversion, and energy transmission. In the past decade, advancements of ambient ionization mass spectrometry (AIMS) afford great opportunities on probing short-lived intermediates and final products, thus expediting our understanding of chemical transformations at electrochemical interfaces. However, most current electrochemical mass spectrometry (EC-MS) approaches focused on capturing and identifying the electrogenerated intermediates by MS snapshot, lacking the ability to trace the dynamics of those metastable species over the reaction processes. Delineating the electrochemical reaction networks on how these unstable intermediates varied and interconnected in both interfacial and subsequent homogeneous processes remains elusive.

Methods

We propose a gas-assisted rapid sampling technique combined with high voltage-free ionization for continuous high-fidelity mass spectrometric readout of reaction intermediates. First, a real-time ECL mass spectrometry platform (RT-Triplex) was developed for synchronization of dynamic electrical, luminescent, and mass spectrometric outputs during ECL events. In this setup, a capillary EC microreactor was coupled with a high voltage-free Venturi easy ambient sonic-spray ionization MS, enabling ultrafast delivery of the newborn electrochemical intermediates from Pt wire electrode to MS inlet. We recently developed the upgraded version by interfacing decoupled electrochemical flow microreactor with sonic spray mass spectrometry (namely DEC-FMR-MS), which spatially separates initial interfacial electrochemical event with subsequent homogeneous reaction, facilitating high-throughput screening and mechanistic dissection of electroorganic synthesis.

Novel Aspect

Unravelling and evidencing hidden interfacial electrochemical mechanisms from identity and dynamic fate of transient intermediates measured by MS.

Preliminary Data or Plenary Speaker Abstract

By using the RT-Triplex, we identified electrochemical short-lived intermediates and radicals (e.g. DMA•+, half-life of sub-millisecond), and tracked multi-step electrochemical redox processes implicating multi-electron transfer with high potential and temporal resolution. By correlating electricity-luminescence-mass triplex channels, two ECL pathways of luminol involving the key intermediates α-hydroxy hydroperoxide and diazaquinone, respectively, were definitely validated at the molecular level, and a “catalytic ECL route” of boron dipyrromethene (BODIPY) involving homogeneous oxidation of the co-reactant tri-n-propylamine (TPrA) with BODIPY radical cation (BODIPY•+) in aprotic solvent was revealed and confirmed. The upgraded DEC-FMR-MS platform combines in-capillary electroorganic conversion and follow-up MS interrogation of desired products in a continuous workflow, enabling rapid online analysis of radical-radical cross-coupling, molecular electrocatalysis, and electrochemical cascade reactions within 4 s per sample on the picomole scale. Moreover, the spatial decoupling of electro-oxidation of reactants allows segmented regulation of interfacial electrochemical and homogenous chemical events, which facilitates MS identification of short-lived radical intermediates and delineation of their roles in each step of electrosynthesis. The merits of this platform are demonstrated by 1) discovery and verification of quasi-electrocatalytic

pathways in electrooxidative C–H/N–H radical cross-coupling, 2) kinetic measurements of TEMPO-mediated dehydrogenation of N-heterocycles, and 3) mapping the intermediate (alkene radical and nitrene) landscape of aziridination of alkenes with molecular evidence.

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Prof. Zheng Ouyang[1], Prof. Xiaoyu Zhou, Prof. Xiaoxiao Ma, Prof. Wenpeng Zhang, Professor Yu Xia
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Mass spectrometry technologies for analysis at high structural specificity

Keynote: Zheng Ouyang Tsinghua University 654 - Mass spectrometry technologies for analysis at high structural specificity, Meeting Room 110, August 22, 2024, 10:00 - 10:25

Mass spectrometry is known for identification of chemical and biological compounds at high specificity. In addition to the measurement of mass-to-charge ratios of the molecular ions, which is associated to the molecular weights, tandem mass spectrometry (MS/MS) is routinely applied to identification and confirmation of the compounds through fragmentation patterns. For real-world applications with complex samples, significant challenges exist and call for new technologies to be developed for both instrumentation and method.

Methods

One of the challenges is the identification of the detailed structures of lipids, which has a huge implication to developing lipidomics for deciphering the complex lipidomes. New methods incorporating photochemical derivatization, gas-phase ion photodissociation or other gas-phase reactions allowed the determination of the C=C bond locations, sn-positions and other structural features that could not be identified through traditional MS/MS methods. By using a photochemical (Paternò-Büchi, PB) reactor in line with LC-MS/MS, large-scale lipidomics methods have been enabled with C=C location specificity. While transferring this type of methods for imaging of tissue sections or single-cell analysis, other challenges need to be deal with, such as lack in sample amounts for carrying out MS/MS analysis of a relatively large number of species.

Novel Aspect

High resolution ion mobility, single-cell lipidomics, lipidomic MS imaging, chiral analysis

Preliminary Data or Plenary Speaker Abstract

In this presentation, instrumental solutions for enabling high resolution ion mobility spectrometry (IMS) and distinction of enantiomers will be introduced for isomer analysis. Trapping-and-MS/MS strategy was implemented using IMS-MS and dual-LIT (linear ion trap), which allowed efficient MS/MS analysis of a large number of lipid species from one sampling point on tissue section or from a single cell. This enables lipidomic mass spectrometry imaging and single-cell lipidomics.

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PHOTOELECTRON SPECTROSCOPY OF SOME COPPER BOROHYDRIDE AND ACETYLIDE ANIONS

Howard Ma 224 - Photoelectron Spectroscopy of Some Copper Borohydride and Acetylide Anions, Meeting Room 110, August 22, 2024, 10:25 - 10:44

Copper reagents have played a significant role in numerous transformations in synthetic organic chemistry, including the Sonogashira reaction and `Click' cycloadditions. In both these reactions, a copper acetylide is formed as an important intermediate. Recently, copper hydrides have enjoyed a renaissance due to their reactivity and potential applications in hydrogen storage. During our studies of copper hydride complexes via negative ion ESI-MS experiments, we observed the formation of multinuclear copper borohydride anions from an acetonitrile solution of copper(I) phenylacetylide and sodium borohydride. To obtain a fundamental understanding of the properties of these anions, here we use negative ion photoelectron spectroscopy (NIPES) in combination with theoretical calculations to investigate a series of copper borohydride and acetylide anions.

Methods

NIPES experiments were carried out at the Pacific Northwest National Laboratory with a magnetic-bottle time-of-flight photoelectron spectrometer coupled to an electrospray ionisation (ESI) source and cryogenically controlled ion trap. Cuprate anions were generated via ESI of an acetonitrile solution (ca. 0.1 mM) of a copper(I) salt and $NaBH_4$ and introduced into the ESI-NIPES apparatus. Either a 266 nm (4.661 eV) Nd:YAG laser or a 157 nm (7.866 eV) F2 Excimer laser was utilised for photodetachment.

Density Functional Theory (DFT) calculations were carried out with Gaussian 16 and ORCA 5.0. Benchmarking calculations were carried out with a suite of functionals whilst key molecular geometries were optimised at the ωB97XD/aug-cc-pVTZ(PP) level of theory.

Novel Aspect

First examples of a combined photoelectron spectroscopy and theoretical investigation into organocuprates and borohydridocuprate anions.

Preliminary Data or Plenary Speaker Abstract

Multinuclear cuprate anions with coordinated hydride (H-), borohydride (BH4-), acetylide (PhCC-), cyano (CN-), cyanoborohydride (BH3CN-), and/or iodide (I-) ligands were formed upon electrospray ionisation of an acetonitrile solution of a copper(I) salt ([Cu(CCPh)]n or CuI) with sodium borohydride (NaBH4) or sodium cyanoborohydride (NaBH3CN). Different electrospray sources were found to produce both common and unique product ions. Notably, the free phenylacetylide ion (PhCC-) was formed and represents a rare example of its observation in the gas-phase via direct ESI.

Photoelectron spectroscopy of the mononuclear copper hydride anions [(H)Cu(I)]- and [(H)Cu(BH4)]- reveal a vibrational progression which likely corresponds to the Cu-H bending mode (ca. 470(40) cm-1 for [(H)Cu(BH4)]-). The vertical detachment energies (VDE) were measured to be 3.785(5) and 3.69(1) eV for [(H)Cu(I)]- and [(H)Cu(BH4)]-, respectively. DFT calculations reveal linear ground state geometries for the [(H)Cu(I)]- and [(H)Cu(BH4)]- anions whilst a bent geometry is predicted for the neutral structures.
PES for the copper borohydride anions [(BH4)Cu(BH4)]-, [(BH4)Cu(CN)]-, [(BH4)Cu(I)]-, and [(BH4)Cu(CCPh)]- all displayed bands with broad features. Further, all have relatively high VDEs which are estimated to be 4.35(2), 4.61(2), 4.115(5), and 3.96(1) eV, respectively. A vibrational progression was revealed for the mixed borohydride/iodide heterocuprate [(BH4)Cu(I)]- with a frequency of 242(20) cm-1. Analysis of vibrational frequencies with the ωB97XD DFT method reveals a Cu-I

stretching frequency of 258 cm-1. Analysis of the highest occupied molecular orbitals (HOMOs) of the anions reveal differences between the copper hydrides and borohydrides, where the HOMO of the copper hydrides corresponds to a σ orbital whilst a π orbital is the HOMO of the borohydrides.

From the PES of PhCC- at 266 nm, we estimate an electron affinity (EA) of 3.07 eV, from which the bond dissociation energy (BDE) of phenylacetylene (PhCCH) was derived to be ca. 127 kcal mol-1 using a gas-phase thermochemical cycle.

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Copper-based mass spectrometry and ion mobility to resolve isomeric barriers of phosphatidylcholines

Salomé Poyer 507 - Copper-based mass spectrometry and ion mobility to resolve isomeric barriers of phosphatidylcholines, Meeting Room 110, August 22, 2024, 10:44 - 11:03

Lipids present a challenging analytical task due to the number of known or potential isomers in biological samples. Currently, many analogs remain undifferentiated, and despite the many technical and methodological advances made in mass spectrometry (MS) for lipid analysis, their characterization at the isomeric level still remains an analytical challenge. Since phosphatidylcholines (PC) bearing particular isomeric patterns are relevant biomarkers, developing a method suitable for their characterization at the fatty acid (FA) level could be an essential advantage for early-stage disease diagnosis. We developed a copper-mediated single method that allows both double bond (DB) and stereo numbering (sn) positioning from MS/MS and identification of E/Z stereochemistry of DB by ion mobility spectrometry (IMS).

Methods

Liquid chromatography (LC)-MS/MS experiments were performed on a Q-TOF instrument (Agilent infinity II coupled to a Q-TOF 6540) equipped with an electrospray ion source operating in positive ion mode. The post-column introduction of CuCl2 and ligands allowed copper complexes to be formed in the ion source. IMS-MS/MS experiments were performed using cyclic IMS (cIMS) technology (Waters select series) by direct introduction of isomeric lipids in mixtures with salts and ligands. Collision-induced dissociation (CID) MSn experiments were performed on a quadrupole ion trap instrument (amazon speed ETD, Bruker) and UVPD action spectroscopy in the 7-14 eV range on a linear ion trap (LTQ, Thermo) coupled to the DESIRS beamline at the SOLEIL synchrotron.

Novel Aspect

In a single experiment using a commercial instrument, E/Z stereochemistry, double bond, and aliphatic chain positions of phosphatidylcholines are obtained.

Preliminary Data or Plenary Speaker Abstract

Since CID-MS/MS of protonated or sodiated PCs does not yield any structural information about the DB or sn locations, we used copper adduction to observe different fragmentation behaviors in MS/MS. Simple copper adduction of PCs in the ESI source produces exclusively Cu(II) reduction to Cu(I) and, consequently, non-reactive species. Adding an electron-rich ligand leads to the formation of a copper-based quaternary PC complex by ESI that allows both DB and sn positioning. Indeed, CID activation of such species yields a series of product ion separated by 14 or 26 Da, diagnostic of the DB position. In addition, the relative ratio of fragment ions resulting from ester-breaking bonds allowed us differentiating sn-1 vs. sn-2 branched FA chains. This methodology allowed us to easily locate DBs on PCs with up to 5 unsaturations and was coupled to LC using post-column introduction to annotate PCs in complex mixtures. In addition, we used cIMS to distinguish E/Z stereoisomers of these quaternary complexes, which showed a significant improvement in separation compared to protonated species. We were then able to identify DB, sn locations, and E/Z stereochemistry from a single IMS-MS/MS experiment.
MSn and UVPD action spectroscopy experiments were performed to gain further insights about the gas-phase behavior. We identified the reactive radical intermediate by MSn and hypothesized a mechanistic fragmentation pattern based on both MSn and UVPD results. To be suitable for Lipidomic workflow, software development for the automation of data treatment is in progress, to annotate PCs at the isomeric level from LC-MS/MS experiments.

Mr Oscar Lloyd Williams[1], Dr. Niklas Geue[2], Ms Claudia Cox[1], Mr Thomas Hoare[2], Mr Hari Newnham[2], Dr David Marshall[3], Dr Berwyck Poad[3], Dr Thierry Brotin[4], Professor Perdita Barran[2], Dr Nicole Rijs[1]
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Of Cryptophanes and Cations: Unravelling structural changes induced by encapsulation or complexation.

Oscar Lloyd Williams 462 - Of Cryptophanes and Cations: Unravelling structural changes induced by encapsulation or complexation, Meeting Room 110, August 22, 2024, 11:03 - 11:22

As supramolecular chemistry matures, increased focus is being placed on “smarter” systems such as self-sorting or selective chemistry. Host-guest chemistry is a form of supramolecular chemistry where a larger “host” molecule encapsulates a smaller “guest” molecule. These systems have a selectivity towards certain cations, providing opportunities for catalysis, molecular recognition or sequestration. Cryptophanes are a class of spherical cage molecules with an internal cavity well-suited to the encapsulation of small, charged guests such as metal ions. This encapsulation behaviour is of considerable interest as a means to remove toxic heavy metals such as thallium from aqueous solutions.

Methods

Solutions of cryptophanes and alkali metal salts were prepared and mixed to give various complexes in solution. Direct spray ESI ion mobility-mass spectrometry was performed on these complexes, utilising both cyclic and travelling wave ion mobility-mass spectrometry. Collision cross sections for all complexes were derived. Structural categorisation based on collision cross sections was carried out. Pre-IM CID was also performed both manually and using automated activated ion mobility-mass spectrometry, to fragment complexes and observe any arrival time differences of the fragments, correlating to changes in structure.

Novel Aspect

Advanced ion mobility and pre-IM-CID techniques revealed the surprising structures of cryptophane-cation complexes and their encapsulation mechanisms.

Preliminary Data or Plenary Speaker Abstract

Some cation and cryptophane complexes exhibited smaller overall collision cross sections than expected, revealing no-periodic size trend. This decrease in experimental collision cross section was compared to predicted collision cross sections with the cation in a variety of positions, as both encapsulated and association ("exclusion") complexes. It was clear that the collision cross section was consistent with the full encapsulation of the cation within the cryptophane.
By pre-IM CID it was observed that the fragments of the encapsulation complexes had longer arrival times than those of their precursors. This is counter to the standard fragmentation behaviour, but linked to an “opening” of the cryptophane sphere as covalent linkers are lost, resulting in a larger cross section.
The smaller cations, lithium and protons, yielded structures that exhibited far later arrival times, consistent with complexation but not with encapsulation. The non-encapsulated association complexes did not exhibit the same changes in collision cross section with pre-IM CID. Instead, the species produced are smaller. The significantly smaller size of these cations means they cannot interact fully with the interior of the cryptophane cavity. Qualitatively, when fragmented, these complexes also exhibited notably different fragmentation behaviour, rather than linker fragmentation.
Finally, a novel computational method for predicting cavity volumes of host molecules was developed and was used to probe the predicted change in structure of the cryptophane,

mechanistically suggesting that the size of the encapsulated complexes represented a lower limit to the size of the cryptophane complexes.

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The Internal Energy of Ions Produced by ESI Depends on the Density of Molecules in Electrodes at First Vacuum Stage

Daiki Asakawa 416 - The Internal Energy of Ions Produced by ESI Depends on the Density of Molecules in Electrodes at First Vacuum Stage, Meeting Room 110, August 22, 2024, 11:22 - 11:41

In a high-pressure environment, electrospray ionization (ESI) can be achieved without discharge between the emitter and counter electrode, allowing to produce gas-phase ions from liquid with high surface tension such as pure water which requires a high onset voltage for stable ESI. The high-pressure ESI has shown an enhanced desolvation while conventional atmospheric pressure ESI has promoted and preserved anion-adducted ions. Those observations are related to the desolvation and the ion dissociation during the transferring of ions/charged droplets from a super-atmospheric pressure environment to a vacuum which has not been systematically investigated. In this study, we have investigated internal energy distribution of ions produced by the HP-ESI source and compared it with conventional atmospheric pressure ESI.

Methods

The benzyl ammonium solutions were deposited in platinum-coated borosilicate capillaries and loaded into a nanoESI source under the atmospheric or super-atmospheric pressure environment. For the experiments performed with the high-pressure ESI source, the ESI emitter was placed inside a high-pressure chamber made of aluminum block and insulating flange. The ion source was pressurized with compressed air from an air compressor. To transfer ions from the high-pressure chamber into the vacuum, two interfacing methods were used using direct and indirect coupling configurations. he generated ions were introduced into a benchtop Orbitrap mass spectrometer through a heated inlet capillary. Three heated capillaries with different inner diameters, 0.25, 0.5, and 0.6 mm were used in this experiment.

Novel Aspect

The increase of the gas throughput into the first vacuum stage in mass spectrometer decrease the internal energy of ions

Preliminary Data or Plenary Speaker Abstract

In this study, we have investigated internal energy distribution of ions produced by the high-pressure ESI source two interfacing methods using direct and indirect coupling configurations and compared it with conventional atmospheric pressure ESI. The benzyl ammonium thermometer ions were used as the probe for the evaluation of the internal energy distribution of ions. According to the results, the ions source pressure did not affect the internal energy distribution of ions. In contrast, the gas throughput into the first vacuum stage clearly influences the internal energy distribution of ions. The heating of ion occurs when ions accelerated by tube lens voltage collide with residual gas in the first vacuum stage. As the density of atoms/molecules in the tube lens was increased, the mean free path of ions decreased. As a result, the collision frequency between the ions and the residue gas molecules increased. However, for a shorter acceleration time, the kinetic energy involved in each collision was smaller, thus resulting in a lower mean internal energy of ions. Instead of using the measured pressure for the vacuum stage, which was different from the effective local pressure, we found that it was better to describe the local condition and the associated ion dissociation in a more quantitative manner using the gas throughput to the first vacuum stage.

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Dr Takemichi Nakamura[1], Dr Toshihiko Nogawa[1], Dr Yayoi Hongo[2]
[1]RIKEN, [2]OIST

Collision-Induced Isomerization of Cyclic Peptide Ions Probed by Energy-Resolved Ion Mobility/Tandem Mass Spectrometry: A Case of Anabaenopeptins

Takemichi Nakamura 441 - Collision-Induced Isomerization of Cyclic Peptide Ions Probed by Energy-Resolved Ion Mobility/Tandem Mass Spectrometry: A Case of Anabaenopeptins, Meeting Room 110, August 22, 2024, 11:41 - 12:00

Structural analysis of macrocyclic peptides by tandem mass spectrometry (MS/MS) can be a rather complicated process compared to that of linear peptides. Anabaenopeptins are a group of hepatotoxic cyclic peptides isolated from cyanobacteria, Anabaena sp. The analogous peptides share a common macrocyclic structure consist of five amino acid residues. However, each analogue has a different amino acid connected to an exocyclic position via a ureido linkage. The collision-induced dissociation (CID) fragmentation patterns anabaenopeptins change dramatically depending on the nature of single exocyclic amino acid, which governs the availability of mobile protons [Nakamura, et al., 2024, DOI: 10.5702/massspectrometry.A0144]. We've looked in this interesting system further by using energy-resolved (ER) ion mobility (IM)/MS/MS.

Methods

Mass spectra were acquired with a Synapt G2 quadrupole/time-of-flight hybrid tandem mass spectrometer (Waters) equipped with a UPLC inlet system and an ESI source. A SELECT SERIES Cyclic IMS system (Waters) was also used for obtaining high-resolution IM spectra. For ER-MS/MS and ER-IM/MS/MS experiments, [M+H]+ ions were selected as the precursor and argon was used for CID before (in trap cell) or after (in transfer cell) ion mobility separation in the traveling-wave analyzer filled with nitrogen. Collision energy was applied one of the collision cells and stepped through from low to high for the ER experiments. The IM spectra corresponding to the protonated molecules of the cyclic peptides were extracted with accurate mass windows in the post-run data processing.

Novel Aspect

ER-IM/MS/MS experiments allowed observation of the energy- and structure-dependent collision-induced isomerization of protonated molecules of cyanobacterial cyclic peptides, anabaenopeptins.

Preliminary Data or Plenary Speaker Abstract

The difference between the structure of anabaenopeptin A (AP-A) and B (AP-B) is the exocyclic amino acid residue; the former has Tyr at the side chain of the macrocyclic peptide structure whereas the latter has Arg. In the CID spectra of their [M+H]+ ions, AP-A showed extensive fragmentation including the cleavages of macrocyclic peptide structure whereas AP-B solely showed product ions corresponding to the loss of the side-chain Arg at higher collision energies. Previous ER-MS/MS experiments have demonstrated that the striking differences in the spectral patterns of AP-A and AP-B were attributable to the presence/absence of mobile protons. Interestingly, the IM spectra of AP-A suggested the presence of isomeric [M+H]+ ion structures prior to fragmentation. In addition, the ER-IM/MS/MS experiments showed that relative abundance of the isomeric ion structures depends on the degree of collisional excitation prior to the IM separation. The other analogues, AP-C and cit-AP-B, the former has side-chain Lys and the latter has citrulline, also show extensive fragmentation like AP-A. With some degree of collisional activation, these two analogue also showed multiple [M+H]+ ion structures. On the other hand, the [M+H]+ ions of AP-B did not show any sign of isomerization even at higher collision energies. These observation was consistent to the fact that ionizing proton is mobile in of AP-A, AP-C, or cit-AP-B whereas the proton is sequestered at the Arg residue in AP-B. Namely, since protons are mobile in the [M+H]+ of AP-A, AP-C, and cit-AP-B, protomers and/or isomeric ion structures were generated prior to dissociation as internal energy

increased by collisional excitation. Since no mobile protons in [M+H]+ of AP-B, no isomerization channels opened but the dissociation at the side-chain occurred at the higher collision energies.

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Dr. Birgit Schilling[1]
[1]Buck Institute for Research On Aging

Cellular senescence and aging in healthy individuals – proteomic analysis of lung and ovary tissues across the age range.

Keynote: Dr Birgit Schilling University of California San Francisco 631 - Cellular senescence and aging in healthy individuals – proteomic analysis of lung and ovary tissues across the age range, Plenary 3, August 22, 2024, 15:00 - 15:25

Aging is a complex biological process associated with progressive loss of physiological function and susceptibility to several diseases, such as cancer and neurodegeneration. As senescence burden increases with aging and becomes a risk factor for many age-related diseases we are specifically interested in senescence-derived aging signatures. We use cutting-edge proteomic workflows to investigate the senescence-associated secretory phenotype (SASP), as well as proteomic technologies to identify intracellular and extracellular proteomes, and protein signatures that change with aging. We will present several studies investigating ovary and lung aging with a particular focus on the extracellular matrix. Overall, we are interested in translational research and research that aims towards therapeutic interventions to improve human aging or age-related diseases.

Methods

We obtained human tissues, such as healthy human ovary and human lung from Northwestern University (Chicago) and University of Pittsburgh (Pittsburgh) and processed samples for mass spectrometric analysis. After proteolytic digestion and sample processing samples were acquired using the Orbitrap Eclipse and the Orbitrap Exploris 480 (Thermo). All obtained data was searched using Spectronaut (Biognosys). R scripts were used for further data processing.

Novel Aspect

Precious human samples were investigated to develop senescence and aging signatures for novel biomarker development and future therapeutic interventions.

Preliminary Data or Plenary Speaker Abstract

The human ovary is one of the first organs to age and exhibit early onset of functional decline that affects reproductive longevity and overall systemic health. The underlying causative mechanisms that drive this functional decline have not been fully elucidated. We hypothesize that cellular senescence plays a major role during ovary aging, including increased fibrosis and inflammation. We developed a senescence signature derived from human ovary explants where we induced senescence in tissue culture using doxorubicin followed by SnucSeq and proteomic secretome (SASP) analysis. The latter senotype contained 25 proteins that overlapped between the ovarian transcriptome and secreted proteome (SASP) including MYH9, SOD2, and Lumican. This senotype is then subsequently used for systematic spatial transcriptomic and mapping senescent cells in the ovarian tissue cohort. We also investigated lung aging: Human lungs are exposed to various stresses throughout lifespan, and age acts as a risk factor for pulmonary physiological function impairment and chronic lung disease development. We aim to develop signatures of lung aging and predict aging trajectories. Frozen native lung tissues were collected from 20 human participants – 8 female and 12 male – aged between 21 months and 95 years (10 old and 10 young). In parallel, human precision-cut lung slices (PCLS) from 3 donors were cultured ex vivo and induced for senescence using bleomycin. All samples were analyzed by mass spectrometry (data-independent acquisition, DIA). We were able to identify and quantify 5,558 protein groups (2 unique peptides) from our human lung tissue. The lung proteome was dramatically remodeled with age with 729 significantly changing protein groups,

featuring up-regulated immune and inflammatory responses and altered extracellular matrix homeostasis. These findings provide novel insights into protein changes throughout health span and upon senescence induction, that can be further monitored in age-related lung diseases, to decipher various aging trajectories in human.

Dr. Martina Marchetti-deschmann[1,2,5], Ralf Haider[1,2], Dr Samuele Zoratto[1,2], Christopher Kremslehner[2,3], Gaelle Gendronneau[2,4], Florian Gruber[2,3]
[1]TU Wien, Institute of Chemical Technologies and Analytics, [2]Christian Doppler Laboratory for Multimodal Imaging of Aging and Senescence - SKINMAGINE, [3]Medical University of Vienna, [4]Chanel PB, [5]Austrian Cluster for Tissue Regeneration

Spatially resolved analyses reveals significant changes of the N-glycome and transcriptome in aging skin

Martina Marchetti-Deschmann 481 - Spatially resolved analyses reveals significant changes of the N-glycome and transcriptome in aging skin, Plenary 3, August 22, 2024, 15:25 - 15:44

We introduce a multimodal imaging workflow to research spatially-resolved senescence-associated changes in the human skin. Changes were studied on skin biopsies from young and aged donors implementing a combined multiple analysis per tissue section: N-glycans by MALDI FTICR MSI, MALDI-IHC on a MALDI-TOF instrument targeting senescene markers, immunofluorescence, autofluorescence, and histological tissue staining. Furthermore, spatial transcriptomics was performed on a consecutive section to validate findings and to cross-correlate the different techniques.
This integrated approach aims for a holistic understanding of senescence, enabling a detailed exploration of the complex interplay between molecular and cellular changes in skin aging.

Methods

Longitudinal FFPE sections (5 µm thickness) were prepared on poly-L-lysine coated ITO slides. Sections were dewaxed and rehydrated with organic solvents, and antigen retrieval was performed in an automated device (2100 Antigen Retriever). An HTX TM sprayer was employed for enzyme (1.04 µg/cm2 PNGase F) and matrix (150 µg/cm2 CHCA) deposition. Spatial transcriptomic (10X Genomics) was performed according to protocol. N-glycans were enzymatically digested overnight (37 °C), followed by matrix application and MALDI FTICR MSI. Post matrix removal, overnight photocleavable mass-tags antibodies (AmberGen) staining (4 °C) preceded matrix application for MALDI-IHC. Immunostaining was performed after matrix removal. MALDI-IHC performed on a Shimadzu 8030. N-glycan performed on a Bruker 7T scimaX FTICR.

Novel Aspect

single-tissue multimodal spatial-omics workflow reveals senescence-related changes in human skin

Preliminary Data or Plenary Speaker Abstract

This study introduces a multi-spatial-omics approach to investigate skin aging, using biopsy sections from young and old donors. Following recent trends in bioanalytics and addressing the challenge of preserving fragile skin tissue integrity, we have developed a workflow that sequentially applies autofluorescence, MALDI-IHC, MALDI FTICR MSI, immunofluorescence, and tissue staining on the same section, with spatial transcriptomic and immunofluorescence conducted on a consecutive section. This approach enables immunofluorescence data to bridge spatial transcriptomic findings with the other analyses, maximizing information density.
We examined 21 samples from three donors, each prepared into 5 µm thick sections. Senescence-focused spatial transcriptomic was conducted at 50 µm to detail transcriptional gene expression. MALDI-IHC and MALDI FTICR MSI were performed at 50 µm to bridge instrument data and ensure effective results correlation. Exclusive MALDI FTICR MSI experiments were done at 10 µm for detailed in-situ N-glycan distribution. The high mass accuracy of FTICR MSI allowed identification of ~30 N-glycans, including sialylated structures, some varying between young/old donors. MALDI-IHC targeted key markers like GLUT1, H2AFX, and COL1A1, correlating them with well-established senescence-related immunofluorescence markers such as K14 and γ-H2AFX to highlight age-related distribution and level variations. Autofluorescence and H&E tissue staining were implemented in

support of feature correlation. The spatial transcriptomics approach targets glycosylation factors and senescence-related genes (e.g., IVL and LOR) to better correlate age-related findings.
This comprehensive multimodal workflow, specifically designed for soft tissue analysis, provides valuable insights into cellular senescence and highlights the dynamic molecular landscape of skin aging.

Dr. Caitlin Randolph[1], Matthew Muhoberac[1], Connor Beveridge[1], Palak Manchanda[1], Sanjay Iyer[1], Dr Berwyck Poad[3], Professor Stephen Blanksby[3], Dr. Shane Tichy[4], **Prof. Gaurav Chopra**[1,2]
[1]Department of Chemistry, Purdue University, [2]Department of Computer Science, Purdue University, [3]Queensland University of Technology, [4]Agilent Technologies

Artificial Intelligent Agents for Automating Deep Lipidomics Workflows to Investigate Alzheimer's Disease and Aging-Related Lipid Droplets

Gaurav Chopra 591 - Artificial Intelligent Agents for Automating Deep Lipidomics Workflows to Investigate Alzheimer's Disease and Aging-Related Lipid Droplets, Plenary 3, August 22, 2024, 15:44 - 16:03

Lipid droplets (LDs), or cellular "fat," have been recently demonstrated by our research to hold significance in Alzheimer’s disease (AD). However, detailed composition of LDs remains unknown to date. Conducting large-scale lipidomics experiments is time-intensive and yields extensive data, complicating annotation and interpretation. Presently, there are no methods to automate workflows, including experiment method generation and bioinformatic analysis. To address this, we've developed artificial intelligent (AI) agents. These agents enable users to interact in a chatbot-style manner, facilitating detailed lipid annotation and identification via multiple reaction monitoring (MRM)-profiling and liquid chromatography ozone electrospray ionization MRM (LC-OzESI-MRM) experiments. The AI-agents decipher the intricate tissue- and region-specific lipidomic profiles of LDs from age-matched AD and aged mouse brains.

Methods

LDs were extracted from diverse brain regions of 13-17 month-old AD (5xFAD) and age-matched WT mice, spanning cortex, hippocampus, diencephalon, and cerebellum. Tailored AI agents were developed, utilizing large language models (LLMs), to comprehend user inputs for experiment planning, execution, and analysis. Guided by an AI manager, these agents employed instrument-specific tools, gleaned insights from scientific literature, and consulted instrument documentation for troubleshooting, thereby streamlining lipidomics profiling and data analysis, interpretation and visualization. Optimized worklists compatible with Agilent MassHunter were generated, facilitating MRM-profiling and LC-OzESI-MRM deep lipidomics profiling with “ozone on” and “ozone off” experiments. The AI agents meticulously processed and annotated lipids, identifying acyl chain composition and unsaturation sites, and conducted comparative bioinformatics/pathway analyses to elucidate lipidomic patterns.

Novel Aspect

AI agents offer expert guidance to plan, execute, analyze and troubleshoot MRM- and LC-OzESI-MRM experiments to profile LDs in AD.

Preliminary Data or Plenary Speaker Abstract

Users interact with AI-agents in a chatbot-manner to do several tasks: (a) generate worklists for the Agilent MassHunter software, (b) identifies lipid species present in samples (e.g. brain and liver) specifying number of replicates for differential analysis including fatty acyl (FA) chains and double bond locations, (c) recommended initial LC parameters and cleaning frequency for the experiments, (d) assisted in generating detailed plots to elucidate the experimental results, and (e) provides insights from mass spectrometry lipidomic literature. The AI-agents were used to schedule experiments for MRM-profiling for LDs from different brain regions in 5xFAD and WT mice. AI agents annotated and identified 3000 lipids from 10 lipid classes in LDs in a region- and tissue-specific manner with triacylglycerols (TGs) and cholesterol esters but also identified a variety of previously unknown lipid species spanning acyl carnitine, sphingomyelin, phosphatidylethanolamine, and ceramide subclasses. Deep profiling of TGs with LC-OzESI-MRM resolved isomeric lipid structures with varying carbon-carbon double bond (C=C) position(s). Forty-seven unsaturated TGs were identified and quantified in liver tissue, with 26 and 22 TGs with specific C=C locations in LDs from AD

and wild-type mice were compared. The agent also utilized OzESI tools to identify mono and polyunsaturated fatty acyl (PUFAs) isomer double bond isomers in LDs from four distinct brain regions of AD mice. Similar to monounsaturated fatty acyls, AI agents compiled a list of PUFAs double bond locations using “ozone off” and “ozone on” mode. Finally, the AI agent iteratively learnt from errors to write custom code for data analysis and visualization. AI-agents answered specific questions about the results and troubleshooted runtime errors, like LC leaks or poor MS signal, providing specific information and citations from the user manuals of the Agilent 6495C QQQ LC-MS, thereby streamlining rapid and detailed characterization of LDs to elucidate its functional role in AD.

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Mr Quang Vinh Phan[1], Mr Connor Karozis[1,2], Dr Benjamin Rowlands[2,3], Prof Kay Double[1,2], **Dr Michael Gotsbacher**[1,2]
[1]School of Medical Sciences, University of Sydney, [2]Brain and Mind Centre, [3]School of Chemistry

Biodistribution of a Copper-Delivering Agent in Mouse Brains

Michael Gotsbacher 636 - Biodistribution of a Copper-Delivering Agent in Mouse Brains, Plenary 3, August 22, 2024, 16:03 - 16:22

Copper is essential for health and function of neurons; regional dyshomeostasis of Cu is a major hallmark of CNS conditions including ALS and Parkinson's. Thus, emerging therapies aim to restore CNS Cu levels from pathological levels. Treatment with Cu-delivering agents (e.g., Cu(II)-ATSM) addresses the localised Cu-deficiency observed within the CNS. However, there is no direct evidence for how the drug or related metabolites biodistribute in, or enter the CNS tissues, but there is only evidence of their effect through detected changes of Cu concentrations within CNS sections, e.g., through LA-ICP-MS.
This project aims to (1) determine all hepatic metabolites of Cu(II)-ATSM and (2) determine their special distribution in individual mouse brain sections derived from a treatment pilot study.

Methods

Aim (1): To determine hepatic metabolites, a liver microsome assay was used on Cu(II)ATSM and [15]N-labelled Cu(II)ATSM, followed by MS-led discovery metabolomics on a Thermo Orbitrap HXF, using the [63/65]Cu and [14N/15N] isotopic patterns to guide scripted metabolite identification on mzXML data.
(2) Dissected mouse brain regions (cerebellum, striatum, cortex midbrain) were extracted for metabolomic and lipidomic analysis (using suitable internal standards) and analysed by discovery (Orbitrap HFX) and targeted (Sciex QTRAP 7500) metabolomics approach to determine the spatial distribution of neuroprotective metal-delivering agents and their metabolic derivatives.

Novel Aspect

Mapping the biodistribution of Cu(II)ATSM and its metabolites is novel and will deepen our understanding of therapies addressing CNS metal-dyshomeostasis.

Preliminary Data or Plenary Speaker Abstract

We have devised a synthesis of [15]N-Cu(II)ATSM using [15]N-methylamine, to yield CuATSM-[15]N or CuATSM-([15]N)2 on gram scale, as required for liver microsome and future mouse model studies. In the microsome assay (0–60 min incubation), six compounds were identified as the main phase I/II metabolites in time-dependent manner, matching some of the transformations predicted in Compound Discoverer. These metabolites are currently analysed from within dissected tissue extracts on recently purchased Sciex QTRAP 7500, after analysis using a Sciex QTRAP 6500 indicated abundance comparable to baseline. Global metabolomics analysis of the brain tissue extracts showed significant metabolomic difference based on principal component analysis. High abundance lipids of each main lipid class are currently analysed to investigate lipid changes correlating to Cu(II)ATSM treatment at 15 or 30 mg/kg versus control.
Future work will see application of [15N]Cu(II)ATSM in a mouse model to provide an isotopic handle on Cu-free metabolites, as likely to occur after CNS biodistribution and Cu(II) transfer to Cu(II)-dependent enzymes. MS imaging of alternate brain hemispheres is intended to provide a true spatial mapping of all related metabolites. Downstream, all disease-relevant transition metals will be mapped in every other tissue slice by LA-ICP-MS. The scoped work will provide complementary data and direct insights into molecular mechanisms underpinning effects of a Cu-delivery agent in the CNS.

Dr Emily Byrd[1], Dr Thomas Minshull[1], Dr Emma Norgate[1], Miss Monika Olejnik[1], Miss Amy McGeoch[1], Dr Chalmers Chau[2,3], Professor Paolo Actis[2,3], Dr Antonio Calabrese[1]
[1]Astbury Centre for Structural Molecular Biology, School of Molecular and Cellular Biology, Faculty of Biological Sciences, University of Leeds, Leeds, LS2 9JT, UK, [2]School of Electronic and Electrical Engineering, University of Leeds, LS2 9JT, UK., [3]Bragg Centre for Materials Research, University of Leeds, LS2 9JT, UK.

Understanding how ALS-associated mutations alter the structure and phase separation propensity of the TDP-43 C-terminal domain using structural mass spectrometry

Emily Byrd 324 - Understanding ow ALS-associated mutations alter the structure and phase separation propensity of the TDP-43 C-terminal domain using structural mass spectrometry, Plenary 3, August 22, 2024, 16:22 - 16:41

TDP-43 is an RNA binding protein involved in RNA processing and stress granule assembly through liquid-liquid phase separation (LLPS). During cell stress, TDP-43 migrates to the cytoplasm for stress granule incorporation but can undergo aberrant liquid-to-solid transitions and form amyloid fibrils. Accumulation of TDP-43 amyloid deposits in the brain results in a toxic gain-of-function associated with Amyotrophic lateral sclerosis (ALS). The disordered C-terminal domain (CTD) of TDP-43 facilitates the protein-protein interactions responsible for LLPS, harbours ALS-associated mutations and truncations comprising the CTD are found in patients. Understanding the structural dynamics of monomeric TDP-43 and its higher order assemblies using structural mass spectrometry (MS) would offer insight its function (RNA binding) and its toxic mechanisms (amyloid assembly, aberrant LLPS) in disease.

Methods

Native MS with ion mobility (IM) using a Synapt G1 HDMS (Waters Corporation, Wilmslow, UK) was used to determine conformational shifts of TDP-43 CTD familial variants and identify relative abundances of higher order assemblies. Here, we use sub µM nano electrospray ionisation (ESI) emitters pulled in house which enabled native MS of the disordered TDP-43 CTD in buffers which mimic the physiological environment, such as MES and PBS. We also deployed HDX-MS on a Synapt G2-Si HDMS (Waters Corporation, UK) to study RNA binding by TDP-43. Our integrative structural proteomics approach was correlated with data from Thioflavin T (ThT) fluorescence amyloid assembly kinetics and differential interference contrast (DIC) microscopy to identify LLPS propensity, along with flow induced dispersion analysis (FIDA).

Novel Aspect

Native IM-MS coupled with sub µM nano ESI emitters captures TDP-43 conformational dynamics to inform on mechanisms of ALS.

Preliminary Data or Plenary Speaker Abstract

We have used native MS and IM-MS to observe discrete conformational changes in the CTD of TDP-43 as a result of three ALS-associated mutations (Q331K, M337V and R361S), and correlated these data with amyloid assembly kinetics determined by ThT fluorescence and LLPS propensity via FIDA and DIC microscopy. We show that charge altering mutations and salt concentrations influence the conformational dynamics of the CTD by shifting the cross section area determined using IM-MS and that this, in turn, regulates both amyloid assembly kinetics and LLPS. Under high salt conditions, the rate of amyloid assembly is increased for all variants which also correlated with the increased propensity to undergo LLPS. The rate of amyloid assembly for each CTD variant does not appear to be dependent on protein concentration, suggesting that different amyloid assembly mechanisms may occur at different protein concentrations. Using sub µM nano ESI emitters for native MS and IM-MS,

the abundance of cluster ions from salts were reduced to produce resolvable charge state distributions of the TDP-43 CTD, which requires salt to remain stable, making TDP-43 a difficult system to analyse in ammonium acetate. Overall, these findings indicate that electrostatic interactions determined by the charge distribution of the CTD play a role in the gain-of-toxic function of TDP-43, and demonstrate the power of sub μM nano ESI emitters to study the conformational ensemble of disordered proteins that would otherwise be intractable. Understanding the molecular mechanisms that govern TDP-43 amyloid assembly and aberrant LLPS is crucial in understanding the role of TDP-43 in ALS and may uncover viable targets for therapeutic design.

Prof. Dr. Emma Schymanski[1], Begoña Talavera Andújar[1], Arnaud Mary[1], Carmen Venegas[1], Tiejun Cheng[2], Leonid Zaslavsky[2], Evan Bolton[2], Michael Heneka[1]
[1]University Of Luxembourg, [2]National Center for Biotechnology Information

Can Small Molecules Provide Clues on Disease Progression in Cerebrospinal Fluid from Mild Cognitive Impairment and Alzheimer's Disease Patients?

Emma Schymanski 173 - Can Small Molecules Provide Clues on Disease Progression in Cerebrospinal Fluid from Mild Cognitive Impairment and Alzheimer's Disease Patients?, Plenary 3, August 22, 2024, 16:41 - 17:00

Alzheimer's Disease (AD) is a complex and multifactorial neurodegenerative disease influenced by genetics, lifestyle, and environmental factors that affects over 50 million people worldwide. AD is often divided into three stages: (1) preclinical stage characterized by normal cognitive ability, (2) prodromal stage characterized by mild cognitive impairment (MCI) and (3) dementia stage. AD is currently diagnosed via clinical symptoms and non-specific biomarkers such as Aβ1-42, t-Tau, and p-Tau measured in cerebrospinal fluid (CSF). Alone, these biomarkers do not provide sufficient insights into disease progression. In this pilot study, these biomarkers were complemented with small molecule analysis using on the CSF of three groups; AD, Mild Cognitive Impairment (MCI) due to AD, and a non-demented control group (ND).

Methods

In total, 30 CSF samples were extracted by lumbar puncture, with 10 available per group (ND, MCI and AD). Informed consent was obtained. The clinical biomarkers measured were Aβ1-40, Aβ1-42, t-Tau, p-Tau and neurofilament light (NfL). Non-target high resolution mass spectrometry (NT-HRMS) coupled to liquid chromatography (LC) was performed using both reverse phase (RP) and hydrophilic liquid interaction chromatography (HILIC) coupled to an Orbitrap Q Exactive HF mass spectrometer using electrospray ionization (ESI) in both positive and negative modes. Highly sensitive bile acid target analysis was performed using a LC-QQQ-MS method on a Sciex 7500 via the Genome BC Proteomics Centre of the University of Victoria (Canada).

Novel Aspect

Combining non-target LC-HRMS and target LC-MS in cerebrospinal fluid revealed potential molecular markers that could indicate Alzheimer's Disease progression

Preliminary Data or Plenary Speaker Abstract

An open source cheminformatics pipeline based on MS-DIAL and patRoon was enhanced using CSF- and AD-specific suspect lists to assist in data interpretation. New disease-specific database (AD-database) and suspect lists (TOP1, SC20 and AD-CTD) were created to explore the CSF metabolome and exposome of MCI and AD subjects via literature mining and datasets integrated within PubChem. These were complemented with the CSF subset of HMDB (HMDB-CSF) and PubChemLite for Exposomics (PCL). All lists are publicly available (DOI: 10.5281/zenodo.8014420 and DOI: 10.5281/zenodo.6936117). NT-HRMS revealed twelve Level 1-2a statistically significant features (ANOVA p-value <0.05), including amino acids (valine, proline), sugar acids (galacturonic acid, threonic acid) and pharmaceuticals (diazepam). ChemRICH analysis revealed a significant increase of hydroxybutyrates in AD, including 3-hydroxy butanoic acid, which was found at higher levels in AD compared to MCI and ND. The highly sensitive target LC-MS method was able to quantify 35 bile acids (BAs) in the CSF, revealing several statistically significant differences in AD including higher levels of dehydrolithocholic acid, the major metabolite of the cytotoxic secondary BA lithocholic acid, and decreased conjugated BAs levels. While MS-DIAL provided a higher number of annotated chemicals, the combination of different software and suspect lists enhanced the annotation of a variety of chemicals, increasing the general understanding of the CSF metabolome/exposome, including new chemicals not yet reported in CSF in HMDB. The results revealed that the HILIC LC method appears to be the most suitable for future non-target experiments, as most significant

differences were observed in these measurements. This work provides several promising small molecule hypotheses, including the potential role of the microbiota-gut-brain-axis, which could be used to help track the progression of AD in CSF samples. This work, published in Talavera Andujar et al (2024) DOI: 10.1021/acs.est.3c10490 is currently being expanded in follow-up experiments.

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Distinguished Professor Nicki Packer[1]
[1]Macquarie University

Analysis of the CD52 glycopeptide shows activity is dependent upon both its N- and O-linked glycan structures

Keynote: Professor Nicki Packer Macquarie University 754 - Analysis of the CD52 glycopeptide showsactivity is dependent upon both its N- and Olinked glycan structures, Meeting Room 105, August 22, 2024, 15:00 - 15:25

Human soluble CD52 is a short glycopeptide comprising 12 amino acids (GQNDTSQTSSPS) which functions to inhibit immune responses by initially sequestering pro-inflammatory high mobility group box protein 1 (HMGB1). Human CD52 is heavily glycosylated, with a complex sialylated N-linked glycan at Asn3 and O-linked glycosylation on several possible serine and threonine residues. Anti-inflammatory activity of CD52 has been linked to its specific glycosylation, with terminal 2,3 linked sialic acids playing a key role in binding to the inhibitory SIGLEC-10 receptor leading to T-cell suppression. It is currently unclear how CD52 O-glycosylation has impact on its immunosuppressive activity.

Methods

A combination of high resolution mass spectrometry and molecular dynamics simulations were used to further elucidate the mechanism by which CD52 interacts with HMGB1 Box B. Soluble recombinant CD52 was produced in Expi293 cells, then purified via affinity chromatography. Subsequent anion exchange chromatography fractionated active and inactive CD52. Following activity testing, both N- and O-glycans were removed from CD52 fractions for released glycan analysis with negative mode PGC-LC-MS/MS to determine glycan structures. Following in-depth glycomic analysis, CD52 fractions were subjected to high resolution C18 (EThcD)-MS/MS, before and after N-glycan removal, to determine the site and occupancy of O-glycosylation on CD52. This acquired information was then used to inform a molecular dynamic model of HMGB1-Box B and SIGLEC-10 interaction with CD52.

Novel Aspect

Multiple mass spectrometry approaches were explored and paired with dynamic modelling to more completely understand the mechanism of action of a therapeutically important glycopeptide.

Preliminary Data or Plenary Speaker Abstract

After released N- and O- glycan structures and their isomers were solved with negative mode PGC-LC tandem mass spectrometry, a glycan-assisted glycoproteomics approach was used to confidently identify high heterogeneity of N-linked and O-linked glycoforms on CD52. Pairing this released glycan information with EThcD-MS/MS, the site of O-glycosylation was able to be confidently localised on CD52. This experimental information was then used to create a molecular dynamics simulation of the interactions between soluble CD52, HMGB1 and its downstream immune receptor, SIGLEC-10. The molecular dynamics model inferred a bi-modal relationship between the O-glycans and N-glycans on CD52, and HMGB1 and SIGLEC-10 respectively. With accurate identification of the active glycoforms of CD52 and effective dynamic modelling, the mechanisms behind CD52 can be more thoroughly understood, further informing development of recombinantly produced CD52 as a therapeutic agent.

We show that a specific combination of O-glycan and N-glycan structures present in low abundance on CD52 is responsible for the downstream immune activity of this molecule, and with modelling infer that α-2,3 sialylation is critical for the O-glycan on rCD52 to bind to HMGB1-Box B (and not to HMGB1- Box A). This adds to the importance of α-2,3 sialylation on the N-glycan required for interaction with SIGLEC-10, and confirms that removal of sialic acid and replacement with only α-2,3 sialylation is sufficient to restore CD52 activity.

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Xue Sun[1]
[1]First School of Clinical Medicine, Peking University

Large-Scale pattern analysis of N- and O-glycoproteomics using ion-mobility assisted mass spectrometry

Xue Sun 616 - Large-Scale pattern analysis of N- and Oglycoproteomics using ion-mobility assisted mass spectrometry, Meeting Room 105, August 22, 2024, 15:25 - 15:44

Glycosylation plays crucial roles in biological processes such as cell signaling, immune response, protein folding, cell adhesion, and recognition. Aberrant glycosylation can significantly affect immune activation, antigen recognition, antigen-antibody binding affinity and malignant cellular immune escape, thus leading to immune disorders and promoting the pathogenesis of diseases. However, a comprehensive and systematic characterization of native glycoproteome encounters great challenges due to multiple factors, such as the heterogeneity of glycosite-occupancies and glycan structures, low abundance of glycoproteins, bias of enrichment methods for glycopeptides, difficulties in differentiating glycopeptide isomers and the elucidation of their spectra. To address these hurdles, there is an urgent need to develop and implement innovative research techniques that enhance the precision and sensitivity of glycan structure analysis.

Methods

Here, we employed trapped ion mobility tandem time-of-flight mass spectrometry (timsTOF MS) to investigate the patterns and associations of protein N- and O-glycosylation. To begin with, we assessed the feasibility of tandem-TIMS platform with optimal PASEF mode utilizing three purified glycoproteins including fetuin, recombinant erythropoietin (rEPO), and Apolipoprotein E (ApoE). Subsequently, we performed a large-scale characterization of N- and O-glycosylation for more complex biological samples, including human lung cancer cells, serum, and exosomes. Next, we delved into serum antibody glycosylation and systematically compared the aberrant glycosylation patterns of serum antibodies in eight immune disorders, including RA, systemic lupus erythematosus (SLE), Sjögren's syndrome (SS), amyloid light-chain amyloidosis, mild IgA nephropathy, severe IgA nephropathy, acute myeloid leukemia and acute lymphoblastic leukemia.

Novel Aspect

Our informative patterns of N- and O-glycosylation facilitated a better understanding of the pathogenesis of immune-mediated disorders.

Preliminary Data or Plenary Speaker Abstract

Indeed, TIMS-assisted MS with the additional dimension enhanced the physical separation of isomeric glycopeptides in the ion mobility space. We demonstrated that this platform could generate high-quality mass spectra and produce abundant information of diagnostic oxonium ions for intact glycopeptides identification. When combined with database search and manual spectra-check for confirmation, we definitely identified more glycosites and glycans than previously reported. The results displayed the presence of numerous glycoproteins exhibiting significant biological functions in A549 cells, A549 cell exosomes, serum, and serum exosomes. We observed unique glycoprotein profiles in each of these four types of samples, showing specific patterns and variations in glycosylation in different biological regions.
Additionally, we validated that N-glycans conservatively occupied the Fc domain which was consistent with pioneer studies, while O-glycans were more flexible and ubiquitous, and distributed across the entire functional regions, suggesting that N- and O-glycosylation have distinct roles in antibody function. The analysis of IgG, IgA, IgM, and IgD demonstrated the glycan heterogeneity and the regional specificities among different antibody classes, potentially contributing to the diverse repertoire of antibodies. Moreover, our results displayed both commonalities and specificities in the antibody glycosylation patterns across the eight immune disorders. The identified disease-differential glycosites and domain-dependent glycan profiles enable us to get more clues about disease

mechanisms. This analysis enables us to offer valuable insights into the pathogenesis of immune disorders and provides a robust tool for further exploring the biological functions of glycosylation.

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Professor Ronghu Wu[1]
[1]Georgia Institute of Technology

Effective Mass Spectrometry-Based Methods for Comprehensive and Site-Specific Analysis of Surface Glycoproteins and Their Dynamics

Ronghu Wu 118 - Effective Mass Spectrometry-Based Methods for Comprehensive and Site-Specific Analysis of Surface Glycoproteins and Their Dynamics, Meeting Room 105, August 22, 2024, 15:44 - 16:03

The cell surface contains many important proteins, such as receptors and transporters, and almost all of them are glycosylated. These surface glycoproteins regulate nearly every extracellular activity, including cell-cell communication, cell-matrix interactions, and cell immune response. Modern mass spectrometry (MS)-based proteomics is very powerful to globally analyze proteins and their modifications. However, it is still exceptionally challenging to globally characterize glycoproteins because of the low abundance of many glycoproteins and the heterogeneity of the glycans. It is even more challenging to comprehensively analyze glycoproteins only on the cell surface. Systematic characterization of surface glycoproteins and their dynamics will advance our understanding of glycoprotein functions, cellular activities, and disease mechanisms.

Methods

In our lab, we have worked on developing novel and effective methods to globally surface glycoproteins. Integrating metabolic labeling and bioorthogonal chemistry, we can selectively separate and enrich glycoproteins only from the cell surface, which allowed us to globally and site-specifically analyze surface glycoproteins. Furthermore, in combination with multiplex proteomics, we systematically investigated the dynamics of glycoproteins on the cell surface. For example, we quantified the dynamics of cell-surface glycoproteins on monocytes and macrophages under the treatment of lipopolysaccharides (LPS) in time-resolved and site-specific manners. Glycopeptides from the quantification experiment were labeled with the TMT reagent for the multiplex quantification. The TMT labeling enabled us to quantify glycopeptides from several samples simultaneously, increasing the throughput and the quantification accuracy.

Novel Aspect

We developed a novel MS-based method integrating metabolic labeling and bioorthogonal chemistry to systematically characterize surface glycoproteins and their dynamics.

Preliminary Data or Plenary Speaker Abstract

First, we verified that the method specifically targeted glycoproteins on the cell surface using the gel and fluorescence experiments. Then surface glycoproteins were selectively enriched for MS analysis. The method was applied to analyze surface glycoproteins in eight different types of human cells. On average, more than 350 surface glycoproteins were characterized in each type of cells. Quantification results reveal that besides cell-specific glycoproteins, the uniqueness of each cell type further arises from differential expression of surface glycoproteins. For the dynamics of surface proteins in monocytes and macrophages with the infection, 764 cell-surface glycoproteins and 2,205 unique glycopeptides were detected from all identification and quantification experiments. Over 400 glycoproteins and 1,000 glycopeptides were found commonly in both monocytes and macrophages. Gene ontology-based clustering of all identified glycoproteins showed that their functions correspond very well with the known functions of cell-surface glycoproteins. It was found that the surface glycoproteomes were remodeled in cells under the LPS treatment, including the expression of new glycoproteins to the surface and the removal/internalization of existing surface glycoproteins. Some surface glycoproteins gradually increased their abundances while the abundances of others abruptly increased before returning to the normal state, which would not be observed in a typical control-versus-treatment experiment. A comparison of the responses to LPS between monocytes and macrophages showed the similarities and differences between their surface glycoproteomes, and the

priming of monocytes for the response during the differentiation process. Previously reported markers for inflammation or differentiation were detected to validate the effectiveness of the current method. At the same time, the results revealed dramatic changes of other surface glycoproteins that have never been reported to play roles in the immune system. Furthermore, site-specific protein glycosylation was also observed where the abundances of specific glycoforms were altered on the cell surface.

Dr Abarna Vidya Mohana Murugan[1], Dr Catherine Hayes[2], Dr Frédérique Lisacek[2], Dr Kiyoko F Aoki-Kinoshita[3], Dr Niclas G Karlsson[4], Dr Kathirvel Alagesan[1], Dr Rebecca E Griffiths[5], Dr Tiago Oliveira[1], Dr Kimberly A Finlayson[6], Dr Eloise Skinner[7], Dr Martin Linster[8], Dr Jason P van de Merwe[6], Dr Gavin J. D. Smith[8], Dr Samantha Richardson[9], Prof. Daniel Kolarich[1]
[1]Griffith University, [2]SIB Swiss Institute of Bioinformatics, [3]GaLSIC, Soka University, [4]Oslo Metropolitan University, [5]Australian Red Cross Lifeblood, [6]Australian Rivers Institute, [7]School of Environment, [8]Emerging Infectious Diseases Program, Duke-NUS Medical School, [9]RMIT University

Phyloglycomics: Mapping the serum glycome in vertebrates to understand the evolution of vertebrate glycosylation

Abarna Murugan 674 - Phyloglycomics: Mapping the serum glycome in vertebrates to understand the evolution of vertebrate glycosylation, Meeting Room 105, August 22, 2024, 16:03 - 16:22

Phyloglycomics allows us to understand the evolutionary relationship of glycosylation features present in a tissue or body fluid across species. While phylogenetic studies have uncovered that numerous key enzymes of the glycosylation machinery are highly conserved across the vertebrate subphylum, these cannot inform on the activity and interplay between the different enzymes, and thus the effective glycome present in a body fluid or tissue of a species. To date, a systematic phyloglycomic understanding across the vertebrate subphylum is still lacking. We have developed the first-ever vertebrate serum glycome database from 75 different species of vertebrate animals using Mass spectrometry-based glycomics technologies.

Methods

Using our porous graphitized carbon (PGC) Liquid Chromatography (LC) Electrospray ionisation (ESI) tandem Mass Spectrometry (MS/MS) technology we performed an in-depth serum glycome characterisation from over 210 different serum samples representative for 70 different vertebrates across different taxa including mammals (46), birds (15), reptiles (7), fish (2) and bats (7). Where possible, we dissected linkage and structure specific features and abundance data and used this information to establish the first comprehensive large-scale phyloglycomic comparison of serum N-glycans. Sialic acids were further analysed by fluorescently labelling them with 1,2- Diamino Methylenedioxybenzene hydrochloride (DMB) and subsequently detected by High Performance Liquid Chromatography on a Reversed Phase C-18 column.

Novel Aspect

The vertebrate serum glycome library understands the intricacies of cross-species boundaries, host-pathogen coevolution contributing to zoonoses.

Preliminary Data or Plenary Speaker Abstract

Out of the 70 species analysed, 62 (~90% of the total vertebrate species analysed in this study) had NeuAc as their predominant SA monosaccharide in their N-glycans. Core fucosylation was a minor in serum N-glycans except for pigs, where 48% of N-glycans were core fucosylated compared to an average 5.5% in most other species. 50% of N-glycans in Green sea turtle serum contained α-Gal epitopes. Water birds such as duck and geese contained at least 10% of N-glycans that expressed sialylated LacDiNAc epitopes. This epitope was rather absent in other land birds besides turkey and emu. Sialic acid O-acetylation was found to play just a minor role across the analysed species. However, the monotreme or egg laying mammal - echidna expressed 44% O-acetylated Neu5,9 Ac followed by rats (35%), donkey (33%) and horse (29%). These data provide a unique opportunity to understand vertebrate phyloglycomic relationships based on glycan structures and their abundances present in vivo. We are currently in the process of finalising the largest vertebrate serum N-glycan and MS/MS spectra database, which will be made publicly available or open access through glycobioinformatics platforms such as Glyconnect, GlyCosmos and UniCarbDB respectively.

Huilin Hao[2], Yvonne Kong[1], Michelle Cielesh[1], Youxi Yuan[2], Atsuko Ito[2], Benjamin Eberand[1], Freda Passam[1], Robert Haltiwanger[2], **A/Prof. Mark Larance**[1]
[1]The University Of Sydney, [2]University of Georgia

Unbiased analysis of the human platelet proteome identifies a new form of domain-specific O-fucosylation generated by FUT10 and FUT11

Mark Larance 22 - Unbiased analysis of the human platelet proteome identifies a new form of domainspecific O-fucosylation generated by FUT10 and FUT11, Meeting Room 105, August 22, 2024, 16:22 - 16:41

Platelet activation induces the secretion of proteins that promote platelet aggregation, blood clotting, inflammation and vascular repair. An overlooked aspect of these secreted proteins is their decoration with post-translational modifications (PTMs) including O-glycosylation, that are important for protein function. Domain-specific O-fucosylation plays critical roles in modulating the biological functions of numerous cell surface or secreted proteins, with the most prominent substrates being the NOTCH receptor and ADAMTS family proteins. To date, only two types of domain-specific O-fucosylation have been characterized: POFUT1-mediated O-fucosylation of Epidermal Growth Factor-like (EGF) repeats and POFUT2-mediated O-fucosylation of Thrombospondin Type 1 Repeats (TSRs). Using unbiased proteomic analysis, we aimed to identify new types of O-glycosylation on platelet proteins.

Methods

To examine the O-glycosylation of platelet secreted proteins, we applied a bottom-up proteomics approach. Purified peptides from the human platelet secretome were analysed by LC-MS/MS using several complementary fragmentation approaches (HCD, EThcD, and EAD). Data-Dependent Analysis (DDA) was performed with either a Neo-Vanquish coupled via a 50cm x 75µm nanoflow C18 column to an Eclipse tribrid (Thermo Scientific), or an Evosep-1 coupled via an 8cm capillary flow column to a 7600 ZenoToF (Evosep, Sciex). Spectra were analysed using Byonic (Protein Metrics) and included “open” searches with a wildcard mass shift of up to 1,000 Da alongside O-glycan specific searches. Downstream characterisation of modified platelet proteins, O-glycosylation enzymes and their mutants utilised HEK-293 cells, or immunoprecipitation of endogenous proteins from tissues.

Novel Aspect

Unbiased analysis identifies a new form of domain-specific O-fucosylation generated by FUT10 and FUT11 important for protein quality control.

Preliminary Data or Plenary Speaker Abstract

Here we detail the most sensitive analysis to date of the platelet secreted proteome with the quantification of >200 significantly secreted proteins after platelet activation. Unbiased scanning for post-translational modifications within these proteins highlighted O-glycosylation as a major component. For the first time, we detected high stoichiometry O-fucosylation on previously uncharacterised sites including multimerin-1 (MMRN1), a major alpha granule protein that supports platelet adhesion. The N-terminal elastin microfibril interface (EMI) domain of MMRN1, a key site for protein-protein interaction, was O-fucosylated at a conserved threonine (T216) with high stoichiometry. Secretion was inhibited of a MMRN1 T216A mutant that could not be O-fucosylated on the EMI domain, supporting a functional role of EMI domain O-fucosylation in protein quality control. Data from interaction predictions using Alphafold2-Multimer suggested that two widely expressed fucosyltransferases, FUT10 and FUT11, annotated in UniProt as α1,3-fucosyltransferases, were responsible for this modification. Co-immunoprecipitation assays, in vitro O-fucosylation assays and knockout cell lines demonstrated FUT10/11 is necessary and sufficient for EMI domain O-fucosylation. Using crosslinking mass spectrometry on purified proteins, we have validated the very high-quality predicted structure from Alphafold2 for the FUT10/FUT11 interaction with the EMI domain. The EMI domain is present in 18 distinct mammalian proteins that are either secreted, or

cell surface integral membrane proteins and produced by a diverse array of tissues such as platelets, neurons and muscle. We have confirmed using electron activated dissociation (EAD) that the EMI domain of 4 other proteins, EMILIN1, EMID1, MMRN2 and COL26A1 were also O-fucosylated one or more times in their EMI domain. This suggests the widespread necessity of this new modification for protein function. Future work will focus on generating a complete map of the prevalence and stoichiometry of EMI domain O-fucosylation across the human proteome.

Mr Kristian Tkalec[1], Mr. Alexander Ziegler[1], Assoc. Prof. Nichollas Scott[1]
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Protein Aggregation Capture enabled carboxylate group derivatisation allows proteome scale peptide supercharging for O-glycoproteomic analysis

Kristian Tkalec 83 - Protein Aggregation Capture enabled carboxylate group derivatisation allows proteome scale peptide supercharging for Oglycoproteomic analysis, Meeting Room 105, August 22, 2024, 16:41 - 17:00

On-bead single-pot solid-phase-enhanced sample preparation, SP3, or Protein Aggregation Capture (APC) (Batth et al., 2019), has become a robust and widely utilized approach for proteomic sample preparation. Recent studies highlight APC as an ideal platform to allow chemical labelling of samples while minimizing sample loss and improving the recovery of labelled peptides for Cysteinome (Desai et al., 2022; Yan et al., 2021) and N-terminome (Weng et al., 2019) analyses. APC's ability to allow labelling of intact proteins under conditions typically associated with the precipitation of proteins, such as high organic solvents, make it uniquely suited for chemical labelling techniques traditionally excluded from proteomic sample preparation due to their cumbersome or challenging nature, such as chemical derivatization of carboxyl groups.

Methods

Here, we establish an APC-based protein-centric carboxyl group derivatization approach to enable peptide supercharging with the goal of streamlining the localization efficiency of low charge density glycopeptides. We demonstrate that robust on bead protein-level derivatization of carboxylate groups can be achieved using the fixed-charged amine (2-aminoethyl) trimethylammonium chloride hydrochloride (AETMA) via EDC/HoBt coupling, simplifying labelling while minimising sample loss, making it amenable to low microgram-scale sample preparation.

Novel Aspect

On-bead protein derivatization demonstrates APC's adaptability, enabling diverse chemical labelling approaches and proteome scale derivatization studies.

Preliminary Data or Plenary Speaker Abstract

Utilising bead-based carboxyl labelling we demonstrate the effective installation of AETMA at D/E amino acids increasing the observed charge state of labelled peptides compared to their unlabelled counterparts. In contrast to previous studies, our optimised labelling approach allows the effective derivatization of multiple amino acids within a given peptide highlighting its efficiency. Electron based fragmentation of AETMA labelled peptides demonstrated a marked improvement in ETD/EThcD efficiency consistent with previous studies (Frey et al., 2013; Ko & Brodbelt, 2012), yet EThcD revealed the presence of ions corresponding to y 59 m/z. Analysis of AETMA derivatised peptides utilising HCD collision-based fragmentation results in the generation of a 59 Da neutral loss from AETMA derivatized amino acids with the integration of this loss into both EThcD and HCD database searching beneficial for peptide scoring. To demonstrate the utility of AETMA peptide supercharging for glycoproteome scale analysis, we assessed the impact of derivatization on the analysis of O-linked bacterial glycopeptides derived from Acinetobacter baumannii, with derivatized glycopeptides demonstrating spectra rich in in c/z• ions, enabling robust localization of labile O-¬linked glycosylation events. To further exemplify the benefit of supercharging the proteome with AETMA derivatization to assess the A. baumannii glycoproteome we digested with alternative proteases such as thermolysin and pepsin which produce many short and low charge state glycopeptides rich in A/E/D amino acids not ideal for ETD/EThcD providing poor localisation of the glycan. ETD/EThcD fragmentation of non-tryptic peptides derivatized with AETMA provided ion rich spectra improving identification and localisation of glycans compared to non-labelled counterparts. Therefore, enabling an improved characterisation of the O-glycoproteome and highlighting the

benefit of increasing the charge state of peptides for ETD/EThcD fragmentation when dealing with large neutral mass PTMs such as glycans.

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Dr. Yu Bai[1]
[1]Peking University

Single Cell Multi-omics Mass Spectrometry Analysis: Methods and Applications

Keynote: Professor Yu Bai Peking University 228 - Single Cell Multi-omics Mass Spectrometry Analysis: Methods and Applications, Meeting Room 106, August 22, 2024, 15:00 - 15:25

Cellular heterogeneity plays an important role in many key biological processes such as tumor, aging, immunity and development, etc. Single cell multi-omics presents global sights of heterogeneous molecular networks and phenotypic differences. Compared to that of single cell genome and transcriptome, single cell proteome and metabolome detection remains challenging because of their large diversity, low abundancy, and lack of amplification ability of proteins and metabolites. The methodology development of single cell multi-omics is essential and highly challenging.

Methods

Ambient sampling combined with high-resolution mass spectrometry plays an important role in in-situ, real-time and high-throughput analysis. Based on this, our experiments conducted single cell multi-omics analysis by using microfluidics and gene-editing techniques and constructing the mass spectrometry immunoassay and the organic mass cytometry and other platforms.

Novel Aspect

Novel strategies for the single multi-omics analysis by using mass spectrometry

Preliminary Data or Plenary Speaker Abstract

Highly sensitive ambient MS immunoassay platform was constructed through combining rhodamine-mass tag based immune signal amplification with electrospray accelerated substrate electrospray ionization device, achieving zmol LOD of thrombin. Cancer biomarkers were further detected in cell, single drop of blood and tissue sample, demonstrating that this strategy is extendable and applicable. A multidimensional organic mass spectrometry flow cytometry platform was built by using Dean-flow microfluidic chip and high-resolution nanoESI-MS. With the help of immune signal- amplification by the above mass probes, simultaneously detection of proteins and hundreds of endogenous metabolites at a single-cell level were achieved, promising in cancer classification and staging, and deep understanding fundamental biological processes, such as differentiation, aging and pathopoiesis. A novel, efficient on-line cell lysis strategy was proposed for the extension of the analysis time for each single cell, which was important for the acquisition of MS/MS data and more accurate metabolites identification. The largest number of identified metabolites (approximately 600) were achieved in single cells and fine subtyping of MCF-7 cells was first demonstrated. Based on the protein signal conversion and amplification through orthogonal exogenous enzymatic reaction, the simultaneous detection of intracellular protein and hundreds of metabolites at single-cell level were realized for the first time, promising for the quantification of the target protein and its functional mechanism study and downstream correlation with the metabolomic phenotype.

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Professor Andrew Ewing[1]
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Correlation of Cellular Measurements of Partial Exocytosis and Nano Vesicular Subcompartments Using NanoSIMS

Andrew Ewing 346 - Correlation of Cellular Measurements of Partial Exocytosis and Nano Vesicular Subcompartments Using NanoSIMS, Meeting Room 106, August 22, 2024, 15:25 - 15:44

Electrochemical evidence since about 2010 has supported the view that most exocytosis from cellular vesicles is partial in nature. The new electrochemical method, intracellular vesicle impact cytometry combined with amperometry has been a key part in this progression. There is a critical need to demonstrate this new concept of release with proven techniques. Quantitative approaches with NanoSIMS have been developed using the gel matrix used to fix the cells as a standard. These approaches can be used to quantitatively measure the content of single nanometer secretory vesicles in cells. The concept is to measure molecules in fixed samples after stimulation where molecular species can leave from (secretion) or enter (capture) vesicles undergoing exocytosis.

Methods

Correlative imaging with transmission electron microscopy and NanoSIMS imaging and dual stable isotopic labelling were used to study the cargo status of vesicles before and after exocytosis; demonstrating a measurable loss of transmitter in individual vesicles following stimulation due to partial release. NanoSIMS imaging of dopamine secretion and drug capture into vesicles quantified the fraction of messenger released and demonstrated drug entry during exocytosis. This provides definitive evidence of partial release and a means to introduce drug into cells. We then combined NanoSIMS imaging with spatial oversampling with transmission electron microscopy imaging to discern the compartments (dense core and halo) of large dense core vesicles in a model cell line to localise ^{13}C dopamine enrichment across nano vesicle domains.

Novel Aspect

NanoSIMS of nanometer subvesicular compartments used to understand and confirm partial exocytotic release from model nerve cells.

Preliminary Data or Plenary Speaker Abstract

We combined NanoSIMS imaging with spatial oversampling with transmission electron microscopy (TEM) imaging to discern the compartments (dense core and halo) of large dense core vesicles in a model cell line to localize ^{13}C dopamine enrichment across nano vesicle domains following 4–6 h of 150 µM ^{13}C L-3,4-dihydroxyphenylalanine incubation. In addition, the absolute concentrations of ^{13}C dopamine in distinct vesicle domains as well as in entire single vesicles were quantified and validated by comparison to the electrochemical data. We found concentrations of 87.5 mM, 16.0 mM and 39.5 mM for the dense core, halo and the whole vesicle, respectively, and the relative amounts of catecholamine in each vesicular compartment were the same as found via the electrochemical sensor. This approach adds to the potential of using combined TEM and NanoSIMS imaging to perform absolute quantification and directly measure the individual contents of nanometer-scale organelles correlating with electrochemical measurements.

Associate Professor David Bishop[1], Monique de Mello[1], Dr Mika Westerhausen[1], Dr Thomas Lockwood[1], Associate Professor Jonathan Wanagat[2]
[1]University Of Technology Sydney, [2]University of California

Quantitative, Multiplexed, Immuno-Mass Spectrometry Imaging Of The Dystrophin-Glycoprotein Complex

David Bishop 70 - Quantitative, Multiplexed, Immuno-Mass Spectrometry Imaging Of The Dystrophin Glycoprotein Complex, Meeting Room 106, August 22, 2024, 15:44 - 16:03

The dystrophin-glycoprotein complex (DGC) is a multi-component, trans-membrane complex in muscle fibres that is responsible for stability during contraction. Mutations to dystrophin result in muscular dystrophies such as Duchenne and Becker muscular dystrophies, which are characterised by an absence or decreased expression of dystrophin. Changes to dystrophin levels affects the other members of the DGC, however these are rarely investigated due to complexities in multiplexed quantitative analyses.
Immuno-mass spectrometry imaging (iMSI) is an emerging alternative for multiplexed, quantitative, spatial analysis of biomolecules. iMSI uses the specificity of metal-conjugated antibodies to a biomolecule, in combination with the sensitive, quantitative imaging of LA-ICP-MS. Here we describe the development of a multiplexed antibody panel to analyse the DGC by iMSI.

Methods

Antibodies were selected to analyse fifteen biomolecules in muscle fibres including major components of the DGC, and were conjugated with unique lanthanide isotopes using the Maxpar™ reagent. The conjugated antibodies were applied to human quadriceps tissues individually and multiplexed before quantitative analysis via LA-ICP-MS using external, matrix-matched calibration standards. Antibodies that exhibited a large quantitative loss when multiplexed were removed from the panel. The optimised antibody panel was then applied to healthy human quadriceps tissue and those obtained from a patient with Becker muscular dystrophy (BMD), k-means image segmentation was employed to remove null signal from the images, and the quantified changes in relative abundance of components of the DGC between healthy and diseased tissue were compared.

Novel Aspect

Immuno-mass spectrometry imaging identifies changes in the abundance of biomolecules hidden by bulk measurements.

Preliminary Data or Plenary Speaker Abstract

Analysis of the multiplexed panel identified five antibodies that were negatively impacted by the multiplexed application. The remaining antibodies were applied to histological sections from healthy and BMD tissues, and significant differences were observed in nine of the ten targets analysed. Converse to the expected results, dystrophin and other DGC members were increased in the BMD tissue. The image segmentation employed removed null values, therefore for a more direct comparison against bulk tissue homogenates used in Western blot or LC-MS, the average values were taken and showed the expected reduction in the abundance of dystrophin in BMD. These results highlight that bulk tissue measurements may mask true changes in abundance of biomolecules. This shows that multiplexed, quantitative localisation of the DGC components with iMSI improves on the non-quantitative or non-multiplexed analyses currently performed, and its use will enhance our understanding of muscle health, as well as inform therapeutic development.

Dr. Reuben Young[1], Dr. Rachelle Balez[1], Miss Tassiani Sarretto[1], Ms. Helena Anastacio[1], Dr. Shadrack Mutuku[1], Marcel Niehaus[2], Dr. Jens Soltwisch[3], Prof Lezanne Ooi[1], A/Prof Shane Ellis[1]
[1]Molecular Horizons and School of Chemical and Molecular Bioscience, University Of Wollongong, [2]Bruker Daltonics GmbH & Co. KG, [3]Institute of Hygiene, University of Münster

Exploring different post-ionisation techniques coupled with MALDI-MSI for single cell lipidomics of neurons and astrocytes

Reuben Young 401 - Exploring different post-ionisation techniques coupled with MALDI-MSI for single cell lipidomics of neurons and astrocytes, Meeting Room 106, August 22, 2024, 16:03 - 16:22

Characterising the lipid composition of cells is paramount to better understanding cell biology and disease. For example, neurons and glial cells, such as astrocytes, become dysfunctional with the onset of neurodegenerative diseases. However, the exact metabolic changes that these cells undergo remain largely unknown. One reason may be the presence of sub-populations with distinct lipidomic phenotypes which, when analysed through bulk analysis, are missed. Recently matrix-assisted laser desorption/ionisation mass spectrometry imaging (MALDI-MSI) has been shown to enable high throughput single cell lipidomic studies. However, conventional MALDI can be limited in the lipids it can detect from single cells. Here, we have utilised and compared MALDI-2 and plasma post-ionisation techniques for the single cell lipidome profiling of neurons and astrocytes.

Methods

iPSC-derived astrocytes and neurons were cultured on-slide, stained with fluorescent dyes and chemically fixed using 4% PFA. Additionally, astrocytes were treated with various inhibitors of lipid biosynthesis (cholesterol and reactive oxygen species). Sample slides underwent brightfield and fluorescence microscopy imaging using a Leica Thunder DMi8 prior to positive polarity MSI using either a MALDI-2 timsTOF Flex or a unique timsTOF Pro equipped with an atmospheric pressure-MALDI and plasma ionisation (SICRIT) source, at 5 and 10 µm pixel sizes, respectively. 2,5-dihydroxyacetaphenone was sublimed onto samples for analysis. Sample data was processed through Bruker SCiLS Lab software, which allowed for MSI processing, co-registration to microscopy images and statistical analysis.

Novel Aspect

Comparison of MALDI-MSI post-ionisation techniques to expand lipid coverage for single cell lipidomics.

Preliminary Data or Plenary Speaker Abstract

Microscopy and MSI data were co-registered to allow for demarcation of on- and off-cell areas. All images showed strong correlation for cell spacing and morphology, with minimal delocalisation of lipids from cells. In the analysis of neurons, MALDI-2 spectra were dominated by phosphatidylcholine (PC) lipids with lower intensity signals observed from phosphatidylethanolamine (PE) and sphingolipids such as hexosylceramides (HexCer) and ceramides (Cer). In comparison, plasma post-ionisation spectra were dominated by PE lipids with other significant signals arising from HexCer, Cer as well as cholesterol. Both techniques thus generated rich lipid profiles that each performed best for different complementary lipid species, including detection of many lipid species not well detected using conventional MALDI analysis of single cells.

To highlight the advantages of single cell MSI using plasma post-ionisation, human iPSC derived astrocytes were investigated. Plated astrocyte cell cultures were either left untreated (control) or were treated with various inhibitors of lipid biosynthesis. Using the co-registered optical images, single cell regions were manually drawn around individual cells and the mass spectral data from these regions was compared through probabilistic latent semantic analysis (pLSA). Statistical analysis of astrocytes solely from the control experiment revealed the presence of no less than two different sub-populations that differed in their lipid profile speciation and abundance. Similarly, pLSA was

conducted on untreated and treated cell MSI data and revealed discrete changes to cellular lipids, in particular sphingolipids such as Cer, HexCer and putative lyso-sphingomyelins, with high-statistical significance ($p < 0.001$).

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Mariachiara Squillaci[1], Michèle Jost[1], Thomas Brunner[1], Prof. Florian Stengel[1]
[1]University Of Konstanz

Single cell proteomics (SCP) analysis to study crosstalk dynamics of signaling complexes in extrinsic and intrinsic cell death pathways.

Mariachiara Squillaci 366 - Single cell proteomics (SCP) analysis to study crosstalk dynamics of signaling complexes in extrinsic and intrinsic cell death pathways, Meeting Room 106, August 22, 2024, 16:22 - 16:41

Since its discovery, apoptosis has been in the focus of cancer research and nowadays many therapies rely on induction of apoptosis to achieve cell death. So far apoptosis-inducing treatments include two very distinct options, either targeting the intrinsic (mitochondrial) pathway via small molecules or the extrinsic (receptors-mediated) pathway via recombinant proteins.
TRAIL-based therapies are clinically relevant in HCC (hepatocellular carcinoma). Despite being part of the TNF (tumor necrosis factor) family ligands, recombinant TRAIL showed little to no off-target effects, nevertheless resistance often occurs. Interestingly, sublethal doses of traditional chemotherapeutics and TRAIL result in synergistic induction of cell death, triggering mechanisms that bypass resistance. We present an SCP approach to study the underlying molecular processes of synergy and drug-resistance.

Methods

First, HCC cells are treated with chemotherapeutics and TRAIL, alone and in combination. TRAIL receptor DISC and BCL-2 specific interaction partners are enriched using tagged versions of TRAIL, TRAIL-R1, R2 and FADD, BCL-2, BCL-xL. Subsequent characterization and quantification of the interactome is achieved by mass spectrometry -based proteomics (LFQ-DIA).
Second, apoptotic and resistant cells after single and combined treatment are harvested and subsequently isolated using a CellenONE dispenser. One-pot sample preparation method combined with LFQ-DIA measurements is used to identify and quantify phenotype-specific proteome features.

Novel Aspect

SCP detection and quantification of proteins relevant for apoptosis and profiling of cell-to-cell variation in HCC.

Preliminary Data or Plenary Speaker Abstract

Previous studies1,2 show that both in HCC cells and in primary hepatocytes c-Jun kinase (JNK) and the BH3-only protein BIM (pro-apoptotic) have a critical role in TRAIL receptor-mediated sensitization of cells to chemotherapeutic drugs and FAS receptor activation. Accordingly, when BIM and BID are silenced, synergistic cell death induction by TRAIL together with chemotherapy ends up being toned down. These data strongly suggest that the parallel activation of different BH3-only proteins by TRAIL and chemotherapy affects the composition of the BCL-2 interactome and favors BAX/BAK-mediated MOMP (mitochondrial outer membrane permeabilization, hallmark of intrinsic pathway mediated apoptosis). Furthermore, TRAIL-R signalling not only enhances chemotherapy-induced mitochondrial apoptosis, but chemotherapeutic drugs appear to also modify TRAIL-R complex formation, resulting in increased caspase 8 activation, BID cleavage and eventually increased activation of the caspase cascade.
A preliminary single cell mass spectrometry study conducted in HeLa WT cells as a model system, allowed us to identify and quantify proteins that are involved both in pro-apoptotic pathways and in anti-apoptotic homeostasis, like BCL-2, BIM, BID, BCL2L13, FAS, MAPK-2 caspase 8 and cytochrome c (overall dataset CV of 13% and data completeness of roughly 75%.). Building on these results, we set out to address heterogeneity and protein dynamics in cancer cells.

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Dr. Nhu Phan[1]
[1]University Of Gothenburg

Targeted molecular imaging with correlative NanoSIMS: Applications to study protein organization and turnover in neuronal cells.

Nhu Phan 370 - Targeted molecular imaging with correlative NanoSIMS: Applications to study protein organization and turnover in neuronal cells, Meeting Room 106, August 22, 2024, 16:41 - 17:00

Biological molecules have been shown to exhibit specific cellular localizations, which relate closely to their functions. To understand the molecular mechanism of a cellular process, it is essential to obtain the spatial information of biomolecules and organelles in the cells, their turnover dynamics and activity. Secondary ion mass spectrometry (SIMS), with a high sensitivity, versatility, and spatial resolution, has been increasingly applied in biological research and neuroscience allowing the visualization of molecular localizations and turnover at the single cell and subcellular resolution. However, applications of SIMS alone is difficult for the biological interpretation due to its limitation in identifying specific cellular structures. Besides, information of large molecules, such as proteins, is lost due to the intense fragmentation, especially in NanoSIMS.

Methods

We resolved these issues using a correlative imaging approach with NanoSIMS and super-resolution stimulate emission depletion (STED) fluorescence microscopy to identify specific protein complexes in single cells. The approach was applied to characterize the protein turnover dynamics of stress granules in human neural progenitor cells (NPCs). Alternatively, we developed dual labelling probes, which are capable of binding to specific cellular proteins, allowing visualization by both fluorescence microscopy and NanoSIMS imaging.

Novel Aspect

Correlative imaging and developed labelling probes enable specific molecular imaging with SIMS extending to large biomolecules beyond metabolites and lipids.

Preliminary Data or Plenary Speaker Abstract

Correlating STED and NanoSIMS imaging, we identified individual stress granules (SGs) in NPCs by STED microscopy and determined their protein turnover by NanoSIMS under the conditions of cellular ER stress (using ER stressor Thapsigargin) and stress recovery. SGs are membraneless organelles formed during cell stress as a cellular defensive mechanism to protect important cellular translational and signalling materials. However, the molecular mechanism of SG assembly and disassembly, and how they affect the cell recovery is not fully known. We found that SGs assemble by recruiting the proteins from the cytoplasm that exist before stress. In addition, the ER stress causes significant protein turnover impairment which could remain longer than commonly expected stress recovery period.
We have developed labelling probes, each containing a fluorophore and an element that is easily ionized by SIMS, particularly fluorine, boron, or iodine. These probes were demonstrated to label specific cellular proteins via click chemistry or immunostaining, and to visualize the proteins' localizations by fluorescence microscopy and NanoSIMS. We also generated a probe containing gold nanoparticles conjugated to nanobodies for specific protein imaging with NanoSIMS at subcellular resolution.

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Dr Oliver Hale[1], Dr Emma Sisley[1], Dr James Hughes[1], **Prof Helen Cooper**[1]
[1]University Of Birmingham

Native ambient mass spectrometry imaging of proteins and their complexes

Keynote: Professor Helen Cooper University of Birmingham 138 - Native ambient mass spectrometry imaging of proteins and their complexes, Meeting Room 109, August 22, 2024, 15:00 - 15:25

Native ambient mass spectrometry (NAMS) combines native mass spectrometry, an established technique in structural biology, and ambient mass spectrometry, in which biological substrates such as thin tissue sections are analysed directly with little or no sample preparation. The combined benefits of NAMS for analysis of protein assemblies and protein-ligand complexes include measurement of accurate mass and stoichiometry, identification of both protein and non-covalently bound ligands, together with information on spatial distribution. Recent efforts using liquid extraction surface analysis (LESA) and nanospray desorption electrospray ionization (nano-DESI), have advanced NAMS for the analysis of fresh frozen issue, allowing the spatial distribution of protein assemblies to be mapped.

Methods

Brain tissue from transgenic mice expressing human SOD1G93A were supplied by Dr Richard Mead, University of Sheffield. Kidney, brain and liver tissue from vehicle-dosed Han-Wistar rats was supplied by Prof Richard Goodwin, AstraZeneca. Brain and kidney tissue sections of 10 µm thickness were thaw mounted onto glass slides. Tissue sections were either sampled by nanospray desorption electrospray ionisation (nano-DESI) or by liquid extraction surface analysis (LESA). The solvent system was 200 mM aqueous ammonium acetate + 0.125% C8E4. Mass spectrometry experiments were performed on an Orbitrap Eclipse mass spectrometer. Intact protein masses were determined by PTCR mass spectrometry. Protein identification was achieved by top-down mass spectrometry with fragmentation induced by higher-energy collision dissociation (HCD) or electron transfer dissociation (ETD).

Novel Aspect

Native ambient mass spectrometry imaging of proteins and their complexes in tissue

Preliminary Data or Plenary Speaker Abstract

In this presentation, recent developments in native ambient mass spectrometry imaging will be discussed, including approaches for the analysis of integral membrane proteins and membrane-associated proteins, improvements in sensitivity, integration of laser capture microdissection to aid protein identification, and applications in drug discovery and molecular pathology associated with neurodegenerative diseases.

Mengze Sun[1], Dr. Yoichi Otsuka[1], Katsuyuki Nagata[2], Hideo Shindou[2], Akiyoshi Hirayama[3], Michisato Toyoda[1]
[1]Graduate School of Science, Osaka University, [2]National Center for Global Health and Medicine Research Institute, [3]Faculty of Environment and Information Studies, Keio University

Mass Spectrometry Imaging of LPLAT8 Knockout Mouse Retina by Tapping-mode Scanning Probe Electrospray Ionization

Mengze Sun 161 - Mass Spectrometry Imaging of LPLAT8 Knockout Mouse Retina by Tapping-mode Scanning Probe Electrospray Ionization, Meeting Room 109, August 22, 2024, 15:25 - 15:44

In the retina, different types of cells form a multilayered structure in which light is converted into neural signals. Saturated phosphatidylcholine (PC) is primarily present in the segment layer of the retina where photoreceptor cells exist. Previous reports have shown that mice with a frameshift mutation and deficiency in the lysophospholipid acyltransferase 8 (LPLAT8) gene, which produces saturated PC, exhibit retinal degeneration. Understanding the distribution of lipids is necessary to clarify and elucidate the status of the retina degeneration. Tapping-mode scanning probe electrospray ionization (t-SPESI) is a technique for direct extraction-ionization of local regions of a sample using small amounts of solvent without sample pretreatment. In this study, mass spectrometry imaging (MSI) of mouse retinal sections was conducted using t-SPESI.

Methods

The retinas of wild-type (WT) and LPLAT8 knockout (KO) mice were sectioned at 10 µm thickness using a cryomicrotome and mounted on a glass slide. MeOH/DMF (1/1 v/v) was flowed through a capillary probe at 5 nL/min. The probe oscillation frequency was approximately 855 Hz, and the high voltage for ESI was +2.0 kV. The pixel pitch of the MSI was 5 µm. A polished probe (INCEMS Technologies), with a flow channel diameter of 5 µm, was used. The retinal ion images were obtained after normalization of the mass spectra by integrated ion intensities over the entire m/z range using IMAGEREVEAL (Shimadzu).

Novel Aspect

This is the first MSI result of spatial phospholipid imaging of LPLAT8-KO retinas using t-SPESI.

Preliminary Data or Plenary Speaker Abstract

Using t-SPESI, we achieved high spatial resolution imaging to clearly distinguish the different layers of the retina. By applying HE staining to retinal sections that had been subjected to MSI, we were able to correlate the cell layers of the retina with the distribution of lipids. PC 32:0, the main product of LPLAT8, mainly existed in the inner segment layer and outer nuclear layers (ISL, ONL). Compared to the WT mouse, the distribution of PC 32:0 showed a reduced thickness of the segment layer of the KO mouse retina. Meanwhile, PC 36:1, a monounsaturated PC, mainly existed on the inner side of the retina, and its distribution was similar in WT and KO mice. PC 44:12, a di-docosahexaenoic acid (DHA)-containing PC , primarily existed in the OSL, particularly in the region of photoreceptor cell discs. A decreased area of PC 44:12 was observed in KO mice.
It was also found that the averaged ion intensities of PC 32:0 and PC 44:12 in LPLAT8 KO mice were decreased compared to WT mice, especially in the OSL, ISL and ONL, where photoreceptor cells exist. On the other hand, for PC 36:1, which mainly existed in the outer and inner plexiform layer (OPL, IPL), the ion intensity of the WT mouse was similar to that of the KO mouse. These results were similar to those of the previous study. As previously reported, the loss of LPLAT8 results in inadequate supplementation of saturated PC and degradation of photoreceptor cells. As the number of photoreceptor cells decreases, DHA-containing PCs, which are also main phosplipids in OSL were decreases. The decrease in ion intensities of PC 32:0 and PC 44:12 in the OSL of KO mice retina could be due to apoptosis of photoreceptor cells.

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Dr. Jianing Wang[1], Mr. Chengyi Xie[1], Prof Zongwei Cai[1]
[1]Hong Kong Baptist University

Subcellular Resolution MALDI Mass Spectrometry Imaging of Lipids

Jianing Wang 127 - Subcellular Resolution MALDI Mass Spectrometry Imaging of Lipids, Meeting Room 109, August 22, 2024, 15:44 - 16:03

In biomedical research, molecular mass spectrometry imaging techniques, especially those based on Matrix-Assisted Laser Desorption/Ionization (MALDI), are becoming increasingly important. The advanced design of the optical system has successfully enhanced the resolution of MALDI imaging to 1.2 µm, with oversampling tested at 0.6 µm intervals. This advancement represents significant progress in technology. However, challenges persist in achieving high-quality matrix deposition at high resolution and maintaining system stability. Currently, the highest resolution available for commercial MALDI MS imaging systems is 5 µm. If unmodified commercial systems could achieve subcellular resolution, it would not only significantly enhance their application but also maintain high stability and usability.

Methods

A ten-fold (10x) expansion MS imaging method was developed. The samples were embedded in an expandable hydrogel and swollen in water to achieve near 10x expansion. The timsTOF flex MALDI-2 imaging mass spectrometer, equipped with a microGRID sample carrier, was utilized to perform MS imaging on these expanded samples, thereby achieving high spatial resolution.

Novel Aspect

This study achieves submicron lipid mass spectrometry imaging with unprecedented detail.

Preliminary Data or Plenary Speaker Abstract

We developed a tenfold (10x) expansion MS imaging technique that has enhanced the imaging resolution of unmodified commercial mass spectrometers to the sub-micrometer level, achieving an imaging resolution of 500 nm. This enhanced resolution allows for the visualization of tissue structures very similar to those observed with optical microscopes, enabling more precise analysis of the correlation between structure and function. Notably, this technology not only identifies individual neurons in mouse brain tissue but also successfully displays the dendritic structure of Purkinje cells with widths less than 1 µm. Additionally, the technology reveals subcellular structural details within single cells, opening new research opportunities to explore the biological functions of lipids at the subcellular level.

Nathan H Patterson[1], Wanqiu Zhang[1], Maria Jose Q Mantas[1], Phuong Thao Tran[1], Reza R Choubeh[1], Baruch Berger[1], Erin Seeley[2], Basant Gamal[3], Akshay Basi[4], Amir Jazaeri[3], Jared Burks[4], Samuel Mok[3], Sammy Ferri-Borgogno[3], Fang Xie[5], M. Reid Groseclose[5], Pedro Machado Almeida[6], **Alice Ly**[1], Peter Roelants[1], Thomas Moerman[1], Nico Verbeeck[1], Marc Claesen[1]
[1]Aspect Analytics NV, [2]Department of Chemistry, University of Texas at Austin, [3]Department of Gynecologic Oncology and Reproductive Medicine, The University of Texas MD Anderson Cancer Center, [4]Department of Leukemia, The University of Texas MD Anderson Cancer Center, [5]GSK, [6]Lunaphore Technologies SA

Weave: A software package for integrated spatial multi omics visualization and data analysis

Alice Ly 212 - Weave: A software package for integrated spatial multi omics visualization and data analysis, Meeting Room 109, August 22, 2024, 16:03 - 16:22

Spatial omics technologies enable molecular analyses directly from tissues and there is a growing demand for integration across various omics levels. For example, combining Mass Spectrometry Imaging (MSI) for lipids with Imaging Mass Cytometry (IMC), multiplexed immunofluorescence (mIF) for proteins, or spatially resolved transcriptomic assays (ST) delivers deeper insights and allows more conclusive findings.
Spatial-multi-omics data analysis is a daunting bioinformatics task: data is typically acquired on serial sections for technical reasons, at different spatial resolutions, and in a variety of data formats. This requires expertise across multiple domains to obtain the best results from each individual technology.
We present software designed to facilitate the seamless integration and joint visualization of different assays, as utilised across a variety of studies.

Methods

Mouse lymph node underwent IMC (Hyperion) and MALDI-IHC (rapifleX) using a 16-marker antibody panel. Frozen mouse brain sections underwent lipid MSI (timsTOF flex) and mIF with a 14-marker antibody panel (COMET). Human ovarian cancer samples underwent metabolite, glycan, and peptide MSI (timsTOF fleX), mIF (COMET), and ST (Visium). All sections were H&E-stained and digitised when possible.
A non-rigid registration pipeline between modalities was used with Gaussian weighted spatial matching to integrate across datasets and fuse each measurement in the “stack” of sections. A common coordinate system was established across the assays that permits direct comparison and downstream analysis of the integrated datasets, e.g. spatial correlation or distance based analysis between analytes, among others.

Novel Aspect

Novel software to organize and integrate data from different spatial omics assays, addressing the need for spatial multi-omics bioinformatics solutions.

Preliminary Data or Plenary Speaker Abstract

We present a suite of bioinformatics tools dedicated to enabling spatial multi-omics data analysis. The software provides efficient integration and joint visualization of readouts from different assays, along with multiple common downstream multimodal analysis pipelines. Our approach for spatial multi-omics data analysis consists of multiple steps. Prior to data analysis, metadata labeling of individual section data informs the creation of stacks and helps identify sequences of tissue sections where stack creation is feasible. In data analysis, first we provide accurate, non-rigid image registration (manual or automatic, where appropriate) to create a single coordinate system across serial sections and their measurements. Second, several data integration approaches are provided to create an integrated data structure across assays of different spatial resolutions. For example, applying Gaussian weighting to account for differences in spatial resolution when fusing ST (55

µm/px), MSI (10-20 µm/px) and mIF (<0.5 µm/px) datasets. Once coordinated, this data structure acts as a foundation for downstream joint data analysis and visualization. A web-based spatial multi-omics visualization tool allows remote, interactive browsing of the combined data, facilitating direct visual comparison of analytes across different omics technologies without distribution or installation of any software on the client side beyond a web-browser. Additionally, for assays in which data is acquired as regions of interest (e.g. IMC), readouts can be stitched together and overlaid onto reference images. Finally, we provide examples of downstream analysis, including spatial correlation between analytes across assays, multi-omics tissue segmentation and differential expression analysis. The broad applicability of our approach and the associated data visualizations are demonstrated via a number of spatial multi-omics use cases which combine MSI with complementary assays. Pursuing data management, analysis and visualization in the cloud helps non-experts to organize, analyze and communicate results in a centralized way.

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A/Prof Shane Ellis[1,2], Mr Andrej Grgic[1], Dr. Benjamin Bartels[1], Mr Alexandros Lekkas[3], Mr Elias Panagiotopoulos[3], Dr. Dimitris Papanastasiou[3], Prof. Dr. Ron M.a. Heeren[1]
[1]Maastricht University, [2]University of Wollongong, [3]Fasmatech Science & Technology

A MALDI-2-MSI source with transmission and reflective mode capabilities that exploits laminar gas flows of novel intermediate pressure ion guides

Shane Ellis 383 - A MALDI-2-MSI source with transmission and reflective mode capabilities that exploits laminar gas flows of novel intermediate pressure ion guides, Meeting Room 109, August 22, 2024, 16:22 - 16:41

As MALDI-MSI pushes spatial resolution towards the low-micron regime by using transmission-mode (t-MALDI) increasing demands are placed on developing new ion optical systems to ensure the limited amount of material is efficiently detected. Laser post-ionization (MALDI-2) improves the sensitivity of t-MALDI but requires elevated source pressure and thus suitable ion optics. We have developed a new ionization source based on novel intermediate pressure ion optics instead of a conventional ion funnel. In this new setup, ions from the MALDI/MALDI-2 plume are captured in a low-pressure flow of laminar gas generated inside a novel RF ion guide. We present the design and optimization of this new ionization source and evaluate its performance in both reflective and t-MALDI geometries for tissue imaging.

Methods

A OrbitrapTM Elite mass spectrometer (Thermo Fisher Scientific GmbH, Bremen, Germany) was heavily modified to include the novel t-MALDI-2 ionization source. The system enables both reflection and transmission mode geometries, the latter assisted by a 50x objective placed beneath the sample holder supported on a system of attocubes for precise positioning with nanometer resolution. A wavelength tunable OP laser (Ekspla NT230) was used for MALDI-2. The parameters of the new ionization source including sample positioning, rastering, sample visualization, laser triggering, and voltage settings are all controlled by software developed in C# language. Tissue samples were coated with matrix using sublimation. Data visualization was performed using Lipostar MSI (Molecular Horizon Srl, Italy).

Novel Aspect

First report of a mass spectrometry imaging ionization source with laminar gas flow for high ion transmission and sensitivity

Preliminary Data or Plenary Speaker Abstract

Extensive ion optics and computational fluid dynamics simulations were carried out to optimize the design of the ionization source in terms of the ion transfer efficiency of ions from the sample surface into the RF ion guide. A system of DC lenses was employed to enable the direct injection of ions produced by a single-step or two-step laser desorption ionization processes into downstream ion optics. The design is gas dynamically optimized to maintain laminar flow conditions and minimize ion losses driven by turbulent flows. Different pressure conditions were simulated and an optimum pressure regime in the range of 10 - 15 mbar was identified which allows lossless ion transfer from the sample surface to the RF ion guide. The higher-pressure regime has also placed new demands in downstream optics and in this new geometry, the S-lens configuration has been replaced by an ion funnel with an axial DC gradient.

The optical set-up made of a polarization-based beam splitter and a λ\2 wave plate enables supply of both reflective and transmission modes with light from a single laser (Explorer One 349-120, Spectra Physics, USA). Alongside 50x magnification, an infinity-corrected objective is used to facilitate diffraction limited laser focus in the transmission geometry with ablation craters ~2 µm in diameter obtained from matrix coated tissues

Experiments on mouse brain and kidney tissue have demonstrated high signal-to-noise detection of lipids, in both reflective and transmission operating mode, the later enabling laser ablation craters as low as ~2 µm in diameter). Following initial optimization using ESI and MALDI the system was coupled with MALDI-2 to further increase ion yields and lipid coverage. Using MALDI-2 S/N values >100 are obtained for lipids from single 2 µm pixels of mouse brain tissue.

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Jan Preisler[1], Antonin Bednarik[1], Marek Stiborek[1], Lenka Jindrichova[1], Vadym Prysiazhnyi[1], Michal Zalud[1], Viktor Kanicky[1], Barbora Adamova[1], Jarmila Navratilova[1,2], Jiri Kroupa[3], Pavel Houska[3]
[1]Masaryk University, [2]St. Anne's University Hospital, [3]Brno University of Technology

Digital Mass Spectrometry Imaging using Nanoparticle Tags

Jan Preisler 41 - Digital Mass Spectrometry Imaging using Nanoparticle Tags, Meeting Room 109, August 22, 2024, 16:41 - 17:00

The detection of single specific microscopic objects has always been a challenging subject. Conventional imaging methods employing selective tags, such as confocal fluorescence microscopy (CFM), imaging mass cytometry, or electron microscopy, are excellent tools for biological tissue imaging. Yet, they are unable to visualize single biomarker molecules or use more tags for multiplex imaging. Laser-assisted mass spectrometry methods offer imaging capabilities as well as chemical information to differentiate between tags. Here, we demonstrate the mass spectrometry imaging of individual gold and silver nanoparticles (NPs) using two ionization techniques: subatmospheric pressure laser desorption/ionization (SubAP LDI) and laser ablation inductively coupled plasma (LA ICP). The detection efficiency of the techniques is discussed, and imaging of an immunohistochemically labeled tissue is demonstrated.

Methods

LDI MSI was performed using a SubAP dual MALDI/ESI ion source (MassTech) and orbital trap (QExactive+, Thermo Fisher Scientific).
LA ICP MSI employed a laboratory-built 2940 nm laser ablation system connected to an ICP mass spectrometer (7900, Agilent). A 213 nm laser ablation system and confocal fluorescence microscope were used for reference.
Model samples were prepared by depositing gold or silver NP suspensions on Si, glass, or gelatin substrates using a piezoelectric dispenser. NP counts on Si were determined using an electron microscope.
Sections of 3D human colorectal carcinoma HT29 cell aggregates were labeled with primary biotinylated anti-Ki-67 monoclonal antibody and by streptavidin conjugates with 20 nm AuNPs or Alexa Fluor® 635 to image the proliferating marker, Ki-67 antigen.

Novel Aspect

Detection of individual nanoparticles using LDI and LA ICP MS allows digital imaging of single biomarker molecules on tissues.

Preliminary Data or Plenary Speaker Abstract

To assess the detection efficiency of individual NPs, a specific number of Au or AgNPs with diameters from 20 to 100 nm were deposited on glass slides, gelatin, and Si using a piezoelectric dispenser for SubAP LDI MSI, LA ICP MSI, and electron microscopy (the reference counting method), respectively.

Gas-phase reactions and ionization processes were utilized to enhance the signal and detect the released Au or Ag ions by SubAP LDI MSI. Individual NPs with a diameter of 40 nm or more could be detected at optimized ion injection time, laser pulse frequency, and pixel size. Using SEM as the reference method, 84% and 95% detection efficiencies were achieved for 100 nm AuNPs and 80 nm AgNPs on glass, respectively. On a porcine brain homogenate section, we detected ~30% of deposited 100 nm AuNPs with a signal intensity of (1.4 ± 0.4) thousand ion counts.

In LA ICP MSI, the 2940 nm laser ablation system featured a simple rectangular ablation cell with a washout time of ~70 ms. Intact AuNPs were desorbed by the IR laser during gelatin ablation and detected by the ICP mass spectrometer running in the single-particle (SP) detection mode. The detection efficiency of 20 nm AuNPs was 83%. A set of sections from the equatorial part of a single

3D cell aggregate with a labeled proliferating marker was imaged by SP IR LA ICP MSI and reference conventional UV LA ICP MSI and CFM. Substituting proportional detection of photons or Au ions (in CFM and UV LA ICP MSI) with NP counting reduced noise and background signal in SP IR LA ICP MSI compared to both reference techniques; individual AuNPs were detected with an accuracy of one AuNP on a pixel.

The financial support of the Czech Science Foundation 24-10924S is gratefully acknowledged.

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Dr. Magnus Palmblad[1]
[1]Leiden University Medical Center

Data Science in Mass Spectrometry: Fascinating Past, Exciting Present and Promising Future

Keynote: Associate Professor Magnus Palmblad Leiden University Medical Center 651 - Data Science in Mass Spectrometry: Fascinating Past, Exciting Present and Promising Future, Meeting Room 110, August 22, 2024, 15:00 - 15:25

The history of computing and the history of mass spectrometry are intertwined, going back at least to the work of Sibyl Rock at Consolidated Engineering Corporation (CEC) compiling the first computing manual for mass spectrometry 1946, and together with Clifford Berry developing the first, or one of the first, commercially available electronic computers, the analog CEC 30-103. This work was driven by the need to solve problems in mass spectrometry. Another intersection is the development of an early expert system, Dendral, by Joshua Lederberg and colleagues at Stanford, for identifying chemical structures from mass spectra, representing a major milestone not only in mass spectrometry, but also in the development of artificial intelligence (AI).

Methods

In recent years, machine learning, in particular deep neural networks trained on thousands or millions of spectra, has significantly improved our ability to identify peptides and small molecules by predicting their observability, chromatographic behavior and fragmentation in tandem mass spectrometry. This keynote will highlight some of these recent developments.

Deep learning models for chromatographic and fragmentation behavior were implemented in Python using TensorFlow and Keras, and compared with simpler regression models implemented in R. All models were trained on publicly available data.

Text mined annotations from the scientific literature were retrieved from Europe PMC using the Annotations API, and combined with predictions of log P from ALogPS 3.0 from OCHEM.

Novel Aspect

This keynote is a brief history of data science in mass spectrometry, with some recent applications and future outlook.

Preliminary Data or Plenary Speaker Abstract

The integration of machine learned models can already be used to generate realistic synthetic data. This has many potentially beneficial applications in system suitability testing, experimental design and method optimization, and providing ground truth for algorithm and computational workflow benchmarking. It also has some significant risks, which must be addressed.

Some of these models are available in tutorials from ProteomicsML (proteomicsml.org), a resource created specifically to lower the barrier for applying machine learning in proteomics.

Large language models able to learn from millions of documents describing mass spectrometers and mass spectrometry data may provide further assistance to human experts in data interpretation, resuming the pioneering work of the DCRT/CIS conversational AI from mass spectrometry search developed at NIH in the early 1970s. They are also likely to improve the named-entity recognition of mass spectrometry analytes from the literature, which in term improves training data for prediction of observability or quantifiability.

Finally, some recent work on using generative AI to explore data analysis workflows for mass spectrometry, built on ELIXIR resources bio.tools and the EDAM ontology. By building and running ensembles of workflows rather than one particular workflow, it may be possible to increase the confidence in biological interpretations, as well as learn something about the combined tools themselves.

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Emma Palm[1], Prof. Dr. Emma Schymanski[1]
[1]University Of Luxembourg

Identifying dead-end pesticide transformation products of potential concern

Emma Palm 221 - Identifying dead-end pesticide transformation products of potential concern, Meeting Room 110, August 22, 2024, 15:25 - 15:44

To address water contamination, identifying persistent, mobile, and toxic (PMT) chemicals, including their transformation products, is crucial for assessing environmental and human health risks. A major challenge in transformation product (TP) identification is the scarcity of public data. For example, among over 140,000 compounds in the global chemical inventory, only 1.3% have open TP data available. To combat this, models to predict TPs are usually applied on-the fly in non-target analysis workflows or stored in databases. Typically, only one or two generations of products are considered due to combinatorial explosion and calculation time, although many known reactions are multi-step. Consequently, a comprehensive database with multiple generations of predicted TPs is needed to identify problematic TPs resulting from longer reaction pathways.

Methods

Environmental TPs were predicted for a list of 321 pesticides detected in ground and surface waters in Europe and Australia using the “env” model in BioTransformer3.0 version 2023-05-23. The unique outputs of the predictions were then used as inputs for the next generation of predictions. This was repeated 4 times to obtain 5 “generations” of TPs. All outputs were stored in an SQL database to enable fast querying of the results.
“Dead-end” TPs, defined here as TPs for which no further TP could be predicted, were then identified. For these TPs, organic carbon partition coefficient (KOC), biodegradability, estrogen and androgen receptor activity were predicted using KOC, ReadyBiodegradable, CEAPP and CoMPARA modules in OPERA to estimate their mobility, persistence, and toxicity.

Novel Aspect

Multiple transformation prediction steps were performed on common pesticides, provided as a prototype database, to determine potentially concerning “dead-end” TPs.

Preliminary Data or Plenary Speaker Abstract

The dataset from five generations of BioTransformer predictions contained 175,659 unique TPs by InChIKey. The calculations took approximately 2 days on a laptop (intel i9 processor, 14 cores). Among the predicted TPs, 884 were identified as "dead-end," suggesting that most are intermediates, which may be less concerning from a persistence standpoint. Most dead-end products required multiple generations of predictions. For simazine, five dead-end products were not predicted until the 4th or 5th generation, four of which retain the triazine moiety. Since the role between “parent” and “TP” is not always distinct (some parents are also TPs and vice versa), creating a database of predicted TPs also allows for longer pathways than the number of prediction steps. In this dataset, the longest generated pathway resulting in a dead-end product was 13 generations for pentachlorobenzene transforming to 2,5-dioxopentanoate.
Several of the dead-end TPs were formed from multiple precursors. For example, 1,3,5-triazine-2,4-diamine was a predicted dead-end TP of 7 compounds including simazine, propazine, sebuthylazine and atrazine, generally formed after 3 generations. 1,3,5-Triazine-2,4-diamine was also predicted to be both persistent and mobile and the precursors were among the most common contaminants in both the EU and Australia. Another example is TFA, which was the predicted dead-end TP of benfluralin, trifluralin, flurtamone and trifloxysulfuron. This is especially noteworthy as TFA has been found to accumulate in some environmental compartments and may potentially result from a vast number of per and poly-fluorinated alkyl substances (PFAS).

Of the dead-end TPs, 235 could potentially be classified as mobile based on their predicted KOC values, while 372 were found to be potentially endocrine disrupting based on predictions.
This current work serves as a prototype to expand this database to the global chemical inventory within the project ZeroPM. The current version of the database can be found here: 10.5281/zenodo.10849343

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Mrs Sarah Bamford[1], Prof Paul Pigram[1], Dr Wil Gardner[1], Dr Benjamin Muir[2], Prof David Winkler[1], Assistant Professor Dilek Yalcin[3]
[1]La Trobe University, [2]CSIRO, [3]Ege University

Self-Organizing Maps with Relational Perspective Mapping (SOM-RPM) Applied to Time-of- Flight Secondary Ion Mass Spectrometry (ToF-SIMS) Data

Sarah Bamford 277 - Self-Organizing Maps with Relational Perspective Mapping (SOM-RPM) Applied to Time-of- Flight Secondary Ion Mass Spectrometry (ToF-SIMS) Data, Meeting Room 110, August 22, 2024, 15:44 - 16:03

Three dimensional (3D) ToF-SIMS depth profiles produce large and complex hyperspectral data sets. Interpretation requires that the complexity of these data sets is reduced. Individual ion peaks are often extracted and displayed in 3D or a handful of peaks are plotted in one dimension as a function of depth. This method works well for known or simple samples, however for complex or unknown samples or for those with important spatial information in the x-y plane, these methods struggle to convey the depth of information captured within the data set. Furthermore, the choice of displayed ion peaks has the potential to impart user bias and make a significant difference to the interpretation of results.

Methods

Data was collected on an IONTOF V ToF-SIMS instrument, using a 30 keV Bismuth primary ion beam and a sputtering beam (cesium, oxygen, or argon). Unsupervised machine learning, specifically self-organizing maps with relational perspective mapping (SOM-RPM), is employed to create a color-coded similarity map in which changes in color are specifically graded to accord with changes in molecular state. The complete 3D depth profile can then be visualized, providing a unique picture of the local and global mass spectral relationships between individual voxels.

Novel Aspect

Data visualization using color to describe chemical similarity captured using ToF-SIMS

Preliminary Data or Plenary Speaker Abstract

This work will present detailed studies of conducting polymer aerospace coatings and double silver low emissivity coatings, illustrating 3D depth profiles which consider the totality of the mass spectrum at every voxel. Using SOM-RPM to analyze the data yields an intuitive visualization of 3D depth profiles, highlights any structural flaws such as pinholes, illustrates the degree of interfacial mixing and allows for in depth spectral analysis of selected regions. The SOM-RPM methodology has proven to be a robust technique that offers a substantial advance in this field.

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Dr Mark Condina[1,2], Dr Anna Quaglieri[1], Mr Aaron Triantafyllidis[1], Mr Bradley Green[1], Mrs Paula Burton Ngov[1], Dr Giuseppe Infusini[1], Assoc. Professor Andrew Webb[1,3,4]
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Streamlining proteomics investigations for drug discovery using Mass Dynamics 2.0's Dataset Service

Mark Condina 397 - Streamlining proteomics investigations for drug discovery using Mass Dynamics 2.0's Dataset Service, Meeting Room 110, August 22, 2024, 16:03 – 16:22

The proliferation of complex proteomics experiments and the development of numerous new workflows tailored for computational biologists present significant challenges in generating meaningful biological results, especially for researcher teams with varying levels of computational expertise. Mass Dynamics 2.0 (MD 2.0) addresses this gap by offering an intuitive web and cloud-based platform that simplifies the adoption of existing and new methodologies for all researchers. We introduce here MD 2.0 Dataset Service, which allows for the straightforward incorporation and comparison of diverse scientific workflows through an accessible user interface designed for users at any computational skill level.

Methods
MD 2.0's Dataset Service revolutionizes the analysis and re-analysis process by encapsulating computational results, such as protein intensities or enrichment analyses, and enabling their integration with any scientific workflow. This is achieved through an intuitive interface, secure data storage for easy evaluation, and a modular visualization workspace that supports interactive exploration. Here we show the dataset service's ability to facilitate dose-response analysis implemented from the drc R package (Ritz et. al. 2015), comparisons between multiple normalization and imputation methods, and dimensionality reduction techniques.

Novel Aspect
MD 2.0 dataset service democratizes proteomics data analyses, enabling researchers to navigate studies efficiently in a highly interactive environment.

Preliminary Data or Plenary Speakers Abstract
This study illustrates the potential of the dataset service to accelerate proteomics investigations and support research in drug discovery. By leveraging the dataset service, the platform facilitates the rapid creation and interrogation of multiple dose-response analyses. Dose-response analyses are widely adopted in the drug discovery pipeline to screen the effect that several doses of compounds of interest have on protein intensities. The ability to automate and standardize the creation of several dose-response analyses and cohesively interrogate the results has the potential to streamline and speed up the process of target compound selection.

Utilizing a proteasome inhibitor dataset (Zecha J et al 2023), we demonstrate how to perform iterative quality control, normalization, and automated generation of dose-response datasets. The proteasome dataset has protein intensities from the Multiple Myeloma cell line RPMI 8226 treated with several doses of two drugs, bortezomib and carfilzomib, and collected at five different time points.

After upload and quality control, the distributions of the protein intensities are assessed using interactive modules, such as box and PCA plots, before and after applying different normalization and imputation methods. Subsequently, the creation of the ten dose-response datasets is triggered simultaneously using the new Dataset Service user interface, which automates sample selection for analyses using the available metadata (e.g. time and compound).

The results are visualized in several dose-response volcano plots summarizing the effect, using various statistics, such as the span, DMAX, EC50, and P-values. Proteins significantly affected by the treatment are prioritized via selection on volcano plots and simultaneously visualized in dose-response curves across all generated datasets.

Mgr. Natan Horacek[1,2], Ph.D Pavlína Kyjaková[2], Ph.D Robert Hanus[2]
[1]Faculty of Science, Charles University, [2]Institute of Organic Chemistry and Biochemistry, CAS

An automatic and unsupervised artificial peak detection approach for preprocessing GC-MS and GC×GC-MS metabolomic data.

Natan Horacek 113 - An automatic and unsupervised artificial peak detection approach for preprocessing GC-MS and GC×GC-MS metabolomic data, Meeting Room 110, August 22, 2024, 16:22 - 16:41

Comprehensive gas chromatography coupled with mass spectrometry (GC×GC-MS) is a powerful tool for metabolomic studies in a gas phase thanks to its sensitivity and separations efficiency. Yet, its inherent drawback is the detection of artificial chromatographic features. These are usually of three origins: peak splitting due to its size or shape, peaks originating from column or sorbent bleeding. Mass spectra of such features belongs to series of homologs or even to the same peak in both cases sharing high degree of similarity between each other. In metabolomics the manual curation of data represents a great workload calling for automated processing techniques for peak filtering. Here, we present a new algorithm for removal of the undesired features.

Methods

In the initial step, the individual mass spectra of detected peaks are compressed by autoencoder. Subsequently, the similarity among the compressed spectra is calculated and expressed as molecular network. Clusters are then defined in this network by Louvian community search. Within-group similarity is evaluated using mean group transitivity. Finaly, the manually optimized cutoff value then serves to exclude groups of artificial peaks.

Novel Aspect

Our unsupervised algorithm offers an convenient way of GC-MS and GC×GC-MS metabolomic data preprocessing prior to their statistical analysis.

Preliminary Data or Plenary Speaker Abstract

Whole workflow described above was written as a function in R and tested on three following datasets. First, the training dataset consisting of linear hydrocarbons (C9-C40), Supelco 37 mix of fatty acyl methyl esters, Grob mixture and lime oil applied on a cotton pad and sampled with dynamic headspace on thermal desorption tube and then desorbed with thermal desorption unit into the GC×GC-MS. Second, human armpit body odour sampled on a cotton pad and then desorbed in dynamic headspace and captured on a TDU tube. And third, hexane extract from the heads of termite soldiers rich in defensive compounds, injected through SSL injector into GC×GC-MS setup. All three datasets were processed by either ChromaTOF or ChromaTOF Tile commercial software. Using our impurity search algorithm on the testing dataset, we achieved 88% sensitivity and 97% selectivity. When used on the dataset of human body odour samples, we achieved sensitivity of 80% and selectivity of 85%. Finaly, testing the function on termite dataset we managed to detect 89% of peaks belonging to tailing peaks.

Dr Ove Johan Ragnar Gustafsson[1,2], Cameron John Hyde[3], Gareth Robert Price[3], Dr Matthew P. Padula[4], Wai Cheng Mike Thang[3], Dr Pawel Sadowski[5], Nigel Ward[1,6]
[1]Australian BioCommons, [2]University of Melbourne, [3]Queensland Cyber Infrastructure Foundation, University of Queensland, [4]School of Life Sciences, University of Technology Sydney, [5]Central Analytical Research Facility, Queensland University of Technology, [6]Research Computing Centre, University of Queensland

Proteomics Lab: streamlining computational proteomics for life scientists

Ove Johan Ragnar Gustafsson 384 - Proteomics Lab: streamlining computational proteomics for life scientists, Meeting Room 110, August 22, 2024, 16:41 - 17:00

Consultations with Australian researchers have established that computational proteomics is going through a major transition, where growth in experimental complexity and data set sizes, coupled with the challenges of navigating, understanding and redeploying software from a diverse and scattered ecosystem are now significant blockers to research programs. There is thus a demonstrable need to 1) accelerate the discovery and onboarding of proteomics researchers to both fit-for-purpose software and scalable computational resources, while 2) democratising the ability to access and reuse these same resources. Key to this is to also remove the need for researchers to worry about managing the underpinning research infrastructure that makes this possible.

Methods

To address the challenges facing computational proteomics in Australia, a working group consisting of members of the Australian proteomics community, the Australian BioCommons and Galaxy Australia has created a shared space on Galaxy Australia called Proteomics Lab (https://proteomics.usegalaxy.org.au/). This gives researchers a proteomics specific lens into the diverse capabilities accessible via Galaxy Australia, including numerous resources created by the Galaxy-P platform, curated to include tools, reference data and training of relevance to proteomics. The Proteomics Lab webpage is built into Galaxy Australia's Media site, which uses a Python framework to render the webpage from a structured data schema. This simplifies the curation and update of content that makes up the functionality of the page.

Novel Aspect

The provision of a national shared computational platform that is being tailored to the needs of Australian proteomics researchers.

Preliminary Data or Plenary Speaker Abstract

The Proteomics Lab (https://proteomics.usegalaxy.org.au/) provides a proteomics-centric tools list, and a central panel curated in consultation with community members. The central panel highlights critical software (including workflows), in-built reference data, and help information (e.g. Galaxy Training Network tutorials), all supported by an on-going collaborative effort to optimise the installed proteomics software using real world data. For example, collaboration with community researchers has allowed Galaxy maintainers to tailor MaxQuant to access more computational resources and accommodate analysis of more typical sample set sizes (n = 120 is the current maximum possible), and in the past six months MaxQuant on Galaxy Australia has supported hundreds of jobs, submitted by researchers from 13 different Australian research institutions. In addition, users can currently access more than 90 additional proteomics software tools, supported by 32 tutorials created by the global Galaxy Training Network. We invite the life science research community to use Proteomics Lab, and to help us to grow the number of optimised tools, workflows and tutorials available. Collectively, we can ensure that the service continues to be fit-for-purpose and can support best practice implementation of proteomics in Australia.

FRIDAY

Distinguished Keynote Speaker – Inventions and Innovation do not occur in a vacuum

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Mr Koichi Tanaka[1]
[1]Executive Research Fellow, Shimadzu Corporation

Mass spectrometry (MS) is a complex process involving sample preparation, ionization and mass separation in a vacuum, detection, measurement, and data analysis. Over the years, numerous methods have been invented to enhance each step of this process. With over a century of advancements since its initial invention, MS has played a pivotal role in advancing various academic and technical fields such as medicine, pharmacy, biology, chemistry, and engineering. Conversely, MS has also experienced substantial growth and expansion, leading to significant improvements in analytical method performance and a broader range of applications. In essence, MS has continually fostered innovation through mutual exchanges.
In this lecture, I will reflect on the history of MS inventions since the 1980s and discuss key points that can serve as references for future developments in this field.

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Conner Harper[1], Zachary Miller[1], Jacob Jordan[1], Ms. Veena Avadhani[1], Mr. Matthew McPartlan[1], **Professor Evan Williams**[1]
[1]University Of California, Berkeley

Charge Detection Mass Spectrometry: Mass and Conformation of Really Big Things

Keynote: Professor Evan Williams University of California, Berkeley 276 - Charge Detection Mass Spectrometry: Mass and Conformation of Really Big Things, Plenary 3, August 23, 2024, 09:30 - 09:55

Heterogeneous mixtures of high mass molecules or molecular complexes can be challenging to analyze with conventional mass spectrometers due to ions with overlapping m/z values from many components and from adduction from salts or other non-specific molecular interactions. One demonstrated solution to this problem of sample heterogeneity for high mass analytes is to measure the charge as well of the m/z of individual ions so that the mass of each ion can be determined without interference from other ions. Single ion mass measurements have been performed with a variety of instrument types, but charge detection mass spectrometry with electrostatic ion traps has the advantage of virtually unlimited mass range, single charge accuracy, and the ability to make dynamic measurements.

Methods

Experiments are performed using a custom-built charge detection mass spectrometer designed to simultaneously weigh multiple individual ions over a broad range of ion energies. Ions are formed by electrospray using borosilicate capillaries with a range of tip sizes from 600 nm to 20 µm. Many ions are trapped in an electrostatic trap for periods of time that can range from a few milliseconds to many seconds. Short-time Fourier transforms are used to track the fundamental frequencies of ion motion, signal amplitude and signal duty cycle continuously throughout the entire time the ions are trapped. These data are used to obtain the energy, mass and charge of each ion.

Novel Aspect

Charge detection mass spectrometry overcomes heterogeneity enabling detailed analysis of viruses, lipid nanoparticles, synthetic nanoparticles, charged droplets and aggregating proteins

Preliminary Data or Plenary Speaker Abstract

Applications of charge detection mass spectrometry to a wide variety of large analytes will be presented, with masses ranging from a few 10's of kDa to over 1 GDa. Aggregation of protein-based therapeutics, including antibodies, can occur during development, production, or storage and can lead to the loss of efficacy and potential toxicity. CDMS can resolve individual higher order aggregates at high mass and provides detailed information about the conformational heterogeneity of these aggregates. The aggregation pathways for fibril-forming proteins and effects of small molecule inhibitors can be readily investigated in a size range that has not been possible previously. Results with adeno-associated viruses (AAVs) show that the stabilities and chemistry of these viruses differs in ammonium acetate buffers compared to a 1x phosphate buffer containing an additional 200 mM additional sodium chloride. These results demonstrate the importance of properly sizing the tip of electrospray emitters to form ions directly from biochemically relevant storage or formulation buffers. Analysis of lipid nanoparticles is challenging due to their size and their heterogeneity. Results comparing cryoTEM and CDMS data will be presented, and the relative merits of these techniques discussed. With dynamic measurements in CDMS, information about ions that change in energy, mass and/or charge during the time that they are trapped in the electrostatic ion trap can be

obtained. This makes it possible to increase the multiplexing capabilities for rapid kinetics of nanoparticle assembly. These dynamic measurements make it possible to extract detailed information about fission dynamics of aqueous nanodrops with diameters between 10 and 120+ nm (GDa). Methodology for analyzing these complex data along with a description of the wide range of fission processes that have been observed will be presented.

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Dr Hamish Stewart[1], Dr Anastassios Giannakopulos[1], Dr Dmitry Grinfeld[1], Professor Alexander Makarov[1], Dr Christian Hock[1]
[1]Thermo Fisher Scientifc

Behind the Curtain: The Long Development of Next Generation HRAM Analyzers.

Hamish Stewart 62 - Behind the Curtain: The Long Development of Next Generation HRAM Analyzers,
Plenary 3, August 23, 2024, 09:55 - 10:14

The invention of the ion mirror enabled time-of-flight (ToF) mass analyzers to reach high resolution. Multi-reflection analyzers further extend the flight path of ions by passing them repeatedly between ion mirrors, enhancing resolving power and relieving the painful focal constraints of prior ToF designs. However, the technological challenge is enormous, and many competing analyzer concepts developed, scrapped, and buried.

Realizing the potential, the Thermo Scientific™ Orbitrap Astral™ mass spectrometer delivers a generational leap in sensitivity and speed, with 200 Hz operation, extremely high ion transmission and single ion sensitivity. It was built upon decades of hidden technological developments, including several distinct analyser concepts. This secret history is explained in the context of advances in the broader HRAM mass spectrometry field.

Methods

A series of next generation mass analyzer concepts were envisaged, of which three were constructed, whereby ions oscillated between a pair of ion mirrors, either cylindrical, asymmetric (Thermo Scientific Astral™ analyzer), or shaped to generate an Orbitrap™-like quadro-logarithmic potential. The first of these operated in a “closed trap” arrangement, with ions admitted or ejected via a switched deflector, while the latter two incorporated a fixed multi-reflection ion track. The mass analyzers were evaluated by a variety of measurements with electrosprayed calibration solution, measuring performance in terms of resolving power, space charge tolerance, ion transmission and spectral artifacts.

Novel Aspect

Introduction and evaluation of multiple previously unreported high-resolution accurate-mass analyzer designs.

Preliminary Data or Plenary Speaker Abstract

All three analyzers proved capable of reaching impressive resolving power, >70k, though shortfalls in other factors were notable for two of the designs. The closed trap analyzer suffered substantial space charge influence due to the focused ion packet, which compromised its intended usage as a high resolution mass filter for MS/MS analysis. Furthermore, closed trap analyzers suffer fundamental limitations in mass range as light ions overtake heavy ions and cause ambiguity in m/z assignment.

The quadro-logarithmic system had difficulty combining ion transmission with high resolution, caused by a limitation in the design of its injection optics, as well as a significant proportion of ions that escaped the bonds of its spatial focusing lenses and reach the detector at the wrong number of oscillations. This shortfall was considered addressable via constructing a larger analyzer that could accommodate direct injection from a pulsed extraction trap. The most promising concept was the competing Astral analyzer, which had already leapfrogged the others in performance, and was adopted for further development. The development and optimization of the Astral analyzer and deployment into a commercial instrument, the Orbitrap Astral mass spectrometer, produces record results in a range of general proteomics, metabolomics, and lipidomics applications.

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Critical factors determining the performance of linear time-of-flight mass spectrometry – theoretical study and experimental validation

Yi-Sheng Wang - 745- Critical factors determining the performance of linear time-of-flight mass spectrometry – theoretical study and experimental validation

Linear time-of-flight (TOF) mass spectrometry (MS) is commonly regarded as a simple technique with limited applications. The limitation is mainly due to its poor mass resolution in comparison to other modern high-resolution MS techniques. This presentation discusses critical factors determining the mass resolving power of linear TOFMS when it is equipped with a matrix-assisted laser desorption/ionization (MALDI) ion source. The study includes comprehensive theoretical and big-data analysis. Experimental validation showed that the predicted instrument parameters are reasonably accurate.

Methods

The study was conducted using a comprehensive calculation model we developed in a series of publications (J. Am. Soc. Mass Spectrom., 2015, 26 (10), 1722-1731; J. Mass Spectrum., 2018, 53 (4), 361-368; Int. J. Mass Spectrum., 2022, 471, 116756; Int. J. Mass Spectrum., 2023, 489, 117052.) taking into account the detailed dimensions and voltages in every section of the instrument, the extraction delay, and ions' m/z ratios. We have developed calculation tools to rapidly find the recommended instrument parameters to achieve the best performance. Experimental validations were conducted using several instruments, including two laboratory-made instruments and a commercial mass spectrometer.

Novel Aspect

Comprehensive calculation improves linear TOFMS performance and provides an important basis for development of novel high-resolution MS

Preliminary Data or Plenary Speakers Abstract

The results of big-data analyses revealed many previously unknown facts about TOFMS, including the impact of initial velocity and spatial spread of ions, the detector's response limit, and the fringe electric field on the mass resolving power. Significant findings include that longer flight tubes do not necessarily provide higher resolution, the important role of the ion acceleration region in MALDI ion source, and the scaling properties of TOF instruments. The prediction showed that the design of conventional instruments cannot deliver a satisfactory performance. Experimental data obtained using a 3-meter-long laboratory-made instrument optimized for the low-mass range indicated that the calculation results were reasonably accurate. It can deliver a mass resolving power of above 20,000, and the differences in the predicted best extraction delay from the observation for ions in the range of m/z 300 - 1,300 were 1 - 15%. The calculation also showed that a fully-optimized miniature linear TOF mass spectrometer can provide higher mass resolving power in the high m/z range than most modern high-resolution MS instruments. Experimental evidences were demonstrated by comparing the results of a 50-cm-long instrument with a Bruker Ultraflex II system. The calculation method can also apply to commercial instruments with classical electrode design, such as Voyager series from ABI. We have summarized the results into principles for designing linear TOF mass spectrometers of any size.

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Miss Taous ABAR[1], Mister Michel Heninger[1], Mister Joel Lemaire[1], Mrs Hélène Mestdagh[1]
[1]Institut de Chimie Physique (ICP), université Paris Saclay

Analysis of VOCs in aqueous solutions using the coupling of an oven with a mobile FT-ICR-MS associated to chemical ionization

Taous Abar 20 - Analysis of VOCs in aqueous solutions using the coupling of an oven with a mobile FT-ICR-MS associated to chemical ionization, Plenary 3, August 23, 2024, 10:33 - 11:00

Mobile FT-ICR-MS based on permanent magnet associated with chemical ionization allows real-time detection of VOCs present in gaseous samples. The high resolution allows us to identify the molecular formula of the detected ions. The concentration of the VOCs in the gas phase is deduced from the mass spectra intensities and the pressure measurements.
We present here their application to the detection of VOCs in trace amounts in small liquid samples, using a tubular oven to vaporize the liquid. One of the possible applications of such a device is to be able to analyze VOC mixtures present in biological liquids such as sweat, and to identify VOC biomarkers of pathologies.

Methods

The method developed is based on coupling a tubular oven to a mobile FT-ICR associated to chemical ionization. Production of the precursor ions, ionization of the sample to analyze, and detection are performed sequentially in the ICR cell. The oven ensures the complete vaporization of an aqueous solution containing VOCs in trace amounts.
A water pulse is used to produce precursor ions H3O+ in the mass spectrometer. A stream of dinitrogen carries the gases produced by the vaporization. Part of the gaseous stream is introduced as a 10-5 mbar short pulse in the ICR cell. The VOCs are then ionized by proton transfer from H3O+, and the product ions are detected. A mass spectrum is obtained every 2.6 s.

Novel Aspect

Our study shows how coupling a tubular oven to a mobile CI-FT-ICR enables the analysis of VOCs in aqueous solutions.

Preliminary Data or Plenary Speaker Abstract

The tubular oven and the mobile FT-ICR were successfully coupled, and the operating parameters of this new experimental set-up were optimized. These parameters include the heating program of samples, temperature ramp, flow rate of N2 carrier gas, and analyzed volume (0.1 to 1 mL).
The optimization of the coupling was done using an aqueous toluene solution. The intensity of the protonated toluene concentration integrated over the vaporization time was proportional to the initial concentration of toluene in the sample solution. We determined a Limit Of Detection (LOD) for toluene of 18 ppm.
To test the applicability of the optimized analytical method, we analyzed two VOC mixtures: one containing 7 VOCs with different molecular weights and a more complex mixture of 15 VOCs also containing isomeric compounds. The method shows a good ability to separate compounds of complex mixtures. The response of the coupling for the analyzed VOCs is linear, and LODs range from 5 to 50 ppm. A comparison will be made with GC results.

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Joachim Kopka
[1]Max-Planck Institute of Molecular Plant Physiology

GCMS-based 13C-positional isotopologue analysis for investigating photosynthetic carbon assimilation fluxes

Keynote: Dr Joachim Kopka Max-Planck-Institute of Molecular Plant Physiology (MPIMP) 697 - GCMS-based 13C-positional isotopologue analysis for investigating photosynthetic carbon assimilation fluxes, Meeting Room 105, August 23, 2024, 09:30 - 09:55

GC-MS technology in contrast to most other MS-technologies induces abundant in source fragmentation where fragments can represent different metabolite substructures. This phenomenon, while potentially limiting compound de novo identification, enables measurements of stable isotope enrichment at substructures of known metabolites and has the potential to resolve even single C- or N-atoms within a molecule. Current developments of 13C-positional analyses extend biochemical in vitro studies of enzyme activities towards in vivo measurements of carbon assimilation. We provide an alternative to 13C-MNR technology that so far has been the technological solution for positional 13C analyses. GC-MS technology allows positional labeling analyses of compounds within highly complex metabolite fractions at low metabolite concentrations but can be limited by compound specific in-source fragmentation reactions.

Methods

- Dynamic photosynthetic stable isotope labeling
- Gas Chromatography-Electron impact Ionization-Time Of Flight-Mass Spectrometry (GC-EI-TOFMS)
- Gas Chromatography-Atmospheric Pressure Chemical Ionization-high resolution Time Of Flight-Mass Spectrometry (GC-APCI-TOFMS)

Novel Aspect

Quantitative measurement of 13C-positional carbon assimilation rates into central metabolites by dynamic photosynthetic labeling and in source fragmentation GC-MS analysis.

Preliminary Data or Plenary Speaker Abstract

Photoautotrophic organisms fix inorganic carbon (Ci) by two enzymes, ribulose-1,5-bisphosphate carboxylase/oxygenase (RUBISCO) and phosphoenolpyruvate carboxylase (PEPC). RUBISCO assimilates Ci (CO_2) into the 1-C position of 3-phosphoglycerate (3PGA). The Calvin-Benson-Basham (CBB) cycle redistributes fixed carbon atoms into 2,3-C2 of the same molecule. PEPC uses phosphoenolpyruvate (PEP) derived from 3PGA and assimilates Ci (HCO_3^-) into 4-C of oxaloacetate (OAA). 1,2,3-C3 of OAA and of its transaminase product aspartate originate from 1,2,3-C3 of 3PGA. Positional isotopologue analysis of aspartate, the main product of OAA in the model cyanobacterium Synechocystis sp. PCC 6803 (Synechocystis), allows differentiation between PEPC, RUBISCO, and CBB cycle activities within one molecule. In source fragmentation of GC-EI-TOFMS at nominal mass resolution and GC-APCI-TOFMS at high mass resolution was explored using chemical derivatization by N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) or by N-methyl-N-(tert.-butyldimethylsilyl)trifluoroacetamide (MBDSTFA).
The analyses enabled the determination of fractional 13C enrichment (E13C) at each carbon position of aspartate. The method was validated by 13C-isotopomer mixtures of positional labeled aspartic acid. Combination with dynamic $13CO_2$ labelling of Synechocystis cultures allowed measurements of PEPC activity in vivo alongside analyses of RUBISCO and CBB cycle activities. Accurate quantification of aspartate concentration and positional fractional carbon enrichment (E13C) provided molar Ci assimilation rates during the day and night phases of photoautotrophic Synechocystis cultures. The

validated method offers several applications to characterize the photosynthetic Ci fixation in different organisms (bioRxiv preprint at https://doi.org/10.1101/2024.05.07.592938).

Dr River Pachulicz[1], Dr Blagojce Jovcevski[1], Prof Vincent Bulone[2], Prof Tara Pukala[1]
[1]University Of Adelaide, [2]Flinders University

Acid-catalysed esterification of anthocyanin glucosyl units in Brassica oleracea

River Pachulicz 42 - Acid-catalysed esterification of anthocyanin glucosyl units in Brassica oleracea, Meeting Room 105, August 23, 2024, 09:55 - 10:14

Anthocyanins are ubiquitous plant pigments that impart red, blue, and purple hues to plant tissues. Demonstrated to exert diverse bioactive effects in vitro and in vivo, anthocyanins are widely utilised as natural food colorants and nutraceuticals. Anthocyanins display immense structural heterogeneity between species, and, in some cases, within the same plant, due to the large number of unique glycosyl and acyl groups that modify anthocyanin structures. To add to this complexity, there is evidence that anthocyanins are modified by organic acids during storage and analysis, an often-neglected fact that has important implications for food formulations and analytical workflows. Understanding the influence of organic acids on anthocyanin structures during storage will inform best practice for anthocyanin handling, analysis and downstream applications.

Methods

A HPLC-QTOF-MS workflow was employed to first investigate the modification of a common anthocyanin standard, cyanidin-3-glucoside by formic, acetic, and citric acid under different pH ranges. To extend this to a biological sample, red cabbage (Brassica oleracea) extract was incubated with formic acid for 6 months and the resultant formylated species, including their MS and MS/MS fragmentation patterns, are characterised.

Novel Aspect

Anthocyanin glucosyl units are readily modified by formic acid to form various isomeric mono and poly-formylated species.

Preliminary Data or Plenary Speaker Abstract

The modification of cyanidin-3-glucoside was found to be acid-catalysed, with formic acid showing the greatest extent of anthocyanin modification in comparison to acetic and citric acid, likely due to its greater steric accessibility. Furthermore, a 7-week time course to monitor cyanidin-3-glucoside formylation was conducted, with roughly 0.7% modification measured after 7 weeks. Next, red cabbage (Brassica oleracea) extract was incubated with formic acid for 6 months under typical storage conditions, with extensive formylation of anthocyanins observed. From 9 naturally occurring anthocyanin, 53 unique structures could be characterised following incubation with formic acid, with this stark increase due to the presence of isomeric mono and poly-formylated species. Differential formylation patterns for isomeric anthocyanins were identified, with these determined to result from the varying position of the hydroxycinnamic acid acyl group in their structure. Key structural determinants to predict the formylation patterns of red cabbage anthocyanins were finally established, which are highlighted to aid in future structural determination.

Dr. Melanie Odenkirk[1], Arpana Vaniya[2], Margaret Read[1], Cole Michel[3], Katrina Doenges[3], Jacqueline Chaparro[1,4], Susan B. Mitchell[1], Nathan Montgomery[4], Corey Broeckling[4], Sarah Brinkley[5], Katrina Leaptrot[6], Stacy Sherrod[6], Jody May[6], Juliana Chaura[7], Gabriel Velez Mejia[7], Ric de Vos[8], Oliver Fiehn[2], Richard Reisdorph[3], Nichole Reisdorph[3], John McLean[6], Andres Jaramillo-Botero[7,9], Robert Hall[8], Chi-Ming Chien[10], Tracy Shafizaheh[10], Jessica Prenni[1], Steve Watkins[10]
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Juicy Insights: A Standardized, Nontargeted Metabolomics Approach to Facilitate the Comparison of Apples to Apples Across the Globe

Melanie Odenkirk 546 - Juicy Insights: A Standardized, Nontargeted Metabolomics Approach to Facilitate the Comparison of Apples to ApplesAcross the Globe, Meeting Room 105, August 23, 2024, 10:14 - 10:33

Small molecules are essential contributors to both the nutritional content and flavor profiles of food. The advancement of our understanding of food composition heavily relies on high-throughput methods and techniques for characterizing these molecules. However, despite individual investigations into how variable practices of extracting, measuring, and annotating compounds bias metabolic results, niche metabolomics methods remain incredibly common across laboratories. In this context, we explore the ramifications of this prevailing landscape of metabolomics for characterizing food within and across three established laboratories. This work is juxtaposed by a standardized, nontargeted metabolomics workflow and custom internal retention time standard (IRTS) mix developed by the Periodic Table of Food Initiative (PTFI) to reproducibly survey the small molecule composition of food across laboratories.

Methods

Two separate experiments were conducted to (1) ascertain the comparability of results from variable nontargeted metabolomics workflows, and (2) to illustrate how standardized methodology and reagents enhance result agreement. The first experiment was completed by three laboratories (two academic and one commercial) following in-house methodologies for extraction, reversed-phase LC-MS data acquisition, and data processing. This was completed with nine foods (in duplicate) to assess intra- and inter-result reproducibility. Conversely, validation of the standardized, nontargeted metabolomics method developed by the PTFI was completed on six identical foods by five laboratories across three continents. In addition to global representation, a wide range of instrumentation (four LC-QTOF instruments from three vendors (Waters, Shimadzu, and Agilent) and one LC-Orbitrap FTMS system (Thermo)) was included.

Novel Aspect

We present a standardized, nontargeted metabolomics strategy for food characterization that enables the generation of comparable data across laboratories.

Preliminary Data or Plenary Speaker Abstract

Our landscape assessment aimed to evaluate both intra- and inter-laboratory agreement of the measured small molecule composition of food using variable, in-house metabolomics methods. Initially, within labs we observed average correlation values of 98%, indicating that each lab was capable of reproducibly characterizing the small molecule composition of nine unique foods. However, across labs, our assessment of overlapping coverage across foods was minimal, with an

average of only 3 formula being observed across all three labs. To mitigate methodological and data processing biases, we next performed an experiment where six foods in duplicate were analyzed by five laboratories across three continents using a standardized extraction, acquisition, and data processing protocol. From standardization alone, we observed several thousand unique formula across labs. However, inconsistencies in retention times as large as +/-25 seconds across laboratories precluded further alignment of data. To circumvent this issue, a standardized methodology was adopted with IRTS containing 34 non-endogenous compounds with elution times spanning the entire chromatographic separation. This resulted in a reduction of cross-laboratory retention time drift to less than 5 seconds. Following the retention time alignment of data, qualitative coverage of small molecule composition across each method was compared. The overlapping formula assignments across labs improved significantly relative to the overlap of results from variable, in-house methodologies. Unique formula assignments across each lab were also common, likely due to instrumentation biases such as in-source fragmentation. Collectively, the application of a standardized, nontargeted metabolomics method, and the incorporation of data alignment with IRTS allowed for significantly improved agreement of data and demonstrates the potential of the PTFI's standardized, methodological approach for the generation of comparable food composition data by multiple laboratories and instruments.

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Prof. Laurent Bigler[1], Dr. Karoline Rehm[1], Luca Bürgi[1], Daphné Golaz[3], Dr. Vera Vollenweider[2], Prof. Gabriella Pessi[3], Prof. Rolf Kuemmerli[2]
[1]University of Zurich, Dept. of Chemistry, [2]University of Zurich, Dept. of Quan. Biomedicine, [3]University of Zurich, Dept. of Plant & Microbial Biology

Structure elucidation of iron chelators produced by microorganisms

Laurent Bigler 297 - Structure elucidation of iron chelators produced by microorganisms, Meeting Room 105, August 23, 2024, 10:33 - 11:00

Most bacteria growing in low iron environments secrete iron-chelating molecules, called siderophores, to scavenge extracellular iron and reabsorb it through membrane transporters. They are specific to each bacterial species and their structural elucidation and characterization require comprehensive analytical methods as bacterial extracts are complex mixtures. Siderophores have great potential in the fields of medicine, industry, and agriculture. For example, their ability to protect plants from pathogen infections or bacterial communities from pathogen invasion was recently demonstrated. To date, several hundreds of chemically distinct siderophores have been characterized.
In our research, we aimed to develop a universal high-throughput pipeline, that is comprehensive and easy to replicate.

Methods

We present a high-throughput UHPLC-MS/MS pipeline and the application of ion mobility spectrometry to facilitate annotation of known and structure elucidation of unknown siderophores. The second part consists of the purification and full structure elucidation by NMR and MS/MS of new siderophores involved in plant protection.

Novel Aspect

Workflow based on UHPLC, HR-MS/MS, and ion mobility to identify known and elucidate the structure of novel siderophores

Preliminary Data or Plenary Speaker Abstract

The high-throughput structural elucidation workflow was validated using 17 different pyoverdine extracts. Although bacterial extracts are complex mixtures, all ion fragmentation (AIF) and/or broadband collision-induced dissociation (bbCID) can be applied to identify pyoverdines or their biological precursor, ferribactin. We also investigated the collision cross-section (CCS) values of apo- and ferripyoverdines by trapped ion mobility spectrometry (TIMS). These CCS values were highly characteristic and demonstrated to be suitable as an alternative identification marker, replacing other analytical methods.
Finally, we discover derivatives of chryseochelin, new citrate-based siderophores secreted by three Chryseobacterium strains involved in plant protection, and phymabactin, an inhibitor of soil bacteria including pathogenic strains.

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Dr. Haofei Zhang[1]
[1]University Of California, Riverside

Understanding organic peroxide formation during multiphase oxidation of organic aerosols using mass spectrometry

Keynote: Professor Haofei Zhang University of California, Riverside 741 - Understanding organic peroxide formation during multiphase oxidation of organic aerosols using mass spectrometry, Meeting Room 106, August 23, 2024, 09:30 - 09:55

Organic aerosols (OA) are ubiquitously present in the Earth's atmosphere and greatly contribute to fine particulate matter. During OA particles' lifetime in the atmosphere, multiphase oxidative aging by gaseous oxidants such as hydroxyl radicals (OH) occurs ubiquitously, largely driven by peroxy radical (RO2) chemistry at the aerosol particle interface. However, the detailed chemical mechanisms and main oxidation products are not well understood.

Methods

Here, new oxidation mechanisms on organic peroxide formation are reported based on our recent results using advanced mass spectrometry measurements. OA model compounds were oxidized by gaseous OH in a flow tube reactor (FTR) and/or a continuous flow stirred tank reactor (CFSTR). We created laboratory oxidation conditions that mimic the atmosphere and are suitable for different RO2 pathways. The oxidized OA particles were analyzed by a thermal desorption iodide-adduct chemical ionization mass spectrometer (TD-CIMS) and an electrospray ionization ion mobility spectrometry time-of-flight mass spectrometer (ESI-IMS-MS). We use these two mass spectrometry methods to characterize the oxidation products.

Novel Aspect

This work provides new mechanistic insights into the OA multiphase oxidation chemistry in the atmosphere.

Preliminary Data or Plenary Speaker Abstract

From laboratory measurements, we elucidated RO2-initiated reactions at the aerosol interface including RO2 + HO2, RO2 dimerization, and RO2 bimolecular autoxidation.
The RO2 + HO2 chemistry was studied in the FTR, where we achieved atmospherically relevant level of HO2/OH concentration ratios. By coupling hydrogen-deuterium exchange (HDX) with TD-CIMS, we provide direct observation of organic hydroperoxide (ROOH) formation through heterogeneous RO2 + HO2 reactions for the first time. The ROOH may contribute substantially to the oxidation products, varied with the parent OA chemical structure. Similarly, RO2 dimerization occurs at the OA particle interface during OH oxidation in the FTR, which produces ROOR peroxides first at a yield of ~ 10%. Chemical structure analysis achieved by the ESI-IMS-MS with collisional induced dissociation (CID) suggests that the peroxides could quickly evolve to more stable ester and ether dimer products. The dimers enriched at the interface are expected to play a crucial role in the reactivity, volatility, and viscosity of aged OA particles. The CFSTR presents lower OH concentrations than the FTR, more like the real atmosphere. This condition allowed us to study OA chemical aging with low OH concentrations. Under these conditions, we found an intermolecular RO2 autoxidation mechanism that greatly accelerates OA oxidation with enhanced ROOH formation. With this mechanism, we estimate that the OA oxidation timescale in the atmosphere may be from less than a day to several days.
Organic peroxides are the common products from these chemical mechanisms, implying their substantial formation during OA aging, which may have important health impacts.

Prof. Dr. Ralf Zimmermann[1], Dr. Johannes Passig[1], Ellen-Iva Rosewig[1], Dr. Haseeb Hakim[1], Dr. Robert Irsig[2], Prof. Dr. Olli Sippula[3], Dr. Mika Ihalainen[3], Saara Peltokorpi[3], Dr. Angela Buchholz[3], Dr. Liqing Hao[3], Prof. Dr. Annele Virtanen[3], Dr. Julian Schade[1,4], Prof. Dr. Ville Vakkari[5]
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Application of a Novel, Field-Deployable Single Particle Mass Spectrometer for Detection of Toxicants and Climate-Relevant Parameters in Wildfire Aerosols

Ralf Zimmermann 335 - Application of a Novel, Field-Deployable Single Particle Mass Spectrometer for Detection of Toxicants and Climate-Relevant Parameters in Wildfire Aerosols, Meeting Room 106, August 23, 2024, 09:55 - 10:14

By emitting large amounts of CO2, reactive trace gases and particulate matter (aerosols), wild fires strongly affect air quality, human health and climate (1). Atmospheric aging and aerosol-distribution of these s are extremely complex and variable. Moreover, the photochemical changes are highly relevant to climate and health by determining the particle's optical- and radiative-effects, cloud condensation-activity and biological effects. To better understand the impact of wildfire emissions (and of other pollution-sources like ship emissions) it's very important to analyze the internal and external mixing-state of relevant components in the aerosol particle distribution. The new technology of field-deployable resonance-enhanced single particle mass spectrometry (2,3,4), giving unsurpassed access to single particle chemical distribution-information, has been applied to wildfire emissions, generating intriguing results.

Methods

In classical on-line single particle mass spectrometry (SPMS), aerosol particles from ambinet air are introduced directly by an aerodynamic-lens into the SPMS. Individual particle sizes and ion source-arrival times are determined laser-velocimetrically and intense UV-laser pulses are triggered to hit selected individual particles for laser desorption/ionization (LDI). Formed anions and cations from individual aerosol particles are detected in a bipolar time-of-flight MS setup. Beyond this, we recently introduced a multi-step laser ionization scheme in SPMS, exploiting on-the-fly IR-laser desorption and resonance ionization of transition metals (using 248 nm atomic iron transition) as well as of the toxic Polycyclic Aromatic Hydrocarbons (PAH). This allows detecting efficiently the most important air (trace-)toxicants (PAH, transition-metals) together with more abundant species on single particle-basis.

Novel Aspect

A novel Single-Particle Mass Spectrometer enables real-time detection of molecular parameters in wildfire-aerosols, relevant for human health and climate change.

Preliminary Data or Plenary Speaker Abstract

Using the new resonant-ionization SPMS-method, metals and PAHs can be detected in real-time (2,4) at much lower quantities and less dependent from the matrix (e.g., sea salt, organics etc.) on particles. This opens the avenue to detect carcinogenic PAHs on single-particle basis. We investigated ambient air (Baltic Sea), revealing sources and carriers of PAHs. The results suggest PAHs as molecular markers to retrieve pollution sources and aerosol transformation-processes in complex environments. Indeed, we observed aerosol aging-processes via PAH-signatures. In addition to local ship emissions also biomass burning particles were observed. In the main study, particles from smoke from wildfire simulation burns were investigated. Typical vegetation from e.g., the boreal forest and African savannah was used as fuel. Fresh and photochemically aged wildfire emissions were investigated. For climate- and health-relevant PAH we found high concentrations of the softwood combustion-marker retene in boreal forest burnings as well as its rapid degradation during aging. The

toxic, larger unsubstituted PAHs show higher stability against photooxidation. Particles with the highest PAH-loads are observed in the long-range transported size mode even after prolonged aging, in accordance with the ambient air study (3). For the water-soluble components, we could show the time-resolved glyoxal/methylglyoxal-formation during photochemical chamber-aging. Interestingly, a non-uniform mixing of water-soluble glyoxal/methylglyoxal and non-polar PAH was observed (i.e., 2 particle-classes with different chemical composition). Counterintuitively, atmospheric aging reduces cloud condensation nucleation-activity (CCN) of wildfire aerosol-particles, which might be explained by the observed sulphatisation of potassium-chloride to the less hygroscopic potassium-sulphate during aging. The CCN-activity of the aerosols, however, has a large impact on the climate impact of wildfire emissions.

(1) Yue, X. et al.(2018), Nat. Commun., 9, 5413.
(2) Passig, J. et al.(2020) Atmos. Chem. Phys. 20, 7139–7152.
(3) Passig, J. et al.(2022) Atmos. Chem. Phys., 22, 1495–1514.
(4) Schade, J. et al.(2019) Anal. Chem. 91,10282–10288.

Prof. Dr. Philippe Schmitt-kopplin[1], Hayabusa2-initial-analysis SOM team
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Molecular atlas of meteorite soluble organic matter using non-targeted ultra)high resolution organic spectroscopy reveals hydrothermal history of asteroid Ryugu samples

Philippe Schmitt-Kopplin 100 -Molecular atlas of meteorite soluble organic matter using non-targeted ultra)high resolution organic spectroscopy reveals hydrothermal history of asteroid Ryugu samples, Meeting Room 106, August 23, 2024, 10:14 - 10:33

Understanding the origin and evolution of organic matter, is linked to observationally-derived astrochemistry (telescopic observations) and the laboratory wet chemical analysis of return objects and meteorites. The molecular composition and diversity of non-terrestrial organic matter in carbonaceous chondrites can be studied by means of both, targeted and non-targeted chemical analytical approaches, leading to new insights [1].

Methods

In a non-targeted approach, all analytes are globally profiled within the analytical possibilities without biased or constrained hypothesis to gain comprehensive information. Ultrahigh-resolving analytics, like high field FT-ICR-MS and UHPLC-IMS-Qtof/MS² represents powerful tool to allow insights into the holistic complex compositional space to tens of thousands of different molecular compositions and functional groups and likely millions of diverse structures [2,3].

Novel Aspect

The MS-results combined with the metadata on meteorite samples were successfully integrated to understand hydrothermalism on samples from asteroid Ryugu.

Preliminary Data or Plenary Speaker Abstract

The sample from the near-Earth carbonaceous asteroid (162173) Ryugu was analyzed in the context of carbonaceous meteorites soluble organic matter. Sequential solvent extracts of increasing polarity of Ryugu samples show a continuum in the molecular size and polarity reflecting a low temperature and water-rich environment on the parent body approving earlier mineralogical and chemical data [4]. High abundance of sulfidic and nitrogen rich compounds as well as high abundance of ammonium ions confirm the water processing. Polycyclic aromatic hydrocarbons were also detected in a structural continuum of carbon saturations and oxidations, implying multiple origins of the observed organic complexity, thus involving generic processes such as earlier carbonization and serpentinization with successive low temperature aqueous alteration [5].

[1] Schmitt-Kopplin P. et al. (2010) Proc. Natl. Acad. Sci. U.S.A., 107, 2763–2768. [2] Ruf A. et al. (2017) Proc. Natl. Acad. Sci. U.S.A., 114, 2819–2824. [3] Matzka M. et al. (2021) Astrophys. J. Lett., 915, L7. [4] Naraoka et al. (2023) Science 2023, 379(6634). [5] Schmitt-Kopplin P. et al. (2023) Nat. Commun., 14, 6525.

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Professor Trevor Ireland[1], Mr Aditya Patkar[2], Dr Janaina Ávila[1], Dr Mark Kendrick[1], Professor Simon Turner[3]
[1]University Of Queensland, [2]The Australian National University, [3]Macquarie University

Highs and Lows in Analysis of Water with SIMS

Trevor Ireland 431 - Highs and Lows in Analysis of Water with SIMS, Meeting Room 106, August 23, 2024, 10:33 - 11:00

Water is everywhere on the surface of the Earth, but where it came from and how it came to be are key geochemical questions. While many crustal minerals contain abundant structural water, minerals from deep Earth contain only low levels of water. Similarly extraterrestrial materials show a wide variety of water concentrations between low-temperature matrix and low water high-temperature inclusions. Tracing of this water can be accomplished through in situ secondary ion mass spectrometry (SIMS) which allows specific mineral domains to be analysed, and isotopic compositions of oxygen can indicate provenance. Oxygen isotopes in particular show that water has a significant role in the processes operating in the early solar system, and before.

Methods

Oxygen isotopes are measured in multiple collection mode with the OH species measured in a separate condition. 16OH and 17O species can be separated at high resolution (5,000 M/ΔM) allowing clean oxygen isotope signals for silicate minerals with low or moderate water concentrations. However, in high water minerals such as serpentines, tailing of the abundant OH species under the 17O is problematical and can result in several permil contribution to 17O abundances. The range of 16OH count rates can be extreme ranging from several thousand counts per second (background) to over hundreds of millions of counts in hydrous phases. Such an extreme range is accessible to us by using an electrometer operating in charge mode.

Novel Aspect

Water concentrations and tracing of oxygen isotopes provides insights to the formation of Earth and the role water has played.

Preliminary Data or Plenary Speaker Abstract

The scattered ions from OH can be monitored at the equivalent 16OH-17O mass offset on the high mass side of 16OH with the counts subtracted from the 17O signal. In doing so, we have achieved high precision d17O measurements with D17O (d17O - 0.5*d18O) near zero in terrestrial minerals. This has allowed us to measure oxygen isotope compositions in hydrated minerals in carbonaceous chondrites, which is highly relevant to the analysis of samples returned recently from asteroids. These matrix minerals generally show compositions close to conventionally measured matrix samples from respective meteorite types. However, the SIMS measurements are rapid, consume little material, and maintain the petrological context.

High temperature inclusions in carbonaceous chondrules are found to typically contain very low levels of water. There are distinct petrological relationships for water in different types of chondrules (ferromagnesian silicates) and calcium aluminium inclusions. For these samples, the sample preparation is a key aspect because water effectively pervades Earth's surface and avoiding such contributions is not possible with standard preparation techniques. In CAIs, a large range of oxygen isotope compositions is found, and is often ascribed to alteration processes on asteroid parent bodies in the early solar system.

In samples from the deep Earth (continental flood basalts, mantle xenoliths) water concentrations are low. However, the source regions are large and volatiles released during eruptions can be significant inputs to the Earth's atmosphere.

Planet Earth provides unique conditions for the formation of life. Water is a key ingredient. Much of the water on Earth appears to be dominantly related to asteroidal contribution.

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Professor Yujia Qing[1]
[1]University of Oxford

Towards nanopore proteomics: Single-molecule analysis of post-translational modifications within full-length proteins

Keynote: Professor Yujia Qing University of Oxford 760 - Towards nanopore proteomics: Singlemolecule analysis of post-translational modifications within full-length proteins, Meeting Room 109, August 23, 2024, 09:30 - 09:55

Nanopore technology offers an exciting capability to analyse ultra-long biopolymers at the single-molecule level. The success with nanopore nucleic acids sequencing is now inspiring the analysis of other complex biopolymers, such as polypeptides. In humans, ~20,000 protein-encoding genes give rise to millions of proteoforms through diversification processes like post-translational modification. The primary goal of nanopore proteomics is to locate and identify these modifications within polypeptide chains, rather than to sequence the polypeptides themselves.

Methods

The ionic current carried by a nanopore registers the characteristics of the peptide segment residing within the sensing region. By translocating a polypeptide chain through the nanopore, we can monitor real-time changes in the current to analyse full-length proteins.

Novel Aspect

Our work advances nanopore technology towards a platform for full-length proteoform characterisation.

[1] P. Martin-Baniandres, W.-H. Lan, S. Board, et al., Enzyme-less nanopore detection of post-translational modifications within long polypeptides. Nat. Nanotechnol. 18, 1335–1340 (2023).
[2] W.-H. Lan, H. He, H. Bayley, Y. Qing, Location of phosphorylation sites within long polypeptide chains by binder-assisted nanopore detection. J. Am. Chem. Soc., (2024) (in press).

Preliminary Data or Plenary Speaker Abstract

Recently, we have engineered protein nanopores to capture, linearise, and drive the translocation of full-length polypeptides, including those of more than 1000 amino acid residues, for single-molecule characterisation [1]. This technology has enabled the detection of various post-translational modifications (PTMs), even those deeply embedded within the polypeptide chain or widely separated along it. Interestingly, we observed that structurally distinct PTMs can produce similar nanopore signals, complicating their accurate single-molecule identification. To address this, we applied PTM-specific “binders” that attach to polypeptide chains during their translocation through nanopores, enabling unambiguous identification and localisation of PTMs [2]. This concept has been successfully demonstrated with phosphorylation and can be extended to other PTMs.

Dr. Niklas Geue[1], Mr Hari Newnham[1], Ms Ellen Liggett[1], Dr Alexey Barkhanskiy[1], Dr Jakub Ujma[2], Professor Perdita Barran[1]
[1]The University Of Manchester, [2]Waters Corporation

Visualizing Conformational Dynamics of Biomacromolecules by Coupling Ion Mobility Mass Spectrometry to Electron Microscopy

Niklas Geue 98 - Visualizing Conformational Dynamics of Biomacromolecules by Coupling Ion Mobility Mass Spectrometry to Electron Microscopy, Meeting Room 109, August 23, 2024, 09:55 - 10:14

The use of mass spectrometry and electrospray deposition to prepare surfaces for microscopy has been pioneered by a number of groups, but has not yet been combined with ion mobility mass spectrometry (IM-MS). The advantage of doing this is evident, as many molecules are conformationally diverse and this flexibility can be essential for function. IM-MS is able to capture this and has delivered substantial insights to the study of disordered and conformationally flexible compounds. In microscopy (STM and cryo-EM) precise structures can be found but chemical identity is not evident, and for proteins disordered regions are not observable in cryo-EM. Combination provides chemical identity, structural resolution and population of conformers.

Methods

We have built a soft-landing stage and integrated it into a Select Series Cyclic IM mass spectrometer. The landing stage, which enters the vacuum via an adapted solids probe with a gate valve to allow the pressure to be reduced, comprises a holder for a microscopy grid. This can be heated to outgas/clean or cooled to minimise structural reorganisation on the surface. The landing stage is located after the quadrupole and cyclic cell, such that ions can be selected by m/z and also by mobility prior to landing on the grid that can be coated in a sugar matrix. Once deposition is complete the grid can be removed prior to interrogation using negative staining TEM.

Novel Aspect

Hyphenation of IM-MS instrumentation with electron microscopy via soft-landing reveals conformational dynamics of biomacromolecules.

Preliminary Data or Plenary Speaker Abstract

We have soft-landed and imaged a range of biomacromolecules, including monoclonal antibodies, ferritin, the chaperone complex GroEL as well as adeno-associated viruses. For monoclonal antibodies, we have previously reported on their conformational diversity ranging from collisional cross sections of 56 – 100 nm2. Here we select conformers across charge state ATDs and use TEM to examine the landed conformers. We will further present data from highly conformationally diverse proteins complexes including those that contain substantial disordered regions. Here the power of the multimodal approach become most apparent. For the dimeric protein UVR8, we have previously shown the functional significance of the N and C disordered termini. We conformationally select from this protein complex and follow up with TEM. It is well known that the extended disordered regions cannot be readily imaged with TEM, but the MS and IM preparation proof that they are there. Taken together these examples provides a prospective for combining datasets from mass spectrometry and microscopy to provide more complete views of conformational occupancy in structurally dynamic biomolecules.

Lars Gruber[1,2], Dr. Stefan Schmidt[1], Thomas Enzlein[1], Huong Giang Vo[3], James-Lucas Cairns[1,2], Yasemin Ucal[1], Dr. Florian Keller[1], Denis Abu Sammour[1], Rüdiger Rudolf[1,4], Matthias Eckhardt[5], Dr. Stefania Alexandra Iakab[1], Laura Bindila[3], Carsten Hopf[1,2,4]
[1]Mannheim University, [2]Heidelberg University, [3]Mainz University, [4]Mannheim University, [5]Bonn University

Guiding imaging mass spectrometry by mid-infrared vibrational spectroscopy for deep lipidomic profiling

Lars Gruber 213 - Guiding imaging mass spectrometry by mid-infrared vibrational spectroscopy for deep lipidomic profiling, Meeting Room 109, August 23, 2024, 10:14 - 10:33

In spatial ‘omics, highly confident molecular identifications are indispensable for the investigation of complex biological samples and for spatial biomarker discovery [1].
However, increasing the level of confidence in molecular identifications is often a time-consuming process. Moreover, the effective region-of-interest (ROI) is often only a fraction of the entire tissue region. We overcome this restriction by the determination of the effective ROIs using Quantum Cascade Laser Mid-Infrared Imaging Microscopy (QCL-MIR) prior to MALDI IMS and guide the acquisition based on the MIR-derived hyperspectral dataset.
This combination enables us to dramatically reduce the ROIs up to 95x-fold and allow for in-depth investigations, which would’ve been hindered by expanding acquisition times and data size, ultimately leading to an increase in analytical confidence.

Methods

We developed our workflow on kidney samples of arylsulfatase-A-deficient (ARSA-/-) mice, representing the murine model of human metachromatic leukodystrophy (MLD), characterized by a selective accumulation of sulfatides. For this purpose, QCL-MIR spectral fingerprints were recorded on a Bruker Hyperion ILIM II and used for the definition of the effective ROIs. These ROIs were translated to the IMS acquisition file and MIR-guided MALDI MSI was carried out on Bruker timsTOF flex and solariX 7T platforms. To benchmark our data, we compared it against LC-TIMS-MS (Bruker timsTOF Pro2). Increased confidence in molecular identifications was achieved by applying prm-PASEF on-tissue MS^2 analysis and ultra-high mass resolution IMS. LipidMaps, and SwissLipids databases were used for lipid annotation.

Novel Aspect

Introduction of MIR-guided IMS to reduce the acquisition time and data size, and description a novel workflow for lipid identification.

Preliminary Data or Plenary Speaker Abstract

By MIR microscopy, we were able to identify two effective ROIs in ARSA-/- kidney, which reduced the total number of pixels for IMS by 6x-fold.
Focusing MIR-guided magnetic resonance (MR) IMS on these specific morphological features enabled us to investigate the sulfo-glyco lipidome of ARSA-/- kidney with a mass resolution of R~1,230,000 at m/z 800. The main advantage is highlighted by the identification of the isotopic fine structures (IFS) for the sulfatides, which allows for the most accurate annotation that is available on the MS1 level.
MIR-guided trapped ion mobility spectrometry (tims) IMS enabled us to identify 121 sulfatides directly on tissue, highlighted by the identification of over 20 odd-chain sulfatides. The complete elucidation of the MALDI prmPASEF-derived MS2 spectra allowed for a detailed description of characteristic fragment ions depending on the sulfatide substructures, e.g. degree of hydroxylation or glycosylation. Without mobility-based MS2 analysis, chimeric MS2 spectra would’ve hindered the identification in many cases. Additionally, we benchmarked our workflow by a comparison of collisional cross section (CCS) values between MIR-guided MALDI-IMS and LC-ESI-TIMS-MS across two MS facilities revealing very high (>0.998) correlation. Based on that, MIR-guided tims¬ IMS enabled

the in-depth investigation of structure-ion mobility-relationships that provide chemical rationales for improvements to current ion mobility prediction algorithms [2].
We expanded our concept to specifically target glomeruli cells in kidney tissue sections, where our workflow reduced the total acquisition time by 95%.On average, more than 85% of the MIR-defined ROIs con¬tained glomeruli, as defined by a characteristic ganglioside marker.

References:
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[2] Gruber L, Schmidt S, et al. Deep MALDI-MS Spatial ‘Omics guided by Quantum Cascade Laser Mid-infrared Imaging Microscopy. biorxiv doi: https://doi.org/10.1101/2023.12.14.571637

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Yanis Zirem[1], Laurine LAGACHE[1], Pr Michel Salzet[1], Professor Isabelle Fournier[1]
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Spatial multi-omics informed by SVD k-means++ clustering and statistical assessment of heterogeneity: Advance dry proteomic guided by lipids MALDI MSI

Yanis Zirem 350 - Spatial multi-omics informed by SVD kmeans++ clustering and statistical assessment of heterogeneity: Advance dry proteomic guided by lipids MALDI MSI, Meeting Room 109, August 23, 2024, 10:33 - 11:00

Mass spectrometry imaging (MSI) has emerged as crucial for studies on biological systems at molecular level. Notably, MALDI-MSI has transformed multi-omics strategies, facilitating thorough analysis of peptides, proteins, lipids and metabolites. This capability is essential for unraveling the intricate molecular compositions of heterogeneous cancer samples, which exhibit diverse cell types and distinct molecular profiles. A comprehensive understanding of diseases necessitates precise characterization of lipidomes and proteomes within identified clusters. However, the complexity of protein annotation and the absence of standardized methodologies hinder the efficiency of MALDI MSI data analysis, especially for multi-omics. To tackle this, we introduce an automated process for dry proteomics guided by lipid MALDI-MSI cluster identification, with the goal of streamlining and fortifying the data analysis process.

Methods

The application of this concept was optimized using consecutive sections of rat brain (RB). Lipid, protein and peptide MALDI-MSI were performed on cerebellum area as well as on glioblastoma tissue. A MatLab script was developed to process omics image datasets, performing unsupervised clustering using k-means++ algorithm. Integration of the Silhouette criterion prediction facilitated the determination of the optimal number of clusters for segmentation. A Python script identified discriminant cluster ions for lipids and proteins, which were validated and supplemented by spatial proteomic analysis. Ultimately, this tool coupled with a custom lipid-associated protein database, enabled the development of a prediction model to blindly identify clusters based on their lipidomic fingerprint. Additionally, it automatically provided associated proteomic annotations for dry proteomic.

Novel Aspect

Accurate segmentation process creation for omics data in MALDI-MSI, enabling the implementation of dry proteomics guided by lipid MSI.

Preliminary Data or Plenary Speaker Abstract

We successfully developped an in-house segmentation pipeline tailored for multi-omics MALDI-MSI datasets. Various data compression techniques, such as SVD, t-SNE or NNMF were examined and compared. Among these methods, SVD compression emerged as the most optimal, particularly suited for omics image datasets. Consequently, the pipeline incorporates SVD data compression pre-processing and k-means++ segmentation processing steps, augmented with Silhouette criterion to refine the process and ascertain the optimal number of clusters for image analysis. This methodology was applied to lipid, protein, and peptide MSI datasets obtained from rat brain cerebellum tissues. Overlaying the resulting images from each omics category revealed consistent spatial localization of identified clusters, corresponding to well-known subregions within the cerebellum, such as the white matter, granular layer and molecular layer. These observations derived from MALDI images were validated through comprehensive protein analysis using spatial proteomics strategies. Following this approach, discriminant lipids and proteins were specifically identified for each subregion, affirming that each heterogeneous subpopulation, as observed by MSI, possesses its unique molecular pathway, comprising lipid and protein networks with well-defined spatial localization. Furthermore,

we successfully integrated the identified lipids and proteins within a same biological pathway. A machine learning-based prediction model was developed to blindly identify distinct cerebellum rat brain clusters from a lipid MSI image, leveraging lipid specific fingerprints, and automatically provide their protein annotations within the same pathways. In summary, our MSI image processing methodology introduces the concept of dry proteomics, offering automated protein annotation associated with the lipid pathways identified by MALDI-MSI. We then used the same strategy on 50 patients with glioblastoma, confirming the ability to associate specific lipids with proteins from lipids MALDI-MSI images, but also correlating with patient prognosis.

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Dr Vaughan Langford[1]
[1]Syft Technologies

SIFT-MS: Ion-Molecule Chemistry Coupled with Mass Spectrometry for Real-World Applications

Keynote: Dr Vaughan Langford Syft Technologies Limited 19 - SIFT-MS: Ion-Molecule Chemistry Coupled with Mass Spectrometry for Real-World Applications, Meeting Room 110, August 23, 2024, 09:30 - 09:55

{I am an invited keynote speaker for the "ion-ion/ion-molecule chemistry" session, so I have completed just the third section.}

Methods

{I am an invited keynote speaker for the "ion-ion/ion-molecule chemistry" session, so I have completed just the third section.}

Novel Aspect

The application of gas-phase ion-molecule chemistry in SIFT-MS has enabled the technique to address diverse real-world applications.

Preliminary Data or Plenary Speaker Abstract

Based on ion-molecule chemistry, selected ion flow tube mass spectroscopy (SIFT-MS) is part of the flowing afterglow and selected ion flow tube (SIFT) lineage that was widely utilized to study the interstellar medium and planetary atmospheres. This work generated large libraries of reaction mechanisms and rate coefficients that were used in computational models for molecule formation in the interstellar medium and in planets of our solar system. The SIFT-MS technique utilises similar reaction databases to perform untargeted and quantitative analyses of more “down-to-earth” matrices.
When applied to air analysis applications, SIFT-MS instrumentation almost exclusively utilizes a small group of ions (so-called reagent ions): three cations (H_3O^+, NO^+, and $O_2^{+\bullet}$) and five anions ($O^{-\bullet}$, OH^-, $O_2^{-\bullet}$, NO_2^-, and NO_3^-). These reagent ions react only very slowly (or not at all) with the bulk components of air, enabling the SIFT-MS technique to analyse volatile organic compounds and a broad range of inorganic gases at parts-per-trillion to parts-per-billion concentrations direct from air in real time. The reagent ions are also generated very conveniently from humidified or dry air, rather than from bottled gases, in a microwave discharge source. Analytical specificity is achieved based on diverse ion-molecule reaction mechanisms provided by the reagent ions (under highly controlled, near-thermal reaction conditions) coupled with mass spectrometry. A unique feature of SIFT-MS is the rapid switching of reagent ions using a quadrupole mass filter that maximizes breadth and specificity of analysis in a single run.
This presentation will summarise the ion-molecule reaction chemistry that the SIFT-MS technique uses for sample analysis and briefly covers the operational principles of modern SIFT-MS instruments. It will conclude with illustrative real-world applications of ion-molecule chemistry to workplace safety, environmental, and consumer and pharmaceutical product screening.

Dr Berwyck Poad[1], Ms Rhiannon McVeigh[1], Dr David Marshall[1], Dr Samuel Brydon[1], Professor Stephen Blanksby[1]
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Hyphenation of chromatography with ion-ion charge inversion chemistries for the resolution and structure elucidation of branched-chain lipids

Berwyck Poad 284 - Hyphenation of chromatography with ion-ion charge inversion chemistries for the resolution and structure elucidation of branched-chain lipids, Meeting Room 110, August 23, 2024, 09:55 - 10:14

While branched chain fatty acids, and lipids carrying them, can often be separated using reversed phase liquid chromatography, contemporary mass spectrometry methods are unable to unequivocally identify site(s) of chain branching using collisional activation alone. Structural characterisation of branched chain fatty acids is essential, as they are postulated to have positive impacts for digestive health yet the precise lipid structures responsible remains an active debate. Recent developments exploiting gas-phase ion-ion reactions have demonstrated the efficacy of this approach for lipid structural elucidation, however signal-to-noise on diagnostic fragments can sometimes be low. Here we introduce a novel reagent for gas phase ion-ion reactions that provides a 50-fold enhancement compared to other reagents.

Methods

Experiments were undertaken on an ion mobility enabled quadrupole time-of-flight mass spectrometer (Waters Synapt G2-Si). Fatty acid samples were introduced by electrospray ionisation through a negatively biased ESI probe, while doubly charged 4'-nitro-2,2':6',2''-terpyridine magnesium (II) reagent ions $[(4\text{-}NO_2Terpy)2Mg]^{2+}$ were introduced through a positively biased ESI probe. The native electron transfer dissociation capability was modified to mass select and store negatively charged fatty acids in the trap region of the instrument, and subsequently combine these with mass selected doubly charged reagent cations. The resulting singly charged ion-ion complexes $[(4\text{-}NO_2Terpy)(FA\text{-}H)Mg]+$ were then separated by ion mobility and collisionally activated in the transfer region prior to mass analysis by time-of-flight.

Novel Aspect

Application of a novel ion-ion reagent for lipid structure elucidation in direct infusion and liquid chromatography workflows

Preliminary Data or Plenary Speaker Abstract

Coupling the ion-ion charge inversion strategy with high performance reversed-phase liquid chromatography (Waters Acquity i-Class) permitted analysis of complex mixtures containing multiple fatty acid isomers. The LC-MS separation was optimised using a mixture of three isomeric fatty acid standards containing methyl branches at either the iso (15-methyl-hexadecanoic acid) or anteiso (14-methyl-hexadecanoic acid) positions, along with the straight chain heptadecanoic acid. Extracted ion chromatograms for the diagnostic ion-ion fragmentation products enabled identification of the separated fatty acids. Application of this method to hydrolysed lipid extracts of fermented dietary supplements from Bacillus subtilis showed that this was significantly enriched in branched chain fatty acids, with the majority being branched at the anteiso position.

Complexation of the dication reagent ions with the deprotonated fatty acid (FA) anions resulted in a singly charged positive adduct of the form $[(4\text{-}NO_2Terpy)–Mg–FA]+$. Collision-induced dissociation (CID) of the resulting ion-ion complexes in the transfer region of the instrument (post ion mobility) yielded rich radical directed fragmentation of the fatty acyl chain with product ion peaks spaced 14 Da apart. For fatty acids containing methyl branching, this regular pattern was interrupted at site(s) of branching with the absence of a product ion peak, enabling explicit identification of the branching sites. For unsaturated fatty acids, an enhancement of the product ions formed by fragmentation

either side of the carbon-carbon double bond permitted identification of the site(s) of unsaturation. Moreover, loss of H_2 was observed for cis double bonds from the precursor ion in significantly higher abundance than trans double bonds, enabling assignment of double bond stereochemistry.

Future coupling of this hyphenated approach with in-source fragmentation of intact negatively charged glycerophospholipids with subsequent mass selection of the liberated fatty acyl chains will enable explicit linking and identification of the branched fatty acids to their respective glycerophospholipid carriers.

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Professor Weiguo Wang[1], Dr. Lei Hua, **Prof. Haiyang Li**
[1]Dalian Institute Of Chemical Physics, Chinese Academy Of Science.

Miniature Continuous Atmospheric Pressure Interfaced Ion Trap Mass Spectrometer with Radial Electric Field Driven Collision-Induced Dissociation and its applications

Haiyang Li 341 - Miniature Continuous Atmospheric Pressure Interfaced Ion Trap Mass Spectrometer with Radial Electric Field Driven Collision-Induced Dissociation and its applications, Meeting Room 110, August 23, 2024, 10:14 - 10:33

Miniature mass spectrometry has gained significant attention in response to the growing need for rapid on-site detection in diverse field. Among the various miniature analyzers, ion traps are favored due to their simple structure and higher-pressure tolerance. Nevertheless, miniaturization of the ion trap has some negative effects on the accuracy of qualitative identification and extensive efforts have been made for improving those performances by unique ability of the ion trap for tandem-in-time mass spectrometry analysis. In this paper, we report a novel method for multigenerational CID with degree of fragmentation of nearly 100% allowing the production of multigenerational fragment ions in a single attempt, which involves modulating the float DC in miniature continuous atmospheric pressure interfaced ion trap mass spectrometer.

Methods

The schematic diagram of the CAPIITMS equipped with a hexapole is used, which featured a two-stage vacuum design. A stainless-steel capillary was used as the sample inlet, and the first vacuum chamber was connected to the second chamber via a pinhole skimmer (0.5 mm in length, 0.4 mm inner diameter). A commercially available VUV krypton discharge lamp (PKS106) and a hexapole with an RF frequency at 2.4 MHz and amplitude at 200 V0-p were located in the first vacuum chamber. To elucidate the underlying mechanism of this novel CID, simulations were conducted using Simion software to analyze the distribution of an electric field and the trajectory of ions within the ion trap mass analyzer under different float DCs.

Novel Aspect

A novel radial electric field driven collision-induced dissociation method with high fragmentation efficiency for different species is presented.

Preliminary Data or Plenary Speaker Abstract

It was discovered that modulation of the float DC can regulate the generation of fragment ions in the homemade CAPI-ITMS. Only parent ions of styrene ([M]+, m/z 104) were observed at Ufloat DC of -5 V, whereas a significant amount of fragment ions ([M-C2H2] +, m/z 78) were observed when Ufloat DC was set to -20 V. By modulating the float DC on the ion trap analyzer, multiple fragment ions such as([M+H-nC2H4] +, where n = 1, 2, 3) of tri-ethyl phosphate were observed with a single injection of ions. The underlying mechanism of CID was elucidated through theoretical simulations. It was revealed that the radial electric field was enhanced by the float DC, guiding ions towards regions of intense RF field where broadband heating and dissociation of ions took place. The degree of fragmentation could be manipulated by adjusting the float DC, the initial kinetic energy of ions and the pressure. The sensitivity of aniline was in-creased by extending the ion injection duration of the CAPI-ITMS, but the existence of reagent ions leaded to severe space charge effect. Through selective fragmentation of reagent ions, peak broadening and mass shift were eliminated and mean-while 28-fold improvement of aniline in signal-to-noise ratio was achieved with the ion injection duration varying from 50 to 2500 ms. Moreover, isomeric illicit drugs (JWH-018 and acetylcodeine) were distinguished by generating multiple characteristic fragment ions, multiple characteristic fragment ions of acetylcodeine (m/z 225 and m/z 282) and JWH-018(m/z 214, m/z 155 and m/z 144) were successfully detected. Mixtures of JWH-018 and acetylcodeine were analyzed under Ufloat DC of -40

V and successful identification of these characteristic fragment ions was achieved demonstrating potential applications in identification of synthetic illicit drugs.

Dr. Lei Hua[1], Prof. Haiyang Li[1]
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Photoionization/photoionization-induced chemical ionization mass spectrometry for operando characterization of catalytic reaction processes: instrumentation and applications

Hua Lei 267 - Photoionization/photoionization-induced chemical ionization mass spectrometry for operando characterization of catalytic reaction processes: instrumentation and applications, Meeting Room 110, August 23, 2024, 10:33 - 11:00

Operando characterization of catalytic reaction processes can provide key evidence for the in-depth study of complex catalytic reaction networks, reaction pathways, and reaction kinetics. As a high-throughput mass spectrometry with the advantages of high molecular ion yield and simple spectrum interpretation, photoionization mass spectrometry (PI-MS) based on vacuum ultraviolet (VUV) lamp has proven itself as a versatile and powerful analytical technique for online and real-time process monitoring. However, due to the large difference of PI cross sections for different compounds and the limitation of photon energy, the ability of lamp-based PI-MS for detection of compounds with low PI cross sections and high ionization energies (IEs) is insufficient.

Methods

A new nonuniform electric field high-pressure photoionization/photoionization-induced chemical ionization (HPPI/PICI) source with multiple ionization zones was developed for time-of-flight mass spectrometry. The photoelectrons emitted from the metal electrode under the radiation of VUV light were accelerated by the electric field in the ionization region and gained sufficient energy to ionize the reagent molecules with IEs higher than the photon energy, e.g., 10.6 eV for krypton lamp. Large numbers of reactant ions, such as O2+, H3O+ and CH2Br2+, were generated in a reactant ion production zone, and reacted with analyte molecules through chemical ionization in another analyte ionization zone. Different operation modes, HPPI and PICI modes, could be rapidly switched by simply adjusting the voltage and changing the reagent gas.

Novel Aspect

PI and CI combined ion source is achieved through the regulation of ionization region structure, electric field, pressure, and atmosphere.

Preliminary Data or Plenary Speaker Abstract

The design of the nonuniform electric field in the multiple ionization zones not only improved the ionization efficiency and ion transmission efficiency, but also diminished the formation of cluster ions, such as [MH]+·(H2O)n (n = 1 and 2) and [M2]+. The sensitivities of benzenes and olefins, such as benzene, p-xylene, tetrachloroethylene, and hexachloro-1,2-butadiene, were 6- to 10-fold higher in the HPPI mode than in the PICI mode. While the signal intensities of oxygenated volatile organic compounds (OVOCs), such as methanol, formic acid, butanone, and diethyl ether, were enhanced by 3-fold to more than 3 orders of magnitude in the PICI mode compared to the HPPI mode. The limits of detection (LODs, S/N = 3) ranged from 0.17 to 13 ppbv.
To continuously monitor the variation trend of liquid-phase products during the methane conversion reaction in a kettle with a pressure of 2 MPa, a fused silica capillary with the i.d. of 50 μm was employed to efficiently and evenly extract the liquid products from the kettle. The OVOC products was continuously monitored in the PICI mode for approximately 10 h. The result suggested a possible reaction path of methane conversion that CH4 was first oxidized to CH3OH and CH3OOH, and then, CH3OH was further oxidized to HOCH2OOH and HCOOH. The hydrocarbon products during the initial stage of the methanol-to-olefins (MTO) reaction were real-time monitored in the HPPI mode with a high temporal resolution. The ion source pressure was decreased to 50 Pa to further reduce the formation of olefin cluster ions. The time course of propylene, butene, pentene, and hexene

indicated that the olefins with a higher carbon number were produced later. The applications on operando monitoring of these catalytic reaction processes exhibited an excellent performance and wide potential applications of PI-MS in process monitoring and reaction mechanism research.

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Prof Zongwei Cai[1]
[1]Hong Kong Baptist University

Mass spectrometry determination of microbiota-mediated biotransformation of triclosan for investigation of colitis induction

Keynote: Zongwei Cai Hong Kong Baptist University 769 - Mass spectrometry determination of microbiota-mediated biotransformation of triclosan for investigation of colitis induction, Plenary 3, August 23, 2024, 11:30 - 11:55

Triclosan (TCS) is an antimicrobial agent and a major environmental pollutant widely used in consumer products. Under normal circumstances, TCS will be rapidly metabolized into non-biologically active metabolites such as "TCS-glucuronide" (TCS-G), which are easily excreted from the body. However, we have found that certain gut microbes can reactivate TCS-G and form to TCS in the gut, resulting in colitis. The new toxicological mechanism of TCS may also be applicable to toxicity studies of other environmental contaminants, and the role of gut microbiota should be considered when assessing the toxicity of environmental pollutants.

Methods

We used LC-MS/MS to profile TCS metabolites in various tissues of exposed mice. We found that, in most organs, such as liver, bile, heart, small intestine (mucosa and digesta), the dominant TCS metabolites were its glucuronide- and sulfate-conjugates. However, in the digesta of cecum and colon tissues, the dominant metabolite was free-form TCS, with very low concentrations of the conjugates, illustrating a different profile of TCS metabolism.

Novel Aspect

To our knowledge, detailed profiling of TCS metabolism in animal tissues has not been attempted.

Preliminary Data or Plenary Speaker Abstract

By using MS/MS-based fragmentation analysis, we identified some novel metabolites, including hydroxyl-TCS, hydroxyl-TCS glucuronide, and hydroxyl-TCS sulfate, in the colon digesta, and found that the profiles of these metabolites were also dependent on gut microbiota. Consistent with our results above, the antibiotic cocktail treatment reduced the concentration of free-form hydroxyl-TCS while increasing the concentration of hydroxyl-TCS glucuronide and hydroxyl-TCS sulfate in the colon digesta. These results supported the roles of gut microbiota in colonic metabolism of TCS. We also showed that intestinal commensal microbes mediate metabolic activation of TCS in the colon and drive its gut toxicology.

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Miss Maya Cameron[1], Mr Jaye Marchiandi[1], Mr Jordan Partington[1], Dr Brad Clarke[1]
[1]The University Of Melbourne

Comprehensive analysis of Endocrine Disrupting Chemicals using High Resolution Mass Spectrometry

Maya Cameron 393 - Comprehensive analysis of Endocrine Disrupting Chemicals using High Resolution Mass Spectrometry, Plenary 3, August 23, 2024, 11:55 - 12:14

Endocrine Disrupting Chemicals (EDCs) ubiquitously pollute the environment, where exposure disrupts regular hormone function resulting in cancers, infertility, and obesity. Despite their toxicity, current human biomonitoring studies employing targeted methods neglect to identify metabolites owing to the lack of synthetic standards, potentially underreporting human exposure to this diverse class of chemicals. This study aims to develop a new approach to identifying parent EDCs and metabolites by in vitro formation with hepatic liver fractions, computational prediction of metabolites, coupled with high resolution mass spectrometry (HRMS) for novel metabolite identification. A suspect screening workflow including EDCs and their biologically relevant metabolites has been applied to a small cohort of couples undergoing fertility treatment.

Methods

EDC metabolites were generated in vitro using 120 parent EDCs spiked into mice hepatic liver fractions. The products of this metabolism assay were analysed using LC-MS QToF (Agilent 1260 Infinity II LC coupled to Agilent 6546A QToF MS) in both data dependent and independent modes.
A suspect list was created that contains 120 parent EDCs and their predicted metabolites generated using open-source prediction software BioTransformer 3.0. Matches between the in silico and in vitro generated metabolites contained the highest level of confidence when screening with the suspect list.
This suspect list was applied the urine, serum, seminal fluid, and follicular fluid of couples seeking fertility treatment following extraction and suspect screening workflow using the LC-MS QToF.

Novel Aspect

One of the largest multiclass EDC analyses of in silico and in vitro generated metabolites applied to human cohort.

Preliminary Data or Plenary Speaker Abstract

The suspect screening library predicted 400 metabolites of the 120 parent EDCs. This suspect screening library was further informed using the liver metabolism assays of the parent EDCs.
The suspect library was applied to a range of human biological fluids to assess EDC exposure and determine relevant metabolites using LC-MS QToF. This includes urine, serum, seminal fluid, and follicular fluid. Both parent and metabolite EDCs were identified to give comprehensive analysis of EDC exposure. Preliminary findings suggest urine to contain majority of metabolites with other biological fluids having metabolites below detectable concentrations. This casts doubt as to the necessity of using enzymatic degradation of biological samples prior to analysis as per current human biomonitoring study workflows.
Future work focusses on investigating the ability to semi-quantitatively analyse EDC metabolite exposure using available reference standards of the parent EDCs. This will confirm the presence or absence of metabolites in the biological fluids and determine the necessity of enzymatic degradation.

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Dr Denise Tran[1], Doctor Samiuela Lee[1], Dr Ben Crossett[1], Professor Gemma Figtree[2], Doctor Owen Tang[2]
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Method Development for High-throughput Quantification of PFAS in Plasma for Correlation to the Omics Profile of Patients with Cardiovascular Disease

Denise Tran 592 - Method Development for High-throughput Quantification of PFAS in Plasma for Correlation to the Omics Profile of Patients with Cardiovascular Disease, Plenary 3, August 23, 2024, 12:14 - 12:33

Per- and polyfluoroalkyl substances (PFAS) represent a class of persistent chemicals extensively utilised in both industrial and consumer products, leading to widespread exposure. Due to their resistance to breakdown, environmental accumulation of PFAS has led to an increase in human exposure through ingestion and inhalation. Long-term exposure and high levels of PFAS have been linked to adverse health outcomes, with recent studies suggesting an increased risk of cardiovascular disease (CVD).
To further validate the correlation of PFAS to CVD, a method to quantify PFAS in plasma was developed utilising liquid chromatography-tandem mass spectrometry (LC-MS/MS). This methodology was then applied to plasma samples collected from patients who underwent computed tomography coronary angiogram (CTCA).

Methods

To determine the best methodology for the quantification of PFAS, control plasma samples underwent various organic protein precipitation methods (combinations of methanol, acetonitrile, water , salts . etc.) and then subsequently, were processed through different combinations and sequences of analytical columns before undergoing mass spectrometry analysis. The results of the different methods were compared, and specificity, sensitivity and resolution were benchmarked against PFAS analogue standards to obtain the methodology with the most quantitative, robust and consistent assay.
Utilising the developed methodology, plasma samples collected from patients referred for a computed tomography coronary angiogram (CTCA) by either a chest pain clinic or a cardiologist, were then analysed and PFAS levels were measured.

Novel Aspect

1. Targeted LC-MS/MS workflow of PFAS in plasma
2. Correlating PFAS levels in patients with CVD to their omics profile

Preliminary Data or Plenary Speaker Abstract

Although LC-MS/MS technology has been widely used for quantitative analysis of PFAS in environmental samples (air, water and soil) it has been a challenging task to quantitatively analyse PFAS in plasma utilising this technology due to the biological matrix effects that can result in poor peak shape (tailing, distortion of peaks), and a loss of resolution, resulting in unquantifiable data.
In this study, we developed a method to detect and quantify 19 PFAS analogues in plasma for a preliminary cohort (n=10) of patients with varying levels of CVD.
For the method development, we report several PFAS analogues with significantly improved detection and quantifiable data with extraction solutions of high methanol and low acetonitrile and LC separations with C18 reverse-phase chemistry proved to yield the best results for peak shape and quantifiable data.
This presentation shows the capabilities of PFAS detection using LC-MS/MS and the limit of quantification (LOQ) at levels of plasma as low as 0.013 ppb. In addition, preliminary statistical analyses were also conducted to study the quantifiable levels of PFAS and their correlation to the omic profiles of patients with associated CVD risk. This data suggests a correlation of increased plasma PFAS abundance to high levels of lipid and protein biomarkers of CVD.

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MARK SARTAIN[1], Lee Bertram[1], James Pyke[1], Andrew McEachran[1], Mr. Ajith Puthan Veedu Subramanian
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Empowering Drug Metabolite Identification with a Novel Software Workflow

Mark Sartain 446 - Empowering Drug Metabolite Identification with a Novel Software Workflow,
Plenary 3, August 23, 2024, 12:33 – 12:42

Drug metabolite identification is a crucial step in drug development, helping researchers understand the behavior of drugs in humans, including their efficacy, toxicity, and potential for drug-drug interactions. A challenge in MS/MS-based identification of drug biotransformation products is these compounds are most often absent from known MS spectral libraries. Here we present a novel strategy combining open-source and vendor software to accurately predict drug metabolite structures, search these structures against MS/MS data through a molecular fingerprinting approach, and finally map identifications to molecular features resulting from a differential analysis of drug-treated liver microsomes.

Methods

Pooled human liver microsomes with buffer were incubated at 37°C with 3 concentrations of losartan (4, 20, and 100 µM). Reactions were quenched by addition of four volumes of methanol with vortexing at time points 0, 5, 10, 15, 30, 45 and 60 minutes followed by centrifugation. Supernatants were separated with RP-LC and eluents were analyzed with an Agilent Revident LC/Q-TOF in positive ion mode. Datasets were analyzed with MassHunter Explorer software and MS/MS were extracted with MassHunter Qualitative Analysis. Biotransformer 3.0 was used to predict losartan metabolites and results were used to create a custom database within SIRIUS/CSI:FingerID software to search the MS/MS spectra. Agilent ChemVista software was used to manage and merge all exported results into a database.

Novel Aspect

Innovative Software Approach for the De-novo Discovery of Drug Metabolites

Preliminary Data or Plenary Speakers Abstract

To demonstrate proof-of-concept for the workflow, samples were prepared by incubating human liver microsomes with the drug losartan. Extracts were analyzed by RP-LC and the eluents were analyzed by LC/Q-TOF. Feature finding and differential analysis were performed within MassHunter Explorer, a complete discovery-based software. Out of 1,803 compounds, 270 significant compounds showed variation between time points and were selected for further interrogation.

The losartan chemical structure (.sdf) was loaded into the web-based Biotransformer software (htttps://biotransformer.ca) that provides fast, accurate, and comprehensive metabolism prediction. Lax prediction settings were applied with a combination of CYP450 (phase I) and phase II enzymes using multiple iterations which led to 1,243 biotransformation products with unique SMILES representing 29 molecular formulas. The exported SMILES were then imported into SIRIUS/CSI:FingerID to create a custom structure database. Molecular formulas were generated from the extracted MS/MS of significant features and MS/MS were also searched against the custom database through molecular fingerprinting. The losartan drug precursor and three metabolites were identified. The top search hits for two of the three metabolites were the pharmacologically active carboxylosartan (E-3174) and the aldehyde intermediate losartan carboxaldehyde (E-3179), demonstrating the MS/MS spectra matched to well-known losartan drug metabolites with discriminating scores over many structurally similar isomers. The resulting metabolite IDs were mapped back to the profiling project and the changes in metabolite levels across time points could be

visualized and the project further analyzed. Similar analyses of microsomes incubated with several other drugs is ongoing and will also be presented.

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Dr Alain Beck[1]
[1]Institut De Recherche Pierre Fabre

Combination of Mass Spectrometry instruments and workflows for complex antibody-based products characterization

Keynote: Dr Alain Beck Laboratoires Pierre Fabre 129 - Combination of Mass Spectrometry instruments and workflows for complex antibody-based products characterization, Meeting Room 105, August 23, 2024, 11:30 - 11:55

Mass spectrometry (MS) offers high selectivity and specificity for the potentially unambiguous identification and comprehensive structural characterization of glycoprotein-based therapeutics. MS-based approaches are playing a central role in the biopharma laboratories, complementing, and advancing traditional biotherapeutics characterization workflows. A combination of MS approaches is required to comprehensively characterize antibody-based structures: the commonly employed bottom-up MS approaches are efficiently complemented with mass measurements at the intact and subunit (middle-up) levels, together with product ion analysis following gas-phase fragmentation of precursor ions performed at the intact (top-down) and subunits (middle-down; IdeS/ KGP-based) levels.

Methods

Among the MS-tool box, advantages of native and ion mobility MS, Collision Induced Unfolding (CIU), Cyclic CIU (cCIU), multiplexed Top and Middle-Down MS, multiple fragmentation techniques, comprising high energy collisional, electron-transfer and UV photo-dissociation (HCD, ETD and UVPD), Parallel Accumulation and Serial Fragmentation (PASEF, de novo sequencing), Capillary Electrophoresis hyphenated to MS (CE-MS) and quantification of trace-level Host Cell Proteins (HCPs) by MS have been used.
Antibody-based products Critical Quality Attributes (CQAs, such as N and O-glycans, size and charge variants) and case studies for FDA, EMA, NMPA approved biologics have been used.

Novel Aspect

Our studies show the capabilities of cIM-MS for therapeutic antibodies, paving the way for HR-IM-MS/CIU characterization of more complex Biologics.

Preliminary Data or Plenary Speaker Abstract

In addition to previous MS methods, we first assessed the extent to which multipass cIM-MS experiments could improve the separation of Antibodies Isotypes and Antibody Drug Conjugates. These initial results evidenced some limitations of HR-IM-MS for large native biomolecules which possess complex conformational species that remain challenging to decipher even with higher IM resolving powers. Conversely, for collision-induced unfolding (CIU) approaches, higher resolution proved to be particularly useful (i) to reveal new unfolding states and (ii) to enhance the separation of coexisting activated states, thus allowing one to apprehend gas phase CIU behaviors of mAbs directly at the intact level.

Prof Magdalena Biesaga, Ph.D, D.Sc. Renata Godlewska, Dr Artur Kulesza, Ph.D, D.Sc. Dorota Korsak
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Identification of surfactants with antifungal activity produced by antarctic bacteria Bacillus subitlis strain.

Magdelena Biesaga 37 - Identification of surfactants with antifungal activity produced by antarctic bacteria Bacillus subitlis strain, Meeting Room 105, August 23, 2024, 11:55 - 12:14

One of the greatest challenges to public health is the growing phenomenon of drug resistance to commonly used antimicrobial drugs. To counteract this, a great deal of research is being undertaken to find new bioactive substances that can find applications in medicine, veterinary medicine, but also in the food industry to ensure the microbiological safety of food. Therefore, scientists are increasingly interested in biosurfactants produced by different microorganisms. It is known that some of them exhibit biological activity, including antibacterial or antifungal properties. Bacillus subtilis strains produce a broad spectrum of bioactive cyclic lipopeptides with potential antibacterial and antifungal properties.
The main goal of the research was to identify the composition of biosurfactants produced by antarctic Bacillus subtilis ANT_WA51.

Methods

A volume of 1 mL of a pre-culture B. subtilis WA_51 using McFarland Standard 0.5 was inoculated into 250 ml shake flask containing 50 mL medium to determine the optimum substrate for surfactant production strain growth, surface tension, were compared. Four different media M9 with glycerol, glucose, molasses, and LB were applied. The experiment was conducted in triplicate. The flasks were shaken at 120 rpm for 7 days at 25o C and the measurements were performed every 24 h. LC-MS/MS and LC-HRMS were used to identify the lipopeptides in such solutions after each day. The inhibitory effect of biosurfactant towards fungal growth was studied using plate diffusion technique.

Novel Aspect

New biosurfactants - lipopeptides produced by B.subtilis ANT_WA51 have been identified. The antifungal lipopeptide properties are first presented.

Preliminary Data or Plenary Speaker Abstract

The comparison of media compositions showed that the best media for surfactants productions were M9 with glucose and LB. In all media surfactin homologues were identified after 48 hours, while fengycines appeared after 36 hours and amount of these compounds was stable for next days. Structures for the detected surfactants were proposed based on the acquired HR-MS and MS/MS spectra and literature [1]. LC–MS analysis of extracts has shown several pseudomolecular ion peaks corresponding to fengycin homologues at retention time range 12-15 minutes at m/z 1448, 1462, 1491.9; 1506, 1518. Whereas, the retention times in the range 23-36 minutes were characteristic for surfactins homologues. Characteristic ions at m/z 1022.7, 1036, 1044.6, 1058.6 correspond to surfactins of different fatty acid chain lengths. LC/MS/MS were applied for the identification of different types of fengycines and surfactins. Based on product ions spectra the class of compounds were identified: surfactins (m/z values of 1022.7, 1036.7 and 1058.6) with fatty acid chains varying from C14 to C16. LC/MS/MS analysis of protonated molecules [M + H]+ of fengycin showed that fengycin A C17, fengycin B C16, fengycin A C19/fengycin B C17, fengycin A C16 and fengycin A C20 unsaturated/fengycin B C18 unsaturated were identified.
Biosurfactants produced by B. subtilis WA_51 were tested for their antifungal activity. Among the species tested, Fusarium oxysporum considered the most sensitive. Biosurfactants showed prominent inhibition against Fusarium spp. and Botrytis cinerea. Alternaria spp. were also inhibited but to a lesser extent.

[1] Krucoń, T., Ruszkowska, Z., Pilecka, W., Szych, A., & Drewniak, Ł. (2023). Bioprospecting of the Antarctic Bacillus subtilis strain for potential application in leaching hydrocarbons and trace elements

from contaminated environments based on functional and genomic analysis. Environmental Research, 227, 115785.

Professor Troy Wood[1], Connor Gould[1], Professor Qing Ma[1], Professor Raymond Cha[1], Dr. Valerie Frerichs[1], Professor Alan Friedman[1], Ming Zhang[1], Professor Jun Qu[1], Robin Difrancesco[1], Professor Gene Morse[1]
[1]University At Buffalo

Quality Assurance Using Mass Spectrometry to Analyze Structural Fidelity of Monoclonal Antibodies in HIV-1 Therapeutics

Troy Wood 230 - Quality Assurance Using Mass Spectrometry to Analyze Structural Fidelity of Monoclonal Antibodies in HIV-1 Therapeutics, Meeting Room 105, August 23, 2024, 12:14 - 12:33

The development of broadly neutralizing antibodies (bNAbs) for the prevention and treatment of HIV-1 infection has gained attention as an alternative to conventional antiretroviral therapy because of their potential for passive immunotherapy and long half-lives. A number of bNAbs targeting the HIV-1 viral envelope are under investigation for their effects on viral suppression in combination. While methods to quantify bNAbs using mass spectrometry are one element of quality assurance, it is also important to validate the structural fidelity of therapeutic antibodies; this includes not only validation of the primary amino acid sequence, but also the presence and location of post-translational modifications (PTMs), many of which are essential for optimal efficacy of the antibody.

Methods

A bottom-up proteomics workflow was developed using the NIST mAb IgG1k reference material, used frequently in development of antibody-based proteomics workflows. The approach uses RapiGest to unfold the antibody, followed by reduction and alkylation steps prior to enzymatic digestion. This approach was then applied to the quality assurance of the primary structure of the therapeutic bNAb PGT 121.414.LS, which is currently being evaluated for efficacy of viral suppression in clinical trials in combination therapies. Enzymatic digests of antibodies were analyzed using Sequest node through Proteome Discoverer ver. 2.3; MS/MS using CID was performed to confirm amino acid sequence and locations of PTMs. Glycan sites on PGT 121 antibodies are essential for recognition of HIV-1 envelope glycoprotein are of particular interest.

Novel Aspect

Application of proteomic workflows to assess structural characteristics of a broadly neutralizing antibody used in treatment and prevention of HIV-1.

Preliminary Data or Plenary Speaker Abstract

Initial tryptic peptide maps of NIST RM 8671 revealed 30 peptides from the heavy chain and 14 from the light chain, accounting for 84% sequence coverage for the light chain and 80% for the heavy chain. Numerous PTMs were identified including oxidation, deamidation and glycosylation throughout the antibody. A number of glycans were determined to be present on Asn-300 in the consensus sequence EEQYNSTR coinciding with glycopeptides previously reported. The two highest abundance glycans present correspond to the glycans HexNAc(4)Hex(3)Fuc(1) and HexNAc(4)Hex(4)Fuc(1). The tryptic peptide mapping method developed for NIST RM 8671 was then applied to PGT121.414.LS. Initial peptide mapping results indicated 97% sequence coverage of the light chain and 80% of the heavy chain. 41 peptides were identified from the heavy chain and 21 peptides were identified from the light chain. Full coverage of the LCDR2, LCDR3, HCDR2 were obtained along with partial coverage of the LCDR1. No tryptic peptides derived from the HCDR1 or HCDR3 regions were detected. Additional digestion utilizing another protease is necessary for mapping these two regions. Numerous PTMs were identified for PGT 121.414.LS including oxidation of methionine and deamidation of asparagine and glutamine. Peptides derived from the complementary determining regions were closely investigated for the presence of PTMs. Peptides identified from the complementary determining region did contain PTMs. The glycosylation profile of PGT 121.414.LS revealed a number of glycopeptides for the backbone sequence EEQYNSTR. The identified glycoforms present included HexNAc(4)Hex(3)Fuc(1) and HexNAc(4)Hex(4)Fuc(1).

Additionally, glycopeptides with masses corresponding to HexNAc(4)Hex(5)Fuc(1), HexNAc(3)Hex(3)Fuc(1), and HexNAc(2)Hex(3)Fuc(1) were identified. The bottom-up results indicate numerous glycoforms present in the sample of PGT 121.414.LS.

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Dr. Adam Pruška[1], Dr. Julian Harrison[1], Dr. Renato Zenobi[1]
[1]ETH Zurich

Temperature-Controlled Mass Spectrometry as a Tool for Structural Characterization of Enzymes and Antibodies

Adam Pruška 345 - Temperature-Controlled Mass Spectrometry as a Tool for Structural Characterization of Enzymes and Antibodies, Meeting Room 105, August 23, 2024, 12:33 - 13:00

Determining the structure-function relationship and degradation pathways of proteins is essential for biotechnology research and industry. However, engineering these proteins is often time-consuming and expensive, as it is challenging to identify conformational and degradation products that occur under conditions for optimal enzyme activity. This gap in analysis necessitates developing inexpensive techniques with low sample consumption that can provide detailed structural information, allowing for efficient protein engineering. To overcome these challenges, we use native mass spectrometry (MS), specifically by coupling a source capable of controlling spray solution temperature in a nanoelectrospray emitter. The temperature-controlled nanoelectrospray mass spectrometry (TC-nESI-MS) has the ability to elucidate the thermal properties of peptides, proteins, and oligonucleotides within hours. (Harrison et al., Chem. - Eur. J., 2021).

Methods

Enzyme samples were prepared at µM concentrations in nuclease-free water with 200 mM ammonium acetate (AA) at pH 6. The temperature-controlled nanoelectrospray ionization (TC-nESI) source consists of the nESI emitter placed between two copper blocks controlled by Peltier element (Marchand et al., J. Am. Chem. Soc. 2018). A Peltier element is used to precisely control the temperature. Ion mobility mass spectra were acquired in positive mode utilizing a SELECT SERIES cyclic IMS mass spectrometer (Waters, Wimslow, UK). For these experiments, β-galactosidase, alcohol oxidase, and standard IgG were heated from 25 to 90 ℃ at a rate of 1 ℃/min. Enzymatic activities was determined using a colorimetric assay.

Novel Aspect

Rapid and detailed MS-based method for determining the thermal denaturation and degradation pathways of enzymes and antibodies.

Preliminary Data or Plenary Speaker Abstract

To demonstrate the utilization of TC-ESI-MS for protein structure-function elucidation, we investigated enzymes and antibodies dissociating into multiple intermediate species and fragments during the heat ramp. The relative abundance of charge state series for monomer and fragmentation products changes significantly, with an apparent Tm between 40 – 50 °C. However, as these species decrease in abundance, fragment ions become dominant. These fragments also match the fragmentation pattern observed over time in the solution and have been confirmed by top-down sequencing. Compared to the enzyme kinetics, the temperatures where fragments are primarily present coincide with the temperature where the highest velocity of reaction (Vmax) was observed for β-galactosidase but where the affinity represented by the Michaelis constant (KM) significantly decreased.
The ion mobility data of β-galactosidase acquired during the TC-nESI-MS indicated that none of the analytes underwent significant unfolding during the heating experiments, suggesting the fragments of the investigated enzyme remain active. The fragment masses indicate peptide cleavage between amino acids Ala-736 and Ile-737 in a disordered region of β-galactosidase. Fragmentation between these amino acids would leave the catalytic site intact on the 83.6 kDa fragment, explaining why this enzyme can maintain its activity despite fragmentation. However, Trp-999, located on the second smaller fragment, is a vital substrate binding residue, explaining the change in the Km as fragmentation occurs. The dissociation of the smaller fragment would then explain the reduction in the change in the ability of the enzyme to bind the substrate.

This study introduces TC-nESI-MS as a novel method, offering fast in-solution structural stability information about enzymes and IgGs. When coupled with enzyme kinetics, it reveals connections between enzymatic activity and structural changes in β-galactosidase and alcohol oxidase. This underscores the potential of applying TC-nESI-MS to enhance the understanding of biomolecules crucial for enzyme engineering and biotherapeutics development.

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Dr. G. Asher Newsome[1], Dr. Tjaša Rijavec[1,2], Leah Bright[3]
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Application and Accessibility Improvements for Real-time, Minimally-invasive, Non-proximate Sampling of Conserved Art Objects

Keynote: Dr G. Asher Newsome Smithsonian Museum Conservation Institute 46 - Application and Accessibility Improvements for Real-time, Minimally-invasive, Non-proximate Sampling of Conserved Art Objects, Meeting Room 106, August 23, 2024, 11:30 - 11:55

Minimally-invasive and noninvasive analysis of intact objects is desirable to avoid compromising aesthetic qualities, cultural sensitivities, and other concerns that frequently attend art and heritage items. Non-proximate desorption photoionization mass spectrometry (NPDPI-MS) was designed expressly for the purpose of accessing objects of moderate and greater-than-moderate size which must be positioned over one meter from the mass spectrometer, performing timed thermal desorption sampling to avoid solvent exposure or any visible effect on the sampled surface. The system was previously demonstrated with relatively dense and heat-resistant wooden objects. Real-time, damage-free analysis of polyvinyl chloride (PVC) art objects more susceptible to thermal deformation is demonstrated here and compared to a swabbing/extraction/gas chromatography (GC)-MS methodology. A new, handheld analyte transfer system is also introduced.

Methods

For NPDPI, test samples and objects were positioned 1.5 m from an Orbitrap Elite (Thermo Fisher). A timed jet of neutral nitrogen was supplied at up to 200 °C and 1.0 L/min from a custom-built probe angled at 45° and mounted from overhead. Thermally desorbed material entered a 198 °C, SilcoNert-coated, 2 m long stainless steel transport line. Anisole dopant was added to the analyte/air mixture in transit by an in-line permeation tube. Analyte was conducted past a krypton vacuum ultraviolet lamp (Syagen) for photoionization at the entrance to the differentially-pumped inlet to the mass spectrometer. For comparative analysis, Texwipe polyester swabs passed over objects were extracted with hexane and analyzed on a 7890-5975B Agilent GCMS.

Novel Aspect

PVC films and art objects are analyzed with NPDPI. A novel transfer tube and surface sensor make the system handheld.

Preliminary Data or Plenary Speaker Abstract

The original NPDPI system with rigid stainless steel transfer line was used to study thermal exposure effects on PVC test films of varying composition, thickness, and texture.
Objects were raised into position below the gas probe and transfer inlet using either an adjustable-height table or a Genie platform load lifter. Depending on the temperature of the gas jet and distance from the film, the surface might exhibit no discernable change, clarify from slight melting, or be fully pitted, producing increasing plasticizer ion signal. A standard exposure of 140 °C for 2 s was employed, as opposed to the previous 150 °C for 3 s with wooden objects. Three PVC art objects from Smithsonian American Art Museum collections with maximum dimensions up to 121 cm were directly analyzed for surface plasticizer in comparison to GCMS results. The analysis produced signal from (di(2-ethylhexyl) phthalate (DEHP), diisodecyl phthalate (DIDP), diisononyl phthalate (DINP), dioctyl terephthalate (DOTP) and other plasticizers, but no visible deformation the objects was recorded. Swabs were also analyzed with NPDPI, taking advantage of the quick analysis time and removed restrictions on invasiveness.

Heated, SilcoNert-coated steel is necessary to prevent desorbed analyte from sticking to the transfer line interior, but multiple engineering practicalities require the line be ¼” od tubing. Such tubing can be shaped but is ultimately rigid, making it unable to be hand-manipulated like polymer tubing. To produce a flexible, coated-steel transfer tube a gooseneck construction was implemented. A contact sensor was also added to the non-proximate end of the transfer, along with a redesigned gas jet probe. The compact assembly was built to be inserted into a blind concavity without stabbing the surface.

MSc. Teodora Raicu[1], Dr. Matea Krmpotić[2], Dr. Zdravko Siketić[2], Dr. Iva Bogdanović Radović[2], Univ.-Prof. Dipl.-Biol. Dr. Katja Sterflinger[1], Dipl.-Ing. Dr.techn. Dubravka Jembrih-Simbürger[1]
[1]Institute for Natural Sciences and Technology in the Arts, Academy of Fine Arts Vienna, [2]Division of Experimental Physics, Laboratory for Ion Beam Interactions, Ruđer Bošković Institute

MeV SIMS Approach for Identifying Colorants in Artists' Modern Inks

Teodora Raicu 506 - MeV SIMS Approach for Identifying Colorants in Artists' Modern Inks, Meeting Room 106, August 23, 2024, 12:14 - 12:33

The MeV SIMS (secondary ion mass spectrometry with MeV primary ions) approach was tested for identifying colorants and colorant mixtures in modern inks found on artistic works such as caricatures, drawings, prints, etc. Unlike other MS-based techniques commonly employed in the analysis of inks, MeV SIMS examines solid ink samples without chemical preparation with solvents or colorant separation from mixtures prior to analysis. This is highly advantageous when working with very small sample sizes, as is often the case in the field of cultural heritage. Moreover, the use of MeV SIMS was encouraged by its proven efficiency in generating high yields of molecular ions and (de)protonated molecules of synthetic organic colorants (SOCs) caused by the soft ionization of the molecules.

Methods

Mockups were prepared on Japanese paper with original modern inks from Erich Sokol's estate (Austrian artist, 1933-2003) also used in his caricatures and kept at the State Collections of Lower Austria. The selected ink materials included acrylic inks (AeroColor/Schimncke and ArtiColor/Rotring) and watercolors (Ecoline/Royal Talens). Samples from dried mockups were directly analyzed with 8 MeV Si^{4+} primary ions. The MeV SIMS setup used is equipped with a linear TOF spectrometer. Spectra were measured in positive- (+5kV) and negative-ion (-4kV) mode. Mass calibration was conducted for each spectrum by selecting lower-mass ions (H^+, Na^+, phthalic anhydride ions in acrylic inks, $^{35}Cl^-$ and $^{37}Cl^-$ in the watercolors) and higher-mass ions (mostly molecular ions) to cover the entire mass range (~m/z 0-1800).

Novel Aspect

Exact identification of colorants/colorant mixtures in artists' modern inks on paper by MeV SIMS without their separation prior to analysis

Preliminary Data or Plenary Speaker Abstract

For acrylic inks, both positive- and negative-ion mode MeV SIMS spectra clearly revealed molecular ions and (de)protonated molecules of synthetic organic pigments (SOPs). In positive-ion mode, a high secondary ion yield was obtained for the species corresponding to SOPs, with peaks surpassing 90% in intensity upon normalization. Red acrylic inks primarily contained Naphthol AS pigments, with the $[M+H]^+$/$[M+H]^-$ of PR112 detected at m/z 484, and the $[M]^+$/$[M]^-$ of PR122 at m/z 340. Large fragment ions indicative of these red pigments were also detected in positive-ion mode, such as m/z 377/378 generated by the cleavage at PR112's amide bond. Blue and green acrylic inks predominantly featured SOPs from the phthalocyanine class, such as PB15:1 ($[M]^+$ at m/z 575, $[M+Cl]^+$ at m/z 609/611), and PG7 and PG36 ($[M]^+$/$[M]^-$ at m/z 1127 and 1838, respectively. In yellow acrylic inks selected from the AeroColor line lightfast SOPs were present, e.g. PY83 ($[M+H]^+$ at m/z 816), a diarylide pigment, whereas those from the ArtiColor line included less lightfast SOPs, such as PY74 ($[M]^+$ at m/z 386), a monoazo pigment. Violet acrylic inks included either one pigment, PV23 ($[M]^+$/$[M]^-$ at m/z 588), a dioxazine pigment, or a mixture of red and blue SOPs, such as PR122 and PB15:1. For watercolors, molecular ions corresponding to the constituent dyes were observed only in negative-ion mode, which suggested their acidic nature. Acid dyes, AR88 ($[M–Na]^-$ at m/z 377) and AY73 ($[M–Na]^-$ at 332), were determined. Despite this, identifying dyes was challenging due to the limited number of MeV SIMS reference spectra available up to now. Nonetheless, the

presented MeV SIMS approach efficiently identified synthetic organic pigments and dyes present in the analyzed artists' modern inks on paper.

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Dr Dylan Multari[1], Geraldine J. Sullivan[1], Mary Hartley[2], Ronika K. Power[2], **Professor Paul Haynes**[1]
[1]School of Natural Sciences, Macquarie University, [2]Department of History and Archaeology, Macquarie University

Proteomic analysis of bone collagen from a collection of worked bone artefacts from Pyrmont, Australia

Paul Haynes 288 - Proteomic analysis of bone collagen from a collection of worked bone artefacts from Pyrmont, Australia, Meeting Room 106, August 23, 2024, 12:33 - 13:00

The analysis of collagen sequences as markers of species identification is becoming increasingly widely used in archaeological sciences. This has advantages over traditional morphology-based identification methods because it works equally well with fragmentary bone samples. In this study, we applied nanoLC-MS/MS to the analysis of collagen peptides extracted from a collection of early colonial (c. 1830) worked bone knife handles excavated from Pyrmont, Sydney, Australia in 2017. These bone handles display no distinguishable morphological features that are typically used in osteoarchaeological analyses of species; thus, a molecular approach is required for their identification.

Methods

We analysed bone collagen from a collection of early colonial bone knife handles using three established collagen extraction protocols - ammonium bicarbonate gelatinisation, and acid demineralisation in 0.6M HCl followed by acid soluble and acid insoluble fractionation using 30kDa ultra centrifuge spin filters. Extracted proteins were digested with trypsin and resultant peptides were analysed via nanoLC-MS/MS on a Thermo Q Exactive Orbitrap coupled with a Thermo Easy-nLC1000 liquid chromatography system. A shotgun proteomic approach was used for nanoLC-MS/MS, consisting of a 1 hour reversed-phase gradient. Database searching was conducted using MaxQuant against the curated SwissProt database supplemented with additional collagen sequences added for domestic species.

Novel Aspect

Identifying the source species of a collection of early colonial bone knife handles from Pyrmont, Australia

Preliminary Data or Plenary Speakers Abstract

All three collagen extraction protocols were successfully able to produce peptides for subsequent analysis via nanoLC-MS/MS. Deamidation assessment of peptide data was performed in RStudio using in-house scripts. The bone knife handle samples were found to be deamidated to a similar degree as was observed for a set of previously analysed modern (<50yo) animal bones prepared using the same extraction protocols. For the modern bone standards, as well as the “usual suspects” such as cows, horses, pigs and sheep, numerous other wild species also represent potential sources of bone for use as materials. These include whale bones from commercial shipping, and also large native animals such as kangaroos, so all of these were included in the set of reference bones analysed.

The results from the knife handles indicated that the bones were from bovine species, and hence most likely from domestic cattle. The ability to identify which species were used to craft these bone artefacts allows us to better understand life in the early colonial era of Sydney. Pyrmont was known to be a working class district in the early settlement of Sydney, and people were often reliant on being resourceful as a means of getting by. As such, it was not uncommon for materials to be recycled and reused since the option to buy new was often unfeasible.

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Isaure Sergent[1], Georgette Obeid[2], Dr Thibault Schutz[2], Dr Jean-François Lutz[2], Pr. Laurence Charles[1]
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Combining Ion Mobility with Molecular Modeling to Rationalize the MS/MS Behavior of Biradical Oligomer Anions

Isaure Sergent 483 - Combining Ion Mobility with Molecular Modeling to Rationalize the MS/MS Behavior of Biradical Oligomer Anions, Meeting Room 109, August 23, 2024, 11:30 - 11:48

The structure of digital polymers is usually optimized to best control their MS/MS fragmentation and so achieve the full sequence coverage requested to read their binary message. In particular, large amounts of information stored in long chains of poly(phosphodiester)s (PPDEs) are best recovered using a byte-truncation strategy. In this block architecture, weak C–ON bonds placed between each group of eight co-monomers are cleaved after soft activation of the chains, so-released coded blocks being small enough to ensure de novo sequencing. Yet, the dissociation behavior of inner blocks was observed to depend on their charge state, most probably due to their biradical character. Here, energy-resolved MS/MS experiments are combined with IMS analysis and molecular modeling to investigate this phenomenon.

Methods

All experiments were performed with a Waters Synapt G2 HDMS instrument where mass selected PPDEs chains are activated in the ion trap in front of the traveling wave ion mobility cell so that blocks released as primary fragments can be investigated by IMS and individually sequenced in the post-IMS collision cell. Polymers were ionized in negative ion mode electrospray and the charge state of precursor ions was selected to enable either two or three negative charges per block. Experimental collision cross sections were derived from arrival times using a calibration procedure developed for polyanions with the IMSCal software. A multi-step protocol based on quantum methods and classical molecular dynamics was implemented for molecular modeling and calculation of theoretical CCS.

Novel Aspect

Cyclization of biradical anions prior to their dissociation as evidenced by ion mobility experiments

Preliminary Data or Plenary Speaker Abstract

In the negative ion mode ESI, preferential charge states observed for block-truncated PPDEs indicate 2 to 3 deprotonated phosphate groups per block. Collision induced dissociation (CID) of triply charged inner blocks produces the eight ion series expected from bond cleavage at each phosphate moieties. In contrast, additional fragment families were produced upon activation of blocks at the 2– charge state. All fragments still holding the nitroxide α termination were also detected with +1 m/z shift whereas product ions containing the carbon-centered radical at the ω end were also observed at –1 m/z. Although not preventing reliable sequencing of the blocks, this complicates MS/MS data and somehow contributes to fragment signal dilution. These results can only be rationalized by considering cyclization of these biradical species followed by H• transfer upon activated reopening of this cycle. This yields close-shell anions with a hydroxylamine α-group and one double bond at the ω side. Strong electrostatic repulsions at work in triply charged blocks would avoid this cyclization process. Owing to the very different conformations expected for cyclic and open blocks, IMS experiments were performed to tentatively support this assumption. IMS experimental conditions could be found to resolve the two isomeric forms, the most compact conformation being observed in soft conditions while signal of the most extended conformation increases as the collision energy is raised. Calculated CCS obtained after molecular modeling of the two isomers are consistent with

experimental data derived from IMS experiments and, as expected, the most compact form corresponds to the cyclic isomer.

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Mr Brett Burns[1], Dr Samuel Marlton[1,2], Dr Boris Ucur[1], Prof Adam Trevitt[1]
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Optimizing the low-lying excited states and photodissociation of Norrish type I photoinitiator Acetophenone using group I cations in the gas-phase

Brett Burns 669 - Optimizing the low-lying excited states and photodissociation of Norrish type I photoinitiator Acetophenone using group I cations in the gasphase, Meeting Room 109, August 23, 2024, 11:48 - 12:06

The Minamata convention targets the reduction of Mercury usage to limit anthropogenic exposure. Consequently, industrial applications of photoinitiated polymer curing, previously utilising broad-band mercury vapor lamps, have shifted to the use of narrow-band light emitting diodes. This has spurred both a need and opportunity to better tailor the electronic properties of the next generation of photoinitiators. Oriented electric fields are a promising avenue to adapt current photoinitiators to tune the order of key excited states and thus promote efficient photopolymerization. This study provides a bottom-up, fundamental understanding of how oriented electric fields influence the electronic properties for a fundamental Norrish type I photoinitiator, acetophenone. Insight to the capabilities and limitations of tuning of photoinitiators using charge-based methods is also reported.

Methods

Gaseous acetophenone cations were generated via electrospray ionisation of a methanol solution containing acetophenone and Group I element acetate salts (e.g. Na+, Li + , K+). These were isolated and held in the ion trap of a ThermoFisher Scientific LTQ XL mass spectrometer and subsequently irradiated with UV wavelengths 350 - 225 nm generated by a tunable Nd:YAG pulsed laser. Spectra of photoproducts were obtained and compared against a reference neutral spectrum. Quantum chemical calculations and electric-field analysis were conducted to rationalise experimental transition ordering with specific focus on the low-lying singlet and triplet excited states, analysed with both time-dependent density functional theory and coupled-cluster methods.

Novel Aspect

This research assists in developing a fundamental understanding of the effects of local, oriented electric fields on photodissociation.

Preliminary Data or Plenary Speaker Abstract

Action spectra were obtained for each acetophenone cation and the maximum wavelengths of dissociation were compared against a reference spectrum of gaseous, neutral acetophenone. These transition wavelength maxima, alongside supporting quantum chemical calculations, reveal the shift in the 1ππ* excitation energy (TD-DFT). The shift is compared with the electric field strength offered by each of the bound cations. The experimental and theoretical results were in good agreement, both showing a linear trend of a stronger electric field correlating to a redshift in the dissociation profile.
A Coulombs Law approach was used to determine the strength of each oriented electric field. This model had not yet been tested with systems with a misalignment between electric field and excited state transition dipole vectors. This perspective was rigorously tested computationally for the acetophenone system, and it was found that there is a non-linear response between this alignment and expected electronic shift, supported by the experimental data.
Furthermore, despite a substantially strong electric field, protonation lead to other pathways that are not the target α-cleavage radical formation. This highlights that while it is possible to tune dissociation maxima using charge, care must be taken to determine if new photodissociation pathways are introduced.
Examining the weaker-bound cations, Cs+ and Rb+, indicated that cation-binding energy is another component that should be considered, as cation-loss is a significant channel in these cation-bound dimers, with the potential to outcompete α-cleavage.

This study determines the limits of optimizing photoinitiators with oriented electric fields, as well as exploring the developing predictive platforms for charge-based tuning methods. This was accomplished using a combination of gas-phase action spectroscopy and high-level quantum chemical methods.

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Ms. Vimanda Chow[1]
[1]York University

Probing the molecular mechanism of Constitutive Androstane Receptor (CAR) transactivation by Hydrogen-Deuterium Exchange Mass Spectrometry

Vimanda Chow 253 - Probing the molecular mechanism of Constitutive Androstane Receptor (CAR) transactivation by Hydrogen-Deuterium Exchange Mass Spectrometry, Meeting Room 109, August 23, 2024, 12:06 - 12:24

Research indicates that microbe-derived metabolites can mediate host gene expression and physiological outcomes. The Constitutive androstane receptor (CAR) is an important protein target of these microbial metabolites, impacting the expression of drug-metabolizing enzymes. Not to mention, CAR has been shown to play a role in numerous processes and diseases, such as diabetes, fatty liver disease, and various inflammatory disorders. However, the mechanism by which these metabolites cause different transactivation of CAR or even molecular switching still needs to be better defined. The discovery of these natural high-affinity ligands offers promising avenues for disease treatments. Compared to x-ray crystallography, amide hydrogen/deuterium exchange provides insight into protein dynamics and detects allosteric effects, aiding in understanding the underlying mechanism.

Methods

Bottom-up hydrogen-deuterium exchange mass spectrometry (HDX-MS) was employed to study the interaction between metabolites and CAR at a 1:10 protein:ligand ratio, where the final concentration of the ligand was 100uM in 100mM ammonium acetate pH 8.0 with 6% DMSO. Experiments were conducted using conventional HDX workflow at labeling times of 0,1,10, and 30 minutes prepared using a PAL3 autosampler coupled to an M-class ACQUITY UPLC and a Waters Cyclic IMS Mass Spectrometer. Peptide identification and uptake were analyzed using Protein Lynx Global Server (PLGS) and DynamX.

Novel Aspect

Microbe-derived metabolites are used to elucidate distinct transactivation mechanisms of Constitutive Androstane Receptor (CAR) using HDX-MS.

Preliminary Data or Plenary Speaker Abstract

As part of the superfamily of nuclear receptors, CAR contains a conserved activation function-2 (AF-2) helix 12 for ligand-induced coactivator/corepressor interaction. The degree of stabilization of the AF-2 surface is thought to correlate to the degree of transactivation; therefore, we probed the structural dynamics of CAR with microbe-derived ligands to examine this mechanism. To understand the role of dynamics in mediating transactivation activity, we performed comprehensive differential HDX-MS experiments on the following ligand-receptor complexes: CAR (1) full agonist, (2) partial agonist, (3) antagonist, (4) partial inverse agonist, and (5) full inverse agonist. Here, we present amide hydrogen-deuterium exchange (HDX) kinetics, revealing that each ligand class induced unique changes to the dynamics of the ligand-binding domain. Notably, the full agonist significantly reduced the rate of amide exchange kinetics for H12. However, the partial agonist significantly increased the rate of exchange for H12. In addition, H3 is another area of the receptor that demonstrated differential HDX following ligand binding.
Consequently, CAR activity may function autonomously from H12, with a noticeable compensatory effect observed in the stabilization of H3. This indicates that subtle changes in the ligand-receptor interaction leads to differences in the pharmacology of these agents, where the full agonist, despite the clinical benefits, has been associated with adverse effects. On the other hand, the partial agonist exhibits potency in terms of lowered toxicity and fast clearance.

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Mr. HIU LOK NGAN[1], Prof. ZONGWEI CAI[1]
[1]Hong Kong Baptist University

Application of Imaging Mass Spectrometry-Based Metabolomics to Early Liver Cancer Diagnosis

Hiu Lok Ngan 8 - Application of Imaging Mass Spectrometry Based Metabolomics to Early Liver Cancer Diagnosis, Meeting Room 109, August 23, 2024, 12:24 - 12:42

Cancer is the leading cause of death worldwide, accounting for nearly 10 million deaths in 2020. Approximately one in six deaths is due to cancer. In 2020, liver cancer was ranked 6th of the most common new cases of cancer, yet it was one of the most common causes of cancer death- 830,180 deaths. It is predicted that global mortality of liver cancer will increase by 55% in 2040. Many cancers can be controlled if detected early. However, it is relatively difficult to cure liver cancer as this disease is usually diagnosed in the middle or late stage. There are no widely recommended screening tests for those at average risk. Research on early liver cancer diagnosis is therefore necessary.

Methods

Fine-needle aspiration biopsy assessment is a test for confirming the presence of the malignant cell. This technique requires needle insertion at a precise position of an observable tumor for valid sampling. In this work, we aim to classify the liver carcinogenesis stages before tumor occurrence. As a proof-of-concept study, a mouse model is adopted. For each stage, 12 thin left liver cryosections are analyzed by imaging mass spectrometry. Each pixel belonging to the liver region is treated as one sample. Discriminative features are further shortlisted. The attained model is validated by an external independent dataset and deployed to test the glass smears of aspirates that are randomly sampled from the right liver to show the feasibility of translation to neoadjuvant.

Novel Aspect

Applications of imaging mass spectrometry and predictive modeling show the feasibility of early liver cancer diagnosis by analyzing glass smears.

Preliminary Data or Plenary Speaker Abstract

Preliminary results show that the 16-molecular feature XGBoost model used to classify age-matched control healthy tissue and hepatocellular carcinoma-bearing tissue has an accuracy of 99.16% and 66.52% for the 5:5 split testing dataset and the external independent dataset, demonstrating great predictive power. Application to glass smear exhibited an accuracy of 68.96% after inter-batch data integration. Another predictive model is going to be constructed for multi-group (healthy liver, liver fibrosis, live cirrhosis, and hepatocellular carcinoma) classification.

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Ing. Zuzana Vaňková[1], Tomáš Hájek[1], Robert Jirásko[1], Ondrej Peterka[1], Petr Šimek[2], Prof. Michal Holčapek[1]
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Continuous Comprehensive Four-Dimensional Lipidomics Approach for Analysis of Human Plasma

Zuzana Vaňková 300 - Continuous Comprehensive Four-Dimensional Lipidomics Approach for Analysis of Human Plasma, Meeting Room 109, August 23, 2024, 12:42 - 13:00

Nowadays, more emphasis is paid to the differentiation of individual lipid species and isomers because their analysis is necessary to understand the dysregulation of lipids within their biosynthetic pathways. Reversed-phase liquid chromatography is the most frequently used separation mode in liquid chromatography due to its high selectivity for various types of isomers. However, lipids are separated according to the fatty acyl/alkyl chain composition, leading to overlapping of lipid species from different lipid classes. On the contrary, supercritical fluid chromatography separates lipids according to the polar headgroup and provides very fast analysis. The combination of both approaches offers a good orthogonality and compatibility. The connection to mass separation brings a rather detailed identification of complex lipidomic samples.

Methods

Lipid extract of human plasma was analyzed by RP-UHPLCxUHPSFC/MS² system. The first dimension (^{1}D) was performed by YMC Triart C18 column (150 x 0.5 mm, 1.9 µm) with the flow rate of 8 µL/min. The gradient elution of acetonitrile/water and acetonitrile/isopropanol with 5 mM ammonium formate and 0.1 % formic acid in both phases was established for the separation with a total run time of 120 min. The second dimension (^{2}D) used Reprospher Si column (10 x 2.1 mm; 1.7 µm) with flow rate 5 mL/min, total run time 35 s, and gradient elution of CO_2 and methanol (used as modifier) with 1 % H2O and 30 mM ammonium formate. Fast DDA and MSE experiments were used for lipid characterization.

Novel Aspect

Continuous 4D lipidomic analysis represents a novel strategy for the comprehensive analysis of lipids in biological samples.

Preliminary Data or Plenary Speaker Abstract

The goal of this work was to develop a new comprehensive multidimensional method including the connection of RP-UHPLCxUHPSFC systems to MS for the complex lipidomic characterization of polar and nonpolar lipid subclasses in human plasma. Reversed-phase UHPLC with C18 column (150 x 0.5 mm, 1.9 µm) applied in ^{1}D allows the separation of lipids with low flow rate of mobile phase (8 µL/min), and UHPSFC in ^{2}D is used due to the speed of the analysis (35 s, 10 x 2.1 mm, 1.7 µm column). The development includes detailed optimization of separation in ^{1}D, matching the loop sizes, and rather fast separation in ^{2}D using the gradient elution. The connection with QTOF-MS allows high-speed scanning (10 Hz), high resolution, and MS/MS experiments (represented by fast DDA and MSE approaches), which provides additional structural identification and can be considered as additional dimensions for the comprehensive lipidomic analysis. The GC Image software was used for the graphical visualization and selected reconstructed ion chromatograms or precursor ion chromatograms were utilized to enhance the visualization of low-abundance lipid classes.
Due to the orthogonality of both systems and their connection to MS, we were able to identify over 300 lipid species from 16 lipid subclasses. Furthermore, the method yielded a larger number of identified non-polar lipids than both of our commonly used methods.
Authors acknowledge the support of Czech Science Foundation (GAČR) project No. 21-20238S and ERC Adv grant No. 101095860 sponsored by the European Research Council.

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Professor Vicki Wysocki1
1Ohio State University

Electrons and/or a surface: characterization of capsids, glycoproteins, and nucleoproteins

Keynote: Professor Vicki Wysocki Ohio State University 749 - Electrons and/or a surface: characterization of capsids, glycoproteins, and nucleoproteins, Meeting Room 110, August 23, 2024, 11:30 - 11:55

Native mass spectrometry has expanded in recent years as instruments have been adapted to transmit and detect larger ions that could not be measured previously. As measured ions increase in size, they also increase in heterogeneity and become more difficult to fragment. Examples of these molecular trends include biomolecular therapeutics, whose use has increased in recent years, driving the need for appropriate measurements.

Methods

Instrument performance will be described for an Ultra High Mass Range Orbitrap with ECCR-SID, a Bruker timsTOF Pro modified to include an extended m/z range quadrupole and SID, and a Waters Cyclic IMS instrument with SID and ECD. Samples are buffer exchanged into ammonium acetate or ammonium acetate plus charge reducing reagents.

Novel Aspect

Electron capture, surface induced dissociation, and charge detection mass spectrometry provide otherwise unavailable characterization details for heterogeneous protein complexes.

Preliminary Data or Plenary Speaker Abstract

This talk will focus on electron capture dissociation, electron capture charge reduction, ECCR, and surface-induced dissociation (SID) as effective techniques that can be coupled with charge detection mass spectrometry to characterize a range of biomolecular classes more effectively. Systems to be presented include viral capsid components bound to ligands or adeno-associated viral capsids, glycoproteins, DNA-protein complexes, and RNA-protein complexes. Electron capture can effectively modify the protein complex's charge or dissociate covalent bonds. SID cleaves non-covalent interfaces with lower energy (smaller interfacial area) preferentially cleaving at lower SID energies. Combinations of ECD/ECCR with SID with and without charge detection mass spectrometry (CDMS) will be illustrated for large complexes that are heterogeneous in glycosylation and protomer types.

Dr Nyasha Munjoma[1], **Dr Lee Gethings**[1], Dr Paolo Tiberi[2], Dr Laura Goracci[2], Dr Jayne Kirk[1], Mr Richard Lock[1]
[1]Waters Corp, [2]Molecular Discovery

Comprehensive discovery lipidomic workflow which utilizes a prototype, multi-reflecting ToF with integrated informatics, providing highly confident lipid characterization and quantification

Lee Gethings 216 - Comprehensive discovery lipidomic workflow which utilizes a prototype, multi-reflecting ToF with integrated informatics, providing highly confident lipid characterization and quantification, Meeting Room 110, August 23, 2024, 11:55 - 12:14

Lipidomics allows researchers to probe changes in the lipidome as a result of disease, treatment, lifestyle, etc. Analysis of these lipids in a discovery mode is normally performed by a combination of liquid chromatography (LC) and accurate mass spectrometry (MS). Despite developments in analytical technology the detection and identification of lipids remains a significant challenge. Here we show the key benefits of a novel, benchtop MS and the features it provides to help overcome some of the drawbacks outlined for lipid analysis. Combining this novel MS data with third-party informatic solutions, demonstrates a powerful lipidomic workflow. The benefits of this approach are demonstrated using plasma samples from colon and rectum cancer and healthy control plasma.

Methods

Lipid standard mixes containing a range of lipid classes and the NIST SRM 1950 plasma, were used to assess instrument performance. As a proof-of-concept (POC) colon and rectum cancer and healthy control plasma were used to demonstrate the workflow. Study samples were prepared using protein precipitation with IPA solvent containing premixed deuterated standards. The resulting supernatant was chromatographically separated using a high throughput lipid profiling method which consisted of a 12-minute gradient. The eluent from the chromatography system was coupled to a prototype, benchtop multi-reflecting ToF mass spectrometer. The subsequent data were then processed using third-party informatics to provide lipid identifications and relative quantification. Statistical analysis involving multivariate analysis (MVA) was used to establish group differences.

Novel Aspect

Enhanced lipidomic workflow using a prototype, benchtop multi-reflectron ToF for comprehensive detection and identification of key biological lipids.

Preliminary Data or Plenary Speaker Abstract

Lipid extracts originating from the standard mixes was first assessed to establish instrument performance. Data were acquired using either data dependent (DDA) or data independent analysis (DIA). Resolution and mass accuracy were initially evaluated, achieving 100,000 (FWHM) and 200 ppb respectively for each lipid component. Based on previous literature, the most commonly identified lipids from human plasma (based on the NIST standard) highlighted the reduction in false positive identifications following database searching due to the high mass accuracy provided. A dilution series of lipid standards (0 to 1000ng/mL) spiked into a matrix of human plasma also benchmarked the level of sensitivity achieved from the MS platform. Data showed that sensitivity levels equivalent to tandem quadrupole measurements were readily achieved, whilst an in-solution dynamic range of >4-orders was also demonstrated. Data acquisition rates of 100Hz were utilized for the MS methods, providing the ability to run with faster gradient profiles. Cancer-based study samples were prepared using the same protocol described for the lipid standards. Data processing via Lipostar software was used for peak picking, data normalisation and lipid identification. Statistical analysis involving a range of MVA tools showed clear differentiation between the cancer types and healthy controls. Identification of the differential markers responsible for the group separation, was conducted using a database comprised of LIPIDMAPS and in-house libraries. The primary lipid classes identified included

ceramide (Cer), phosphocholine (PC) and sphingomyelin (SM). Integrated pathway analysis revealed that these lipid species were implicated in the signalling and cell proliferation pathways.

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Comparison of high-resolution mass spectrometry acquisition methods for the simultaneous quantification and identification of per- and polyfluoroalkyl substances (PFAS).

Jordan Partington 459 - Comparison of high-resolution mass spectrometry acquisition methods for the simultaneous quantification and identification of per- and polyfluoroalkyl substances (PFAS), Meeting Room 110, August 23, 2024, 12:14 - 12:33

Per- and polyfluoroalkyl substances (PFAS) are ubiquitous environmental contaminants that amass more than 6.5 million individual compounds and are present in the blood of more than 99% of the population. High-resolution mass spectrometry (HRMS) enables the simultaneous quantification and identification of these chemicals. HRMS can acquire both MS1 and MS2 data, with MS2 data acquired by data-dependent acquisition (DDA) or data-independent acquisition (DIA). DDA acquires MS2 data of isolated precursor ions that satisfy predetermined thresholds or rules, such as abundance thresholds and inclusion lists. DIA acquires MS2 data of all precursor ions indiscriminately, without precursor isolation. Hence, with HRMS extensively employed for the analysis of PFAS a fundamental comparison of HRMS data acquisition methods was undertaken.

Methods

The 25 PFAS of U.S. EPA Method 533 were used for the comparison of HRMS acquisition methods. HRMS data was acquired on an Agilent 1260 Infinity II LC system coupled to an Agilent 6546 quadrupole time-of-flight mass spectrometer. The acquisition methods investigated were MS-Only (MS1), DIA: all-ion fragmentation (All-Ions, DIA), and automated tandem mass spectrometry (Auto-MS/MS, DDA). Acquisition methods were compared by individual analyte response, quantitative accuracy and precision, limit of quantification (LOQ), and identification limit (IL) in various identification workflows (target-suspect screening and MS/MS spectra match). LOQs were also compared to those achieved by a triple quadrupole mass spectrometer acquiring data in a multiple reaction monitoring (MRM) method (Agilent 1260 Infinity II LC system coupled to an Agilent 6495C).

Novel Aspect

Fundamental comparison of HRMS acquisition methods, aiding researchers transition from TQ-MS to HRMS as the number of unique PFAS increase.

Preliminary Data or Plenary Speaker Abstract

PFAS are an expanding chemical class, more than 6.5 million PFAS exist in the PubChem tree, and as a result researchers are shifting from using triple quadrupole (TQ) mass spectrometry to HRMS for the detection and measurement of PFAS. A fundamental comparison of HRMS data acquisition methods (MS1, DDA and DIA) was undertaken and further compared to TQ MRM.

MS1 achieved the lowest mean LOQ at 1.3 (range: 0.34–5.1) pg on column, DDA at 1.5 (0.20–5.1) pg on column, and DIA the highest at 2.1 (0.49–5.1) pg on column. However, the differences in the LOQ of individual PFAS studied between the acquisition methods were all within an order of magnitude. The MS1 LOQs were then compared with the TQ MRM LOQs, where it was revealed that TQ MRM achieved statistically significant lower LOQs for all PFAS with a mean of 0.047 (0.016–0.20) pg on column. Furthermore, HRMS LOQs were compared to historical TQ MRM methods and found to be comparable to those of published methods 10 years ago. Through the suspect screening workflow, MS1 achieved the lowest average IL of 0.99 (0.41–2.0) pg on column, then DIA 1.3 (0.20–5.1) pg on column and DDA 1.7 (0.50–10) pg on column.

Overall, all three acquisition methods demonstrated ultratrace PFAS measurements and are suitable for both the identification and quantification of PFAS. MS1 acquisition provided marginally lower LOQs, despite coming at the cost of levels of confidence in analyte identification. DIA is best suited to suspect screening workflows where library fragment data is available, whereas DDA is suitable for building libraries with fragment data and for screening against them.

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Effects of sustainable rejuvenator on aged British roads compared with commercial bitumen binder rejuvenator by FT-ICR MS

Benedict Gannon 560 - Effects of sustainable rejuvenator on aged British roads compared with commercial bitumen binder rejuvenator by FT-ICR MS, Meeting Room 110, August 23, 2024, 12:33 - 13:00

Bitumen binder holds together the aggregates in road surfaces. The bitumen binder ages when laid at high temperatures and over time with exposure to the environment due to the release of its volatile components and by oxidation, causing rutting and cracking. Replacing road requires new bitumen, a derivative of crude oil, which has environmental implications. A rejuvenator is a substance that can be applied to bitumen binder to restore its desirable qualities and make it fit for re-use. Waste vegetable cooking oil is produced at high rates and is desirable for use as a sustainable rejuvenator. Presented here is Fourier transform ion cyclotron mass spectrometry (FT-ICR MS) analysis of waste cooking oil as a rejuvenator compared with a commercial rejuvenator.

Methods

British road bitumen was aged for 20 hours in a Pressure Aging Vessel (PAV). Six rejuvenated samples were produced by mixing in commercial rejuvenation products (making up 3%, 4%, 5% of total volume) or waste cooking oil (making up 6%, 8%, 10% of total volume). All samples were dissolved in toluene, then diluted to 0.05 mg/ml in 70:30 propanol:toluene (v/v). Samples were introduced to 15 T solariX 2XR instrument by direct infusion, ionised by positive mode atmospheric pressure photoionisation (APPI).
Resulting spectra were calibrated using Bruker Daltonic's DataAnalysis assigned using Sierra Analytics' Composer and subsequently visualised and analysed using KairosMS, where assigned compositions were sorted by heteroatom content. For example, C10H40S1 [H] was sorted to the S1 [H] class.

Novel Aspect

FT-ICR MS analysis of original, aged, and rejuvenated bitumen for sustainable use in construction of British roads

Preliminary Data or Plenary Speaker Abstract

Between 12,000 and 18,000 peaks were assigned in each spectrum with less than 0.2 root mean squared error (RMS) ppm. The m/z range for all spectra was between m/z 185-1200. Average m/z of all spectra remained similar at around m/z 640. Resolving power was sufficient to separate OxSx species from Ox species at m/z 1000.
The assigned data was sorted into heteroatom classes to facilitate analysis. These were then plotted in class comparison plots as well as used to separate double bond equivalents (DBE) by heteroatom content to determine changes between samples. Aromaticity Index (AI) based plots were used to determine shifts in aromaticity between samples. Changes in class were represented as percentage contribution to overall change, making significant changes between samples easier to identify even in cases where most assignments between samples remained similar.
Preliminary data showed that aging results in smaller changes in odd-electron forming species, a trend seen in previous literature surrounding aging of petroleum-derived materials. An increase in combined relative intensity of OxSx [H] containing molecules and a corresponding decrease in the combined relative intensity of the Sx [H] containing molecules can be observed, especially in species

with lower DBE, implying selective oxidation of these species has occurred during aging. Additionally, both N1 and N1 [H] classes are slightly decreased during aging by relative intensity. Both rejuvenators appear to reverse the impacts of aging. Neither rejuvenator replenishes the missing nitrogen containing species, which might indicate an avenue for improvement.