1. Abstract: Keerthi Appala

Assessing the effects of exogenous fatty acids and FASII inhibitors on lipid profiles and growth patterns of *Staphylococcus aureus*

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Fatty acid synthesis (FASII) FASII inhibitors have garnered significant attention due to their ability to suppress FASII-dependent lipid synthesis in *S. aureus.* The incorporation of exogenous fatty acids (FA) from the infection resulted in therapeutic failure. Our research investigated the lipidomic changes in *S. aureus* parent strains after treatment with both saturated and unsaturated fatty acids (SFA &UFA) in the presence of the FASII inhibitor AFN-1252.

For lipid analysis, modified Bligh and Dyer technique was used, and the lipid profiles were analyzed using both hydrophilic interaction liquid chromatography (HILIC and reversed-phase liquid chromatography (RPLC) methods coupled with a Waters Acquity UPLC connected to a Synapt XS TWIM-MS. The lipidomics data underwent evaluation through Progenesis QI, Skyline, and EZ Info.
In Oleic acid (OA)treated JE2 strains, a tiny but significant ***(*P=0.034***) increase in PGs was detected, while AFN-1252 resulted in a statistically insignificant decrease in overall PG abundance relative to JE2 controls. The combination of oleic acid and AFN-1252 restored the PG levels to those seen in JE2 treated with oleic acid. Palmitic acid alone, on the other hand, showed no effect on PG abundances, but AFN-1252 supplementation influenced PG abundances. The combination of AFN-1252 and palmitic acid restored the PG levels closer to the EtOH controls but did not vary statistically from either FA or AFN-1252 treatment alone. These data show that, in the presence of exogenous SFAs, AFN-1252 has no effect on the amount of PGs that daptomycin targets but does influence PG abundances in the presence of exogenous UFAs. Overall, our findings indicate that the addition of an FA-rich medium reversed the *S. aureus'* fatty acid dependent- auxotrophy caused by the FASII inhibitor.

2. Abstract: Sophia Bamishaye

Identification of E. Coli Contaminant Protein During the Expression and Purification of MRI contrast agent hProCA32.Collagen1 Using Bottom-Up Proteomics Approach

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Magnetic Resonance Imaging (MRI) as a diagnostic and therapeutic tool has revolutionized medical imaging by providing non-invasive and detailed visualization of tissues and organs,

providing insight into many biological processes. To overcome the limitations of low sensitivity and specificity of Clinically approved MRI contrast agents, the development of contrast agents that possess high relaxivities and targeting capabilities for molecular imaging of disease biomarkers is imperative. The protein-based MRI contrast agent, hProCA32. Collagen1 is developed from scaffold protein parvalbumin engineered to a collagen type I targeting peptide with a flexible linker. The developed MRI contrast agent was expressed in the periplasmic and cytoplasmic space of bacterial cell E. coli. However, during the purification process, additional contamination proteins are observed along with hProCA32.Collagen1. Identification of such co-purified proteins is challenging by traditional methods. In this study, we employed LC-MS and bottom-up proteomics methods to identify the co-purified proteins. Initially, three sample fractions (A, B, and C) were characterized by LC-MS method. Sample fraction B showed a single protein of hProCA32.Collagen1 with an experimental mass of 14,394 Da which agrees with the theoretical mass. However, sample fractions A and C showed the presence of additional proteins along with hProCA32.Collagen1. To identify the co-purified protein, the samples were digested with trypsin, and peptides were separated by RP-LC using Vanquish Flex HPLC with a 90-minute gradient. An Orbitrap Exploris 240 Mass Spectrometer was used to identify the peptides employing a data-dependent analysis method. Proteome Discovery Software was used to search the LC-MS/MS data against the E. coli proteome from UniProt using the SEQUEST algorithm. Our preliminary results identified the hProCA32.Collagen1 protein with 66% sequence coverage with an additional periplasmic chaperone protein from E. Coli with high confidence. This study showed bottom-up proteomics can identify the co-purified contaminant proteins when they display a size analogous to the recombinant protein.

3. Abstract: Sydney Bedillion

Optimization of a multi-omic workflow for biomarker discovery in a medaka fish model of chronic low dose ionizing radiation exposure

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The effects of high dose ionizing radiation have been extensively characterized to include single and double stranded breaks in DNA, protein damage, metabolic complications, and severe risk for cancer. Human exposure to high dose ionizing radiation is relatively rare; however, exposure to low dose ionizing radiation (LDIR) is common through both medical and environmental sources. The impact of LDIR is much less understood and has been difficult to assess without the identification of an informative set of biomarkers. The development of efficient and reproducible multi-omic workflows for proteomic, lipidomic, and metabolomic analyses will facilitate the elucidation of LDIR specific biomarkers. We have conducted a large-scale LDIR exposure study with the model teleost fish, medaka. Previously established extraction protocols taking advantage of readily available solvents are applied to wild type medaka fish liver and brain tissue to determine suitability for multi-omic analysis. The first protocol utilizes a two-step extraction process that isolates lipid and metabolite samples individually while precipitating proteins. A second protocol employs a single-phase extraction that precipitates proteins and forms a metabolite rich supernatant that undergoes liquid biphasic separation to isolate lipids and metabolites. Lastly, a

triphasic extraction, known as the MPLEx method, allows for simultaneous isolation of polar metabolites, lipids, and proteins. Prepared samples are analyzed by liquid chromatography tandem mass spectrometry methods optimized for specific molecular types via Orbitrap MS platforms followed by data processing completed with Thermo Fisher Discoverer software. All extraction protocols yielded materials amenable to MS-based analysis where differences in yield and recovery are presented for specific classes of molecules. We will extend our optimized multi-omic protocol to irradiated fish tissue that has been harvested as well-defined organs, which expands on previous work completed by our group examining singular omic changes in medaka fish body regions and organ sets post LDIR exposure.

4. Abstract: Karie E. Behm

Quantification of Parathyroid Hormone Fragments from Serum by Magnetic Bead Immunoprecipitation and UHPLC-MS

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Parathyroid Hormone (PTH) is a low-abundance, key diagnostic biomarker for Chronic Kidney Disease Mineral and Bone Disorder and hypo- and hyperparathyroidism.[1,2] Full-length PTH (1-84) is proteolytically cleaved into multiple fragments *in vivo*.[1] Fragment physiologic and pathophysiologic functions are under investigation. Current clinical assays measuring PTH (1-84) may cross-react with PTH fragments, leading to incorrect measurements and patient misclassification or erroneous diagnoses. The CDC is developing an UHPLC-MS/MS method to accurately quantify PTH (1-84) and 8 fragments to gather reliable information about their abundances in health and disease.

A magnetic bead-based immunoprecipitation method was developed to isolate PTH (1-84) and 8 PTH fragments (7-84, 28-84, 34-84, 35-84, 37-84, 38-84, 45-84, 48-84) and corresponding stable isotope labeled standards (SILs) from human serum. Magnetic beads were coated with C- and N-terminal epitope PTH antibodies and incubated with serum for 1 hour. Beads were washed and all PTH forms, including SILs, were eluted and analyzed by UHPLC-MS/MS.

The UHPLC-MS/MS method detects PTH (1-84) and 8 PTH fragments down to 10 pg/mL which is below the normal physiologic range of 15-65 pg/mL. Using the immunoprecipitation method on neat calibrator solutions, all 9 PTH analytes are detected down to 7.5-16 pg/mL. Preliminary results from immunoprecipitation of serum samples indicate ≥90% recovery of 8 PTH targets at 200 and 1000 pg/mL compared to neat calibrators. Bead removal conditions from final samples were optimized to 1) prevent bead contamination of UHPLC-MS/MS instrumentation and 2) minimize PTH loss with sample clean-up. Two magnet clean-up steps and 1 filtration step are optimal for bead removal and PTH recovery.

Magnetic bead-based immunoprecipitation, coupled with a sensitive UHPLC-MS/MS method, allows for effective enrichment and accurate quantification of PTH (1-84) and 8 PTH fragments

with reliable sensitivity. This method will be used to generate data about PTH (1-84) and 8 PTH fragments in health and disease.

Disclaimer
The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of the Centers for Disease Control and Prevention/the Agency for Toxic Substances and Disease Registry. Use of trade names is for identification only and does not imply endorsement by the Centers for Disease Control and Prevention, the Public Health Service, and the US Department of Health and Human Services.

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5. Abstract: Anusha Bhattarai

Amino Acid Based Deep Eutectic Solvents Improve the Charge Reduction of Proteins: An Insight from Mass Spectrometry Study

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Electrospray ionization (ESI) generates multiple gas phase charge states of a protein which often disrupts the noncovalent interactions and ultimately unfolds and denatures the protein and protein complexes. Various charge reducing agents including triethylamine, trimethylamine oxide, imidazole, and alkali metal salts were used to reduce the charge states of gas phase protein during the ESI process. In this study, we investigate the impact of amino acid-based deep eutectic solvents (DES) to improve the charge reduction of proteins and enzymes. Deep eutectic solvents (DESs) known as green solvents, are formed by mixing two or more components at a certain composition. DESs are eco-friendly, sustainable alternative to traditional organic solvents, biodegradable, and non-toxic in nature. For amino acid-based DES, proline: urea was synthesized by mixing them in a 1:1 molar ratio at 80 °C for 3 h in a covered beaker under constant stirring 600 rpm until a homogeneous liquid was obtained. The DES formation was confirmed by the IR spectroscopy, differential scanning calorimetry and DART mass spectrometry. DART-MS showed the formation of hetero molecular cluster of proline: urea at m/z 176.08. In addition to the hetero molecular cluster, several homo molecular clusters of proline and urea were detected. To examine the charge reduction capacity of proline: urea DES, three model proteins such as Lysozyme, α-Lactalbumin and Trypsin were used. When proline: urea DES was added to Lysozyme solution, only three charge states (6+, 7+ and 8+) were noticed compared to seven charge states that observed in Lysozyme when prepared it in water. A similar charge reduction pattern is noticed for α-Lactalbumin. Adding different percentage of proline: urea DESs to Trypsin produced few charges distribution from 6+ to 9+ on which 8+ is the most dominant peak. Our study showed that 1-5%

proline: urea DES can significantly reduce the charge states of the model proteins and can retain the noncovalent interactions and native structure of the protein in gas phase.

6. Abstract: Olatomiwa Bifarin

Automated machine learning/explainable AI (AutoML/XAI) for metabolomics: improving cancer diagnostic models.

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Metabolomics generates complex data necessitating advanced computational methods for analysis and biological insight. Machine learning (ML) shows promise but selecting optimal algorithms and tuning hyperparameters presents challenges, especially for non-experts. Automated machine learning (AutoML) can simplify this process, but interpretability remains a concern. This study demonstrates an integrated pipeline combining AutoML and explainable AI (XAI) techniques for robust metabolomics analysis.
Results: Using a renal cell carcinoma (RCC) urine metabolomics dataset, AutoML (via auto-sklearn) outperformed standalone ML algorithms like SVM and random forest in discriminating between RCC patients and healthy controls. Auto-sklearn leveraged diverse algorithms and ensemble construction to achieve superior performance (AUC 0.97). Furthermore, SHAP provided global feature importance rankings, revealing dibutylamine as the top discriminative metabolite. Local explanations were generated through waterfall plots showing how each metabolite impacted individual predictions. Dependence plots highlighted interactions between metabolites like hippuric acid and a derivative, suggesting related biological pathways. Detailed error analysis *via* decision plots compared feature importance between correctly and incorrectly classified samples. Our pipeline demonstrates the value of combining AutoML to simplify ML and XAI for enhanced interpretability in metabolomics data science.

7. Abstract: Kingsley Bimpeh

An Efficient Monophasic Extraction Method for High-Throughput Bacterial Lipidomics

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Biphasic lipid extraction methods have downsides such as poor reproducibility and generally unable to support high-throughput experiments. A monophasic extraction method that uses methanol, acetonitrile, and water (MAW) as solvents was evaluated as an alternative to a biphasic lipid extraction method.

Different combinations of acetonitrile/methanol were used to extract lipids from the S. aureus model system. For further analytical quantitation, we determined the absolute lipid recovery using nonendogenous internal standard lipids (ISLs) and the limit of detection/quantitation following the MAW extraction method and the biphasic Bligh and Dyer lipid extraction method (B&D). To demonstrate the suitability of the high-throughput implementation of the MAW method for

profiling, we scaled down the MAW extraction and performed untargeted lipidomics on daptomycin-resistant (n=3) and susceptible strains (n=3) that were extracted in 96-well microplates. All analyses were performed with online HILIC coupled to a Waters Synapt XS traveling-wave ion mobility-mass spectrometer.

The optimal solvent conditions for extracting the endogenous lipids in S. aureus were 1:4 (%v/v) acetonitrile/methanol. A comparison of the MAW method with the B&D extraction showed the MAW method extracted lysylphosphatidylglycerol (LysylPGs), diglucosyldiacylglycerol (DGDGs), and phosphatidylglycerol (PGs) than the B&D method.

The extraction recovery of the ISLs confirmed that the MAW method provided comparable recoveries (ca. 90%) to the B&D method. Calibration curves generated for the limit of detection and the limit of quantification of PGs further revealed that the MAW method is sensitive and requires less bacteria for lipid analysis. Furthermore, the lipid peak areas of the small-scale extraction approach were statistically equivalent and strongly correlated with those of the 3.5 mL large-scale extraction when using a total volume of 175 μL ($R2 > 0.99$; Pearson $P < 0.0001$). The results from the small-scale extraction experiment reflected those of already published literature results.

8. Abstract: David Brewer

Analysis of Membrane Composition and Concentrations for Three E. coli Strains

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When it comes to describing different membranes, there are two primary approaches: categorizing the type of lipids that dominate the membrane or analyzing the actual lipid makeup of the membrane. In both scenarios, the lipid category present in the cell membrane is identified. However, delving into the precise lipids and their corresponding fatty acid compositions provides a deeper comprehension of the bacterial pathways being undertaken.

In this study, we performed lipid analysis of three strains of E. coli (BW25113, W3110, and MG1655) using the Bligh-Dyer extraction method with deuterium-labeled 15:0-18:1 phosphatidylglycerol (PG) and phosphatidylethanolamine (PE) internal standards (ISLs). The extracted samples were analyzed using Hydrophilic Interaction Liquid Chromatography (HILIC) and Reverse-Phase Liquid Chromatography (RPLC) coupled with Waters Synapt XS traveling-wave ion mobility-mass spectrometer. To benchmark, E. coli Total Lipid Extracts were spiked with the ISLs and were analyzed together with the extracted samples. We used mixtures of ISLs with PG 30:0, 32:0, 34:0, 36:0, 36:1, 36:2, PE 32:0, 36:0, 34:1, 36:1, 36:2, and Cardiolipin (CL) 72:4 to determine response factors for lipid quantification. Progenesis Q1 and Skyline were used for data analysis. The analysis of results showed that phosphatidylglycerols (PGs) and phosphatidylethanolamines (PEs) were the most abundant lipids in all three E. coli strains.

Within the HILIC data, it was found that the prominent lipid classes were PGs and PEs with primarily mono-unsaturated fatty acids. Using these response factors, concentrations of found

lipids were then determined. It was found that PE and PG 33:1 were the primarily present phospholipids within E. coli across all strains. Following C18 RPLC analysis using Skyline, it was determined that fatty acid 16:1 or 17:1 were prominent acyl chains in the sn2 position throughout the fatty acid compositions.

9. Abstract: William Bryant

Mass Spectrometry Investigation of Therapeutic Deep Eutectic Solvent

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The therapeutic deep eutectic solvent is a new class of deep eutectic solvent (DES), which includes at least an active pharmaceutical ingredient (API) as one of its components. Therapeutic DESs are emerging alternatives which improve the bioavailability, solubility, delivery, and pharmacokinetics properties of drugs. Traditional solvents are homogeneous, having only one component, and their chemistry is relatively simple. One the other hand, DESs comprise two components, generally hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD), with varying ratio. The interaction chemistry between HBA: HBD in DESs is complex. Moreover, stoichiometry and cluster formation of DES at the molecular level received little attention. Mass spectrometry (MS) is an attractive technique for studying isolated gas phase molecules, however, such investigations have not been implemented for DESs. Compared to other techniques, MS is unique to provide the gas phase stoichiometry, cluster formation, and interaction network between two components of DESs. In addition, computational modelling assists to visualize the isolated DES clusters and unravel deeper understanding of structure-property relationship. This study reported the stable gas phase cluster structure of a menthol: ibuprofen DES using electrospray ionization (ESI) and direct analysis in real-time (DART) coupled with mass spectrometry. The most intense peak in the ESI-MS and DART-MS spectrum was detected at m/z 362.83, corresponding to the hetero molecular cluster of 1:1 menthol:ibuprofen complex. In addition to the hetero cluster, three intense peaks appeared at m/z 312.75, 412.83, and 468.25 confirm the formation of homo cluster of two-menthol, two-ibuprofen, and three-menthol, respectively. Density functional theory (DFT) was employed to investigate the possible gas phase structures of the selected clusters obtained from MS. The DFT results exhibit that most of the clusters stabilized by hydrogen bonds between the constituents. MS-guided computational model visualized detailed microstructures and provided insights into formation mechanism and intermolecular interaction of therapeutic DES.

10. Abstract: Jana Maure Carpenter

A Multi-Omics Approach for Microorganism Identification

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The emergence of multi-omics approaches has empowered scientists to answer complex systems biology questions. The genomic and proteomic information of Staphylococcus aureus, Acinetobacter baumanii, Enterococcus faecium, and Pseudomonas aeruginosa, has been thoroughly explored. However, there remains a significant lack of knowledge of the downstream metabolomic and lipidomic profiles of these small organisms. Here, we have optimized a high throughput multi-omics approach that can isolate the constituent parts of the whole biological system that are not otherwise explained by the template-driven aspects of genomics and proteomics. We showcase the potential for a streamlined method for managing multi-omics experiments of diverse microbe populations using an optimized workflow. We analyzed three different extraction methods for their abilities to simultaneously recover lipid and metabolites from Gram-positive and Gram-negative bacteria. The presence of the outer membrane in Gram-negative organisms did influence the recovery of lipids from those organisms relative to the Gram-positives. Using multivariate analyses revealed that a key distinguishing feature between the Gram-negatives and Gram-positives was in their metabolite and lipid profiles. While presence of phosphatidylethanolamines (PEs) were found only in gram-negative, all organisms shared the presence of phosphatidylglycerols (PGs). The fatty acyl composition of the phospholipids further distinguished organisms to the genus level. Metabolite profiles and levels also varied between the organisms. We detected 370 and 264 features with high significance in the positive and negative mode datasets, respectively. Features identified to date include adenosine, pyocyanin, betaine, and 2-heptyl-4(1H)-hydroxyquinoline. In addition to improved extraction techniques, we optimized a mass spectrometry method coupled to ion mobility for multidimensional separation with the capability to identify significant features that separate each strain based on organism-specific lipid and metabolite profiles. Our findings indicate a new use for a well-organized extraction and quantification workflow for widespread applications within lipidomic and metabolomic endeavors in microbial research.

11. Abstract: Jonathan Choi

Adduct Activity of Sheath liquid CE-MS interface Depends on the Sheath Liquid Content for Glycosaminoglycans

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The sheath liquid CE-MS interface has been used to couple capillary electrophoresis and mass spectrometry for the analysis of complex GAG structure. The most compatible fragmentation method to characterize GAG structures have been negative electron transfer dissociation (NETD) and collision induced dissociation (CID). However, both methods pose challenges as NETD requires a high charge state precursor to fragment while CID causes sulfo-degradation if the

precursor is not fully ionized. In order to fully ionize the GAG precursors, different metal ions have been added to produce informative fragments while minimizing sulfo-degradation with nanospray ionization.

A GAG standard was run through CEMS with ammonium acetate as the sheath liquid and triethylammonium (TEA) acetate as the background electrolyte and vice versa to determine where the cation-proton exchange was happening. The results showed that the TEA adduct only showed up when it was placed in the sheath liquid and not the background electrolyte. Then, different amounts of sodium acetate were mixed in with the ammonium acetate to try to produce a fully ionized precursor for CID. The results show that while producing a fully ionized precursor was possible, it was difficult to produce a meaningful fragmentation spectrum for structural characterization because the intensity of the precursor was too low. A fragmentation spectrum for partially ionized precursor shows promise, however, as it produced a similar fragmentation spectrum using CEMS as the spectrum produced by nanospray ionization previously. Further research is required to apply the cation-proton exchange method for structural characterization of complex GAGs.

12. Abstract: Joseph L. Corstvet

Leveraging Non-targeted Lipidomics to Study Mesenchymal Stromal Cell Senescence at Low Sample Volumes

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Mesenchymal stromal cells (MSCs) have emerged as a promising candidate for cell-based therapies due to their low immunogenicity. However, on standard culture substrates, MSC senescence constrains expansion. Senescence also alters therapeutic potency of these cells, raising the interest to understand its molecular mechanisms and markers. Previous work has shown varying culture substrate stiffness can delay senescence, with earlier passages exhibiting more potent cells. Therefore, we sought to identify molecular features that are differentially regulated on distinct culture surfaces over time.

Human bone-marrow MSC from two donors were cultured for three passages on two distinct substrates: tissue culture plastic (TCP) and 100kPa polyethylene glycol diacrylate hydrogels (RGD). Whole lipid extraction was performed in isopropanol followed by SpeedVac drying and resuspension in IPA and ammonium acetate. Samples were analyzed via reverse phase LC-MS on a Thermo ID-X instrument in addition to a triboelectric nanogenerator (TENG) ion source coupled to a Q-Exactive plus instrument. Both analyses were performed only in positive ion mode.

Over 2700 unique m/z signals were extracted after background subtraction and drift correction of the reverse phase LC/MS data using Compound Discoverer. This list was reduced to 617 features using VIP variable selection and annotations were performed on the reduced list. In addition,

TENG-MS data was also analyzed and a list of ~800 unique m/z features was created. Multivariate analysis was utilized to discriminate between TCP and RGD conditions as well as passages. Triglycerides appear upregulated in RGD samples and the late passage, while ceramides were upregulated in passage A, and sphingomyelins were key lipids in TCP cultured samples. Phosphatidylserines were significantly upregulated in later passages as well. Most of these lipids were also present in the TENG-MS dataset, highlighting the viability of the TENG-MS platform for untargeted lipidomics and subsequent annotation.

13. Abstract: Jessica M. Deutsch

Metabolome variation within and between stony coral species susceptible to stony coral tissue loss disease

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Caribbean and Florida coral reefs are currently experiencing the catastrophic stony coral tissue loss disease (SCTLD), which has resulted in the loss of at least 30% of stony corals within the Florida coral reef system. With such loss, the characterization of disease susceptibility and resilience mechanism(s) is vital. Although the SCTLD etiological agent is yet to be confirmed, breakdown in relationship(s) between endosymbiotic algae Symbiodiniaceae and the coral host is observed with SCTLD onset. Metabolomics facilitates identification of small molecules and their biological source and serves as an important hypothesis generation tool in examining disease susceptibility phenotypes. An untargeted liquid-chromatography mass spectrometry approach was utilized to acquire metabolomics data on organic extracts of four healthy coral species with variable SCTLD susceptibility and cultured Symbiodiniaceae. We observed intra- and interspecies metabolome variation for the healthy corals, with *Meandrina meandrites* metabolomes showing relatively lower intraspecies variability compared to the other species included in this study. Herein, we present the application of advanced metabolite annotation data analysis methods to identify host and symbiont-derived metabolites that varied between coral species. Such tools include MassQL, a recently developed supervised chemical substructure query language platform, which enables users to search for sets of defined fragment peaks. Acylcarnitines, vitamin E derivatives, and betaine lipids were identified as variably detected between the coral species. Partitioning metabolome extracts in organic solvents facilitated the identification of natural product-like metabolites with the assistance of annotation tools including DEREPLICATOR, which predicts annotations for analogues of known natural products. Finally, we searched for the presence of these metabolites in organic extracts of *Orbicella faveolata* corals to determine if the presence of SCTLD affected these pathways. The *O. faveolata* corals, variably affected by SCTLD

at the time of sampling, were collected from reefs within the Florida Lower Keys and Coral Reef Ecosystem Conservation Area.

14. Abstract: Christian Freeman

Revealing Altered Branched Fatty Acid Isomer Distributions Affecting Membrane Characteristics in Daptomycin-Resistant Staphylococcus aureus

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Staphylococcus aureus is a common pathogen for hospital and community-acquired infections. Although uncommon, resistance to daptomycin is mediated by mutations in genes associated with the regulation, synthesis, or relocation of fatty acids and membrane lipids. These mutations result in alteration of the structure and distribution of lipids within the membrane, as well as cell surface charge and cell envelope thickness, which prevents daptomycin from causing cell lysis. Here, we paired high-resolution chromatographic separations and IM-MS measurements of branched fatty acid lipid isomers with determinations of membrane physiology to evaluate lipid-mediated daptomycin resistance in Staphylococcus aureus. The daptomycin resistant (Dap-R) strain evaluated here was previously reported to have an elevated level of LysylPGs and decreased PGs due to mutations in the genes that encode for their respective synthases, mprF and pgsA. Intact lipid isomers resulting from isobaric branched- and straight-chain fatty acids were separated and evaluated within each of the major lipid classes of S. aureus utilizing RPLC-IM-MS. RPLC-IM-MS data of Dap-R revealed numerous differences from the daptomycin susceptible (Dap-S) strain, including an increased ratio of branched-chain fatty acid (BCFA) isomers to straight-chain fatty acids (SCFA) isomers across all lipid species (PGs, LysylPGs, DGDGs, and PAs) compared to Dap-S, supportive evidence of a more fluid cell membrane through lipid composition, and LysylPG isomer production. Fluorescence anisotropy and imaging confirmed that the membrane of the Dap-R strain was markedly more fluid due to the net increase in branched lipids, and transmission electron microscopy confirmed a large increase in cell well size in Dap-R. Through our method, we've been able to draw the connection between intact lipid isomers and daptomycin resistance characteristics. Through supplementation of FA 15:0, FA 16:0 or plasmids containing unmodified pgsA, significant alterations to lipid distribution occur, promoting the potential idea of reversing resistance in non-susceptible strains.

15. Abstract: David A. Gaul

Mitochondria Lipidomic Profile Alterations in a Mouse Model of Energy Dysfunction

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Mitochondria's function is to produce the essential energy for the cell. Mitochondrial disease directly affects cellular energy, which often leads to alteration in tissue function. In high-energy

tissues like the heart, mitochondrial response to energy defects is not fully understood. A mouse model was developed with the systemic knockout of the mitochondrial citrate carrier (SLC25A1). In the embryonic heart, SLC25A1 loss leads to impaired mitochondrial respiration and mitochondria with highly aberrant ultrastructure. As SLC25A1 is thought to play a role in lipid biosynthesis through its role in cytosolic acetyl-CoA supply via citrate, our data lead to a focus on lipidomic perturbations.

Cardiac mitochondria were isolated from 2-month-old Slc25a1 heterozygous knockout and wild type controls. Metabolites were extracted with iso-propanol:methanol (90:10) with bead homogenization. The supernatant was analyzed with high-performance liquid chromatography – high resolution mass spectrometry instrument. Data processing with Compound Discoverer yielded features 6,188 features in both positive and negative ionization mode. Among the most intense lipid signals detected in mitochondria extracts were glycerophospholipids containing one FA 22:6 alkyl chain (6 of top 10 features in negative ionization mode). DHA has been indicated to increase the fluidity of membranes and has been noted for cardioprotective effects. In association with knocking out SLC25A1, we observed a general reduction in the lipid classes including carnitines, sphingolipids, and triglycerides. Lipidomic pathway investigation into mitochondrial energy defects can illuminate how membrane composition affects the health and the death of the organelle as well as its properties affecting molecule transport.

16. Abstract: Lauren Heidenreich

Optimization of PRM-LCMS/MS for Glycosaminoglycan Disaccharide Profiling

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Glycosaminoglycans (GAGs) are ubiquitous linear oligosaccharides that constructed on the surface of proteoglycans (PGs) in a non-template driven manner that can be classified based on modifications and include Heparan Sulfate (HS) and chondroitin sulfate (CS). Modifications, like sulfation patterns, are responsible for vast heterogeneity and many interactions of cells within biological processes. GAG expression influences metastasis, homeostasis, pathogenesis, etc. with changes in the entire cellular genome or glycocalyx are often associated with diseases. Disaccharide analysis by MRM is an established technique, yet the extension to a PRM approach on Orbitrap instrumentation has not been demonstrated.

Modern mass spectrometry demands high throughput approaches to satisfy the large sample numbers provided in a typical biological study. Standard approaches for LCMS-based GAG quantitation rely on extended gradients of 30 to 60 minutes that ensure the baseline separation of all potential GAG disaccharides for accurate peak-area based quantitation. Recent MRM-based strategies on triple quadrupole mass spectrometers have reduced this acquisition time, although QQQ systems are not always widely available. Orbitrap FTMS platforms are increasingly omnipresent in most laboratories and provide a single system to explore analytical endpoints ranging from untargeted discovery to targeted assays. New approaches are required to satisfy these demands from a single instrument and meet throughout. Modest enhancement has afforded the

ability to quantify CS/HS dp2 profiles with a detection limit on the order of ng/uL and been able to reduce a standard LC-MS run to nearly 20 minutes with baseline chromatographic separation.

The ability to perform parallel reaction monitoring on the QE has provided the unique ability to monitor all fragment ions for each target disaccharide and identify unique targets for quantitation within the context of our standard dilution series while maintaining our limit of quantitation. Based on elution order, these are largely glycosidic bond cleavages achieved by HCD fragmentation.

17. Abstract: Hannah Hynds

TWIM Calibration Method for Biomolecular Class Assignment and High-Throughput Multi-Omic Collision Cross Section Calculation

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In the field of omics, while single omic techniques have proven adequate, it has been observed that using multiple omic techniques in conjunction yields more thorough and informative sample profiles than any one alone. The rapid gas-phase and structural separation of ion mobility-mass spectrometry (IM-MS) is a promising approach for high-throughput multi-omics. Analysis of complex mixtures using IM-MS is possible because each class of biomolecule separates based on mass, charge state, and three-dimensional shape, yielding unique trends in IM-MS space. However, for traveling-wave ion mobility-mass spectrometry (TWIM-MS) platforms, analyzing multi-omics mixtures poses many challenges such as analysis of high-dimensionality data and generating accurate calibrated collision cross section (CCS) values. It has been illustrated in literature that TWIM-MS CCS values for small molecules and lipids are most accurate when calculated using a calibration curve of structurally similar compounds. To alleviate these challenges, we propose a novel, unbiased, front-end method that can be used to assign multi-omic features to their corresponding biomolecular class for accurate class-specific CCS calculation without identification.
To accomplish this, calibration curves were developed for lipids, metabolites, and peptides using mixtures of reference standards optimized based on the mass and CCS ranges of each biomolecular class. A python-based classification model that contains the calibration curves built upon the reference standards has been developed to assign unknown features in experimental data to a biomolecular class and calculate their corresponding CCS values. The accuracy of the classification and CCS calibration approach has been tested against a single-phase extract of the human serum reference material, NIST SRM 1950, analyzed using liquid-chromatography and flow-injection coupled to IM-MS. The results from the class-specific CCS calibration approach have also been compared against the recently developed analyte-neutral strategy for TWIM.

18. Abstract: Jiangpeiyun (Joy) Jin

Quorum Sensing Systems Influence the Trimethoprim Induced Secondary Metabolite Production in Burkholderia Cenocepacia

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Burkholderia cenocepacia can cause chronic infections in immunocompromised individuals, such as cystic fibrosis patients. Due to the inherent resistance to many commonly used antibiotics in clinical treatment, infections by *B. cenocepacia* are challenging to treat and life-threatening for CF patients. *B. cenocepacia* is known to produce a variety of small molecules, also known as natural products, some of which serve as virulence factors during infections, providing a fitness benefit to survive and adapt to the environmental changes.[1] Production of many of these natural products is dependent on the expression of their biosynthetic gene clusters, which are typically silent under normal growth conditions.[2] *B. cenocepacia* utilizes quorum sensing (QS) system to control gene expression, including genes involved in virulence.[3] Previous studies revealed that exposure to sublethal trimethoprim concentrations mediate production of secondary metabolites including QS signals and other compounds regulated by the QS systems in the epidemic strain *B. cenocepacia* K56-2.[4,5] Thus, there is a high possibility that trimethoprim-mediated gene expression is related to QS. To understand the interplay between QS and trimethoprim-mediated secondary metabolite production, an untargeted, mass spectrometry-based metabolomics approach was used to study the metabolomic profiles of *B. cenocepacia* K56-2 wild-type and loss-of-function mutations in QS genes under exposure to sublethal antibiotics. We found that induction of several secondary metabolites by trimethoprim were repressed in the strains with loss-of-function mutations in QS genes, suggesting that induction of secondary metabolite production by trimethoprim is linked to QS-controlled gene regulation. This study will improve understanding of antibiotic resistance mechanisms of *B. cenocepacia*, seeding the discovery of potential therapeutic targets to improve treatment options for CF patients.

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19. Abstract: Katherine Kenney

Optimizing Mass Spectrometry Assays for Tree Gum Discrimination

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Plant gums are naturally occurring polymers formed commonly by plant cell walls and trees. Due to their chemical and mechanical properties many plant gums (*e.g.*, gum arabic, locust bean gum (LBG), *etc*.) are widely employed in food, cosmetics, textile, biomedical and pharmaceutical industries. As binding mediums and adhesives, they are also found in cultural heritage objects (CHO). Discrimination between these gums is significant for determining conservation efforts for CHO, however characterization of gums is challenging due to the complex or branched structures formed by these polysaccharides, their high molecular weight, and high polydispersity. Recent efforts have been made to improve the discrimination of gums through fingerprinting approaches with mass spectrometry (MS). By optimizing these technologies, we will not only improve the throughput of analysis, but determine the level of confidence that can be achieved with identifying plant gums.

We reproduced a MS assay for characterizing three different gums: Arabic Gum (AG), Tragacanth Gum (TG), and Locust Bean Gum (LBG). Exo-β-1,3-galactanase from Clostridium thermocellum was used to digest AG and TG and endo-1,4-β-mannanase from Cellvibrio japonicus was used for digesting LBG. The enzymes target oligosaccharidic units into repeating patterns to be analyzed using matrix-assisted laser desorption/ionization (MALDI) MS. The gums can be qualitatively and quantitatively identified by the range of characteristic oligosaccharidic signals used to fingerprint each gum sample. The current assay requires a 5-hour digestion; further development is needed for routine analysis of gums, but by optimizing the enzymatic digestion to improve throughput the potential to generate a comprehensive library of plant gums becomes increasingly reasonable. This library would then enable next-generation MS applications such as MALDI imaging on high resolution instrumentation, and greatly improve the confidence in gum identification for CHO for provenance or conservation efforts.

20. Abstract: Jandi Kim

Probing the Biosynthetic Pathway of Heparan Sulfate Using Capillary Electrophoresis Zone Mass Spectrometry

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Glycosaminoglycans (GAGs) are linear anionic polysaccharides present on the surface of mammalian cells and in the extracellular matrix, which regulate a variety of biological processes. During development and under some pathological conditions, the expression of their biosynthetic enzymes can be altered, impacting the structure and function of their glycan chains.

Our research focuses on the analysis of GAG mixtures varying in extent and position of sulfation by CZE-MS. The CZE-MS platform for GAG sequencing was first evaluated by analyzing the molecular composition of HS standards from human CHO cells. A comparison of CZE-MS versus direct infusion with nano-ESI-MS demonstrated that the CZE-MS method yielded a 75% increase in HS characterization even though the sample concentration in the nano-ESI-MS experiment was higher by two orders of magnitude. The composition including was assigned using a custom script.

A knockout cell line deficient in an epigenetic regulator was prepared from A375 human melanoma cells to examine HS biosynthesis, CZE-MS successfully separated complex HS features for both the wild type (WT) and the knockout (KO) cell samples, assigning the compositional characteristics of each. Notably, there was no significant change in chain length between WT and KO samples, but sulfate group occupancy and N-acetylation modification differed significantly. These data suggest that 6-O sulfation on the amino sugar residues in the KO sample increases because of the lack of sulfatase remodeling enzymes. The extent of N-acetyl group modification in the WT sample was, furthermore, wider with a higher average value than that of in the KO sample.

21. Abstract: Jessica Eyram Kugblenu

Mutation of the active site residue H_{421} alters catalysis of *Pseudomonas aeruginosa* D-2-Hydroxyglutarate Dehydrogenase.

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Pseudomonas aeruginosa is an opportunistic gram-negative bacterium responsible for 10% of all hospital-acquired infections in the US[1]. *P. aeruginosa* depends on D-2-hydroxyglutarate dehydrogenase (*Pa*D2HGDH), which oxidizes D-2-hydroxyglutarate to 2-ketoglutarate, to drive L-serine biosynthesis for survival[3-5]. Knockout of the *Pa*D2HGDH gene inhibits *P. aeruginosa*

growth, making *Pa*D2HGDH a therapeutic target against *P. aeruginosa*[2,3]. After recombinant expression of *Pa*D2HGDH, there was only 15% flavin reduction. Previous studies on closely related FAD-dependent dehydrogenases, reported increased enzyme activity upon exogenous metal addition[6]. When *Pa*D2HGDH was analyzed using ICP-MS, there were significant but non-stoichiometric amounts of Mg^{2+} and Zn^{2+} bound to *Pa*D2HGDH; however, only zinc could increase *Pa*D2HGDH activity[4]. Upon *Pa*D2HGDH purification in 1 mM $ZnCl_2$, flavin reduction increased to 92% and zinc was identified as a required metal cofactor that binds, orients, and activates the D-2-hydroxyglutarate substrate for catalysis[4,5]. However, in closely related flavin dependent enzymes such as the glucose-methanol-choline (GMC) enzymes, a catalytic base is responsible for activating the substrate for catalysis[7]. Amino acid sequence comparison of *Pa*D2HGDH with other GMC enzymes reveals a fully conserved Histidine-421 residue, whose topology in the *Pa*D2HGDH active site is conserved for catalytic bases in other GMC protein structures[3]. However, the role of H^{421} in *Pa*D2HGDH has not been established.

In this study, site-directed mutagenesis was used to replace H^{421} with glutamine, asparagine, phenylalanine or cysteine. The variant enzymes were purified in the presence of 1 mM $ZnCl_2$ and investigated for their kinetic properties. For all variant enzymes flavin reduction was only ≤28% with ~0.1 s^{-1} enzyme activity irrespective of substrate concentration and exogenous metal addition, preventing the determination of any kinetic parameters for the variants. The 400-fold decrease in activities for the variant enzymes in comparison to the turnover rate of the wild type suggests that the fully conserved H^{421} residue is important for *Pa*D2HGDH catalysis.

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22. Abstract: Dmitry Leontyev

Desorption Electrospray Ionization Cyclic Ion Mobility Mass Spectrometry Imaging Uncovers New Tissue-level Alterations in Traumatic Brain Injury.

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Mass spectrometry imaging (MSI) enables detection of metabolites in-situ without labels. It has been extensively used to study a variety of metabolic alterations in tissues. One of the most challenging and time-consuming steps in MSI is annotating the massive amounts of data generated. This is particularly challenging in MSI experiments, as a front-end separation technique is not employed and hundreds of thousands of mass spectra are collected. Once averaged, these spectra can suffer from small but significant mass drifts over time, leading to elemental formula mismatches when annotating unknowns. Isobaric ions exacerbate these challenges as they are co-selected when attempting MS/MS experiments, leading to less-robust database matches. Ion mobility (IM) can separate isobaric ions by their collision cross-section values and is now often coupled with MSI to provide drift time data that aids in distinguishing possible structural matches.

Matrix-assisted laser desorption ionization (MALDI) is often chosen as the de facto ionization source for MSI due to its high spatial resolution and widely available instrumentation. MALDI, however, has drawbacks. Matrix signals can interfere with detection of low mass metabolites, and detection of specific compound classes is matrix dependent. Desorption electrospray ionization (DESI) is a complementary ionization method that does not rely on a matrix to induce ionization and is suitable for detecting low molecular weight metabolites and metabolite classes that do not ionize well by MALDI.

In this study, DESI-cyclic IM-MSI was used to investigate metabolic alterations in rat brains following traumatic brain injury. Various amino acids, nucleotides, antioxidants, phospholipids and sphingolipids were found to be altered in the injured cortex. Cyclic IM was found beneficial in separating interfering isobaric species that distort the ion images of species of interest, such as doubly charged cardiolipins that overlap with singly charged lipid ions.

23. Abstract: Wen Lu

Determination of polyphenols and their metabolites in Blueberry extract by HPLC-MS/MS

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Blueberries are one of the richest dietary sources of polyphenols, such as flavonoids and phenolic acids. Polyphenols, as natural compounds abundant in various fruits and vegetables, have gained significant attention due to their potential health benefits such as strong antioxidant and anti-inflammatory properties. This study presents a comprehensive analysis of polyphenols and their metabolites in blueberry extract and biological fluids using High-Performance Liquid Chromatography-Tandem Mass Spectrometry (HPLC-MS/MS).

We established an optimized HPLC-MS/MS method to quantify a panel of polyphenols present in blueberry extract. LC_ESI-MRM analyses were carried out on an Agilent 1200 liquid chromatography (LC) system coupled to an API 3200 electrospray-ionization triple-quadrupole mass spectrometer (AB SCIEX, Framingham, MA). 28 polyphenols were categorized into three groups based on their chemical structures, three LC separation profiles and MS methods were developed, respectively. The detection limit and standard curve were established for each of the analytes of interest. The methods developed will be used for characterization and quantification of polyphenols and their metabolites found in berry extracts.

This research contributes to the growing body of knowledge surrounding polyphenols and their role in health and disease and to guide dietary recommendations and inform the development of polyphenol-based interventions for various health conditions. This highlights the significance of a holistic approach to nutrition and wellness, and the study of functional foods such as blueberries as a therapeutic intervention for cardiovascular diseases including coronary microvascular dysfunction (CMD).

24. Abstract: Ziad Mahmoud

Controlled synthesis of oligopeptides from simple amino acids in aqueous microdroplets *via* triboelectric nanogenerator mass spectrometry (TENG-MS)

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Origins of life chemistry is particularly challenging due to the very large number of reactions leading to the synthesis of extremely complex mixtures. Some theories propose that life emerged from the self-assembly of organics in complex molecule mixtures via condensation reactions. However, the processes leading to these assemblies remain uncertain. Amide bond formation, an example of these reactions, requires specific enzymes in vivo. Here, aqueous microdroplet chemistry monitored with mass spectrometry was used to investigate this reaction under ambient conditions without enzymes. Mass spectra for L-alanine and glycine were first recorded using classical electrospray and

nanoelectrospray ionization. A triboelectric-powered nanoelectrospray ion source was also used. Aqueous microdroplets of L-alanine or glycine were generated by loading monomer solutions in glass capillary emitters and ionized using either constant or pulsed voltages produced by a DC power supply or by triboelectric nanogenerators (TENG), respectively. In the case of standard electrospray ionization at microflow rates, no dipeptides or oligopeptides were detected; with nanoelectrospray, only dipeptides were produced. The high voltage supplied by TENG yielded higher droplet numbers, allowing analysis at ultra-low concentrations and more efficient formation of oligopeptides ions from a single spray source. To further investigate the reactivity of amino acids in charged microdroplets, different experimental conditions were tested. For example, the effect of spray distance between the nanoelectrospray emitter and the inlet of the mass spectrometer and the spray voltage were carefully studied. Tandem mass spectrometry (MS/MS) was used to verify the stability and composition of produced oligopeptides by applying collision-induced dissociation and producing specific fragments. Ion mobility spectrometry was used to evaluate structural differences between commercial oligopeptides and microdroplet-generated isomers. Two-dimensional plots (drift time vs *m/z*) revealed important information on the intensity and variation of oligopeptides drift times. Chemical reactions in microdroplets produced by TENG MS offer an efficient approach to understand the origin of biopolymers and matter-to-life transitions.

25. Abstract: Lester S. Manly

Optimization of CID, HCD, EThcD, and UVPD fragmentation methods for intact protein analysis of human metallothionein

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Metallothionein (MT) is a family of small, cysteine rich, and conserved proteins that are known to participate in heavy metal detoxification, metal homeostasis, and oxidative stress response. Within humans, the MTs exist within four primary isoforms consisting of MT1, MT2, MT3, and MT4. MT1 has further subisoforms of MT1A, MT1B, MT1E, MT1F, MT1G, MT1M, and MT1X. MT expression has found to be altered in numerous diseases, such as with Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis. However, a caveat to quantifying MTs within tissues is that isoforms share a high degree of sequence similarity, especially with MT1 and MT2. Immunoassays and bottom-up proteomics currently have difficulty confidently differentiating the MT isoforms. Thus, we are currently developing a MT quantification method for human tissue utilizing top-down proteomics. Here, we highlight our initial results evaluating several fragmentation methods of intact recombinantly expressed human MT3. The recombinant human MT3 was analyzed via Thermo Scientific Oribtrap Eclipse Tribrid mass spectrometer via hESI coupled to a syringe pump for direct infusion of samples at concentration of 10 µM in 50% acetonitrile. MS1 intact spectra were analyzed by Thermo Scientific BioPharma Finder, while MS2 fragmentation spectra were analyzed by e-MSion ExDViewer. We observed up to 40-50% fragmentation of the total peptide bonds for optimized CID, HCD, EThcD, and UVPD methods resulting in complete sequencing of MT3. EThcD was found to produce the highest coverage and produced the widest range of ions. In conclusion, all these fragmentation methods could be leveraged to fragment MTs with considerations for what type of ion populations are produced. An MS^n method could be leveraged to further increase total fragmentation of all amino acids in the

MTs. Optimization of fragmentation of MT allows researchers to confidently determine which isoform is quantified and allows for localization of potential post-translational modifications.

26. Abstract: Mónica Monge-Loría

Coculture Approaches for Marine Natural Product Discovery

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SCTLD (Stony Coral Tissue Loss Disease) is a white plague type disease that has affected corals in Florida and the Caribbean since 2014. It is a rapidly spreading and highly prevalent disease that affects over 20 species of *Scleractinia* corals[1]. In the search for a SCTLD treatment we have turned to the Coral Probiotic Hypothesis, which states that resistance to disease arises from microbial community changes that aid the coral holobiont fitness[2]. Production of secondary metabolites with antimicrobial properties is one such mechanism to achieve immunity[3]. However, the genes that encode for secondary metabolites are frequently silent[4] since they are not necessary for growth. About 10-20% of biosynthetic gene clusters (BGCs) produce detectable amounts of secondary metabolites under normal laboratory conditions[4], rendering many compounds undiscovered. One approach to activate silent BGCs is coculturing microorganisms to induce crosstalk[5], which we use in this work. Here, we assay *Acropora cervicornis* bacterial isolates' bioactivity against putative SCTLD pathogens to identify interesting candidates. To induce secondary metabolite production, we coculture the coral-associated bacteria with a strain of *Vibrio coralliilyticus* that produces Andrimid, a known natural product elicitor[6]. We employ mass spectrometry-based metabolomics to characterize metabolic shifts that arise from coculture and to guide natural product dereplication.

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27. Abstract: Bilkis Mehrin Moni

Metal ion-induced alterations in the biophysical and kinetic properties of bacterial D-2-hydroxyglutarate dehydrogenase from *Pseudomonas aeruginosa* PA01

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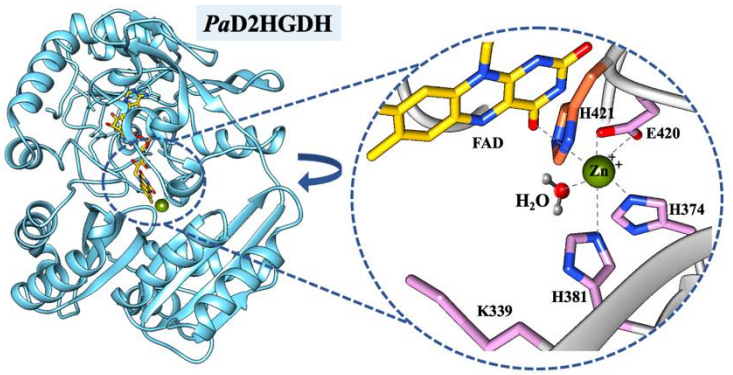
D-2-hydroxyglutarate dehydrogenase from *Pseudomonas aeruginosa* (*Pa*D2HGDH) is classified as a metallo-flavoenzyme due to its reliance on Zn^{2+} for catalyzing the conversion of D-2-hydroxyglutarate to 2-ketoglutarate (1,2). *Pa*D2HGDH is also active with Co^{2+}, Ni^{2+}, Mn^{2+}, and Cd^{2+} as alternative metal cofactors (1,2). However, the role of the alternative metals in *Pa*D2HGDH enzyme remains inadequately investigated. To study the effect of alternative metal ions on the function of the enzyme, the recombinant His-tagged *Pa*D2HGDH was purified in the presence of 1 mM chloride salts of M^{2+} (M^{2+} = Zn^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+}, or Cd^{2+}). Upon purifying *Pa*D2HGDH with 1 mM M^{2+}, the M^{2+}:protein ratio was determined by inductively coupled plasma mass spectrometry (ICP-MS) to assess the binding of metals to the enzyme. The mol Zn^{2+}:protein stoichiometry for E-Zn^{2+} was 2:1 with k_{cat} ~40 s^{-1} for D-malate. Conversely, the E-Co^{2+} displayed a molar Co^{2+}:protein ratio of 0.5:1, while Zn^{2+} was also present in a 0.5:1 ratio, resulting k_{cat} was 45 s^{-1}. The E-Ni^{2+} complex exhibited a molar Ni^{2+}:protein stoichiometry of 2:1, with a k_{cat} of 5 s^{-1}. E-Cd^{2+} was characterized by a molar Cd^{2+}:protein ratio of 0.5:1 and a k_{cat} of 8 s^{-1}. The E-Mn^{2+} complex demonstrated a molar Mn^{2+}:protein stoichiometry of 0.3:1, with a coexistent presence of Mg^{2+} at a ratio of 0.7:1, and a k_{cat} of 17 s^{-1}. These findings indicate that the alternative metal ions influence the catalytic behavior of *Pa*D2HGDH. Interestingly, the inclusion of Zn^{2+}, Co^{2+}, Mn^{2+}, or Cd^{2+} resulted in ~ 1 unit increase in the pK_a value of the flavin N_3 atom (≥12.0), while Ni^{2+} induced a 1-unit decrease (10.4) compared to the E-FAD (pK_a 10.7). Moreover, the exogenous addition of the chloride salts of Zn^{2+} to the inactive metallo-apoenzyme using fluorescence metal binding assay resulted in enzyme reactivation in both hyperbolic and inverse sigmoidal behavior, indicated the enzyme's existence in two distinct conformations. These results underscore that the alternative metal ions modulate the biophysical properties of the of *Pa*D2HGDH.



Active site of *Pa*D2HGDH

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28. Abstract: Shaima Muhammed Nazaar

Development of red blood cells and plasma-based diagnostic lipid biomarker panel for Parkinson's disease.

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Parkinson's disease (PD) is a multifactorial neurodegenerative disease whose etiopathology is largely unknown. One of the pathological hallmarks is the abnormal intracellular aggregation of alpha-synuclein, known as Lewy bodies, in the PD brain. The process of Lewy-body formation is not clearly delineated, but lipids are implicated. The diagnosis of PD mainly relies on clinical symptoms, which hampers the early detection of the disease. Hence, reliable and diagnostic biomarkers are needed for managing the disease. Recent studies suggest that dysregulations in lipid metabolism play a crucial role in PD progression. Red blood cells (RBC) are the most abundant source of alpha-synuclein outside the brain and are rich in lipids. Our study aimed to identify the unique lipid signatures in a sample cohort of 248 from the Victorian Parkinson's disease registry by conducting lipidomic analysis via mass spectrometry on RBC and coupled plasma. Total lipids from RBC and plasma were extracted with methanol/butanol solvent and detected using data-dependent analysis mode on a TIMS-TOF mass spectrometer. Feature extraction and lipid identification were carried out by MS Dial software. Verification and relative quantification by standard internal normalization were performed in Skyline data analysis software. We discovered over 150 significantly ($p<0.05$) altered lipids from 28 different lipid classes. At least 10 lipids from various classes, including phosphatidylethanolamines, phosphatidylcholines, triacylglycerols, sphingomyelins, ceramides, and lysophosphatidylcholines, with individual ROC-area under the curve of approximately 0.7 have potential as PD biomarkers. The findings suggest that dysregulated lipids are from functionally related pathways, offering a novel possibility for employing RBC lipid profiles as diagnostic biomarkers for Parkinson's disease.

29. Abstract: Rebekah Phelan

Quantification of *Enterococcus faecalis* Glycerophospholipids and Fatty Acid Incorporation by Ion Mobility-Mass Spectrometry and Paternò-Büchi C=C analysis

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Enterococcus faecalis is a gram-positive bacterium that is known to cause infection when too abundant in the gastrointestinal tract. *E. faecalis* can become resistant to antibiotics due to gene mutations effecting the lipid profile of the membrane. Phosphatidylglycerol (PG) is a major phospholipid (PL) class found in bacterial membranes of *E. faecalis*, which is comprised of a polar head group and two fatty acid (FA) chains that can be saturated or unsaturated. Unsaturated fatty acid chains increase the fluidity of the membrane which can aid in the incorporation of fatty acids from the environment into the membrane. This uptake modifies the lipid profile which in turn alters the resistance of the bacteria to certain antibiotics. *E. faecalis* is known to produce *cis*-vaccenic acid (C18:1-*11z*), but not its isomer, oleic acid (C18:1-*9z*). However, it is known that oleic acid increases resistance of *E. faecalis* against the antibiotic daptomycin, whose mechanism of action involves the destruction of the membrane. Here, I investigated the incorporation of oleic and *cis*-vaccenic acid into daptomycin susceptible and resistant strains of *E. faecalis* and the effects on the FA isomer abundances of PGs containing FA 18:1 using C30 RPLC-IM-MS. Due to co-elution of unsaturated PGs found in *E. faecalis*, C30 RPLC method optimization is being explored in order to better understand the lipid modifications. Furthermore, the Paternó-Büchi (PB) reaction's cycloaddition at the place of the carbon-carbon double bond (C=C) will be utilized to determine accurate C=C placement of unsaturated FA chains. With this determination, it will be clear which isomer, oleic acid or *cis*-vaccenic acid, was incorporated and to what degree in *E. faecalis*.

30. Abstract: Christina C. Ramelow

Paired proteomic and transcriptomic profiling of LPS-stimulated astrocytes in vivo using TurboID

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Despite decades of research into Alzheimer's disease (AD) pathogenesis, there are no glial-specific disease-modifying therapies. To identify therapeutic targets, our group has developed the novel cell-specific in vivo biotinylation of proteins (CIBOP) approach to quantify astrocyte-specific in vivo proteomic and transcriptomic contributions to AD under homeostatic and neuroinflammatory conditions. CIBOP uses TurboID, a biotin ligase, selectively expressed in the cell type of interest

using a conditional Cre/lox genetic strategy to label the cytosolic proteome. Using mass spectrometry (MS)-based proteomics, we have found that TurboID biotinylates many RNA-binding and ribosomal proteins. Leveraging this, we extended the CIBOP approach to obtain representative cell type-specific transcriptomes, along with the proteome. We crossed astrocyte specific Cre (Aldh1l1-Cre-ert2) and Rosa26$^{TurboID/wt}$ floxed mice for astrocyte-specific proteomic labeling (astrocyte-CIBOP). Astrocyte-CIBOP and control (Cre-only) mice received tamoxifen, followed by biotin-containing water and systemic lipopolysaccharide (via intraperitoneal injections) to induce a neuroinflammatory state. While maintaining RNA-protein interactions, cortical tissue was lysed, biotinylated proteins were enriched via streptavidin beads, and then RNA and protein were eluted. Immunofluorescent microscopy (IF), immunoblotting, MS-based proteomics and RNA-sequencing was completed. Immunoblotting results confirmed biotinylation of the cellular proteome of astrocyte-CIBOP cortex, compared to controls. IF images show that the biotinylation signal is astrocyte specific. RNA gel electrophoresis displays low RNA yield from control streptavidin pulldowns, and high levels of RNA from astrocyte-CIBOP pulldowns. MS-based proteomics and RNA-sequencing confirm enriched proteins and RNA are astrocyte specific. We validated the CIBOP approach to capture the cortical Aldh1l1-positive astrocyte proteome and transcriptome under homeostatic and neuroinflammatory conditions. Our innovative in vivo cell type-specific and native-state dual -omics approach provides complementary transcriptomic and proteomic information to investigate disease mechanisms, discover new biomarkers, and identify therapeutic targets.

31. Abstract: Samrat Sahu

Expanding Methionine Chemoproteomics via the Copper(I)-Nitrene Platform (CuNiP)

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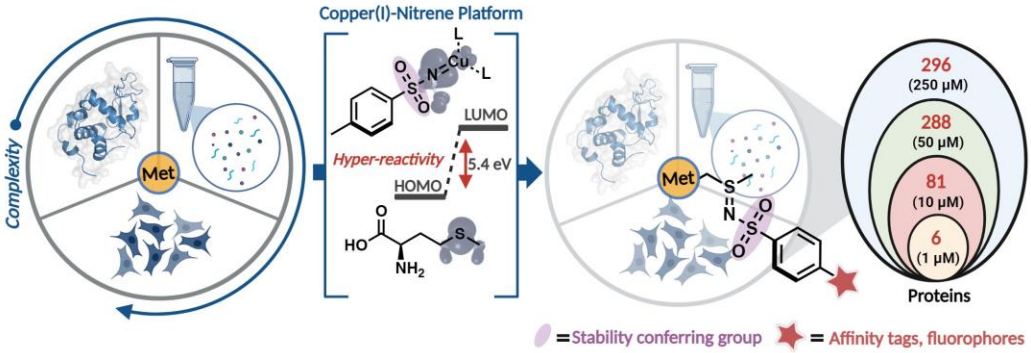

Methionine, constituting a meager 2% of amino acids in proteins, is nevertheless pivotal in cell-regulatory functions, underpinning processes that stave off diseases like neurodegeneration and cancer. Historically, methionine's low surface accessibility in proteins and intrinsic rapid oxidation owing to its condensed Lowest Unoccupied Molecular Orbital (LUMO) and Highest Occupied Molecular Orbital (HOMO) energy gap, presented substantial challenges in its chemoproteomic profiling, a concern further exacerbated by the paucity of efficacious methionine bioconjugation methods. Contemporary techniques have been inhibited by multiple limitations, including requisite acidic conditions, unstable resultant conjugates, and extensive probe usage. Riding on methionine's

high kinetic reactivity and discerning a potential in its differential HOMO-LUMO gap, our study introduces the Copper(I)-Nitrene Platform (CuNiP) as a groundbreaking means to achieve chemoselective sulfonyl sulfimide conjugation of methionine under benign physiological parameters. Crucially, the sulfonyl element augments sulfimide's stability, countering potential hydrolytic tendencies. Our trials with CuNiP showed an impressive spectrum of methionine labeling across a variety of bioactive peptides, intact proteins with diverse molecular weights (6.5-79.5 kDa), and proteins in cell lysate combinations. Further, it was optimally harnessed to label methionines in live T47D breast and LnCap prostate cancer cells, with minimal cytotoxic repercussions. Remarkably, employing nitrene-reactivity-focused probes, our research uncovered an ensemble of previously undiscovered ligandable protein sites endowed with hyperreactive methionine in the human proteome matrix. These revelations, combined with the insights from live-cell methionine labeling, not only enrich our comprehension of protein biochemistry but also set the stage for revolutionary strides in drug discovery and the expansive domain of protein therapeutics.

32. Abstract: Juliet V. Santiago

Identification of state-specific proteomic and transcriptomic signatures of microglia-derived extracellular vesicles

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Microglia are resident immune cells of the brain that play important roles in mediating inflammatory responses in several neurological diseases via direct and indirect mechanisms. One indirect mechanism may involve extracellular vesicle (EV) release, so that the molecular cargo transported by microglia-derived EVs can have functional effects by facilitating intercellular communication. The molecular composition of microglia-derived EVs, and how microglial activation states impacts EV composition and EV-mediated effects in neuroinflammation, remain poorly understood. We hypothesize that microglia-derived EVs have unique molecular profiles that are determined by microglial activation state. Using size exclusion chromatography to purify EVs from BV2 microglia, combined with proteomic (label-free quantitative mass spectrometry or LFQ-MS) and transcriptomic (mRNA and small RNA sequencing) methods, we obtained comprehensive molecular profiles of microglia-derived EVs. LFQ-MS identified several classic EV proteins (tetraspanins, ESCRT machinery, and heat shock proteins), in addition to over 200 proteins not previously reported in the literature. Unique mRNA and microRNA signatures of microglia-derived EVs were also identified. After treating BV2 microglia with lipopolysaccharide (LPS), interleukin-10, or transforming growth factor beta, to mimic pro-inflammatory, anti-inflammatory, or homeostatic states, respectively, LFQ-MS and RNA sequencing revealed novel state-specific proteomic and transcriptomic signatures of microglia-derived EVs. Particularly, LPS treatment had the most profound impact on proteomic and transcriptomic compositions of

microglia-derived EVs. Furthermore, we found that EVs derived from LPS-activated microglia were able to induce pro-inflammatory transcriptomic changes in resting responder microglia, confirming the ability of microglia-derived EVs to relay functionally-relevant inflammatory signals. These comprehensive microglia-EV molecular datasets represent important resources for the neuroscience and glial communities and provide novel insights into the role of microglia-derived EVs in neuroinflammation.

33. Abstract: Mubassarah Munjirin Sazmi

Gas Phase Interaction of Glyphosate Herbicide with Heme-Containing Proteins Investigated by Electrospray Ionization Coupled Orbitrap Exploris 240 Mass Spectrometer

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Pesticides are chemical compounds used to eliminate pests such as insects, rodents, fungi, and unwanted or invasive species of vegetation or weeds. Chemical pesticides have detrimental effects on the ecosystem, animal, and human health. Organophosphates are phosphoric acid esters that are primarily insecticides and are classified as broad-spectrum pesticides. Among Organophosphates, glyphosate is the most commonly used herbicide in the United States which blocks essential enzyme for plant growth. It is a widely used herbicide for controlling broadleaf weeds and grasses. Recently there are significant public concerns about glyphosate as the International Agency for Research on Cancer (IARC) declared it as probably carcinogenic. In this study, we explore the gas phase interaction of glyphosate with various model proteins. The heme-containing cytochrome C, myoglobin, and non-heme containing Lysozyme proteins were utilized as model systems in this work to anticipate binding and interaction caused by glyphosate. In presence of water, mass spectrum of cytochrome C shows the charge state distribution from 5+ to 15+, in which 8+ is the most intense peak. When low concentrations of glyphosate were used, the charge state distribution of cytochrome C was reduced significantly, however, no glyphosate was abducted with the protein. At relatively higher concentrations (>25 micromolar) of the herbicide, a series of glyphosate from one to nine were adducted with cytochrome C. In addition, a series of eight phosphates also bind with the protein. However, such series of adduct formation was not observed for when glyphosate was mixed with myoglobin, only magic number of four and eight glyphosates were adducted with the protein. In the case of non-heme containing protein (Lysozyme), a series of four glyphosate is interacted with protein as very high concentration. Our results showed that glyphosate and phosphate strongly bind and interact with heme containing proteins compared to the non-heme containing protein.

34. Abstract: Elisabeth Schwiebert

Simultaneous Quantitation and Discovery (SQUAD) Liquid Chromatography Mass Spectrometry of Progesterone Steroids Relevant to Ovarian Cancer.

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High-grade serous carcinoma (HGSC), the most common and lethal subtype of ovarian cancer, is an aggressive subtype, often going undiagnosed until the advanced stages. The risk of HGSC and other subtypes of ovarian cancer grows with age, and women with predispositions, including familial cancer history and breast cancer susceptibility gene (BRCA1/2) mutations, are at greater risk. Patients with BRCA mutations are often advised to undergo prophylactic removal of the ovaries and fallopian tubes to reduce their lifetime risk, yet a large proportion of them will never develop ovarian cancer. These surgeries are invasive and could be avoided in lower risk individuals with an alternative risk assessment that evaluates genomic and metabolomic factors. Building on previous work that demonstrated that progesterone (P4) treatment induced tumorigenesis and metastasis of HGSC in an ovariectomized BRCA1 mouse model, we hypothesize that ovarian P4 is an essential early factor in determining ovarian cancer risk in BRCA1/2-mutation carriers. To test this hypothesis, circulating P4 was analyzed as an essential biomarker in the early development of HGSC and its subsequent metastasis. Concentrations of P4 and its metabolites were measured from serum samples taken every two weeks over the lifetime of these mice. To measure these concentrations, we have developed a SQUAD LC-MS method for a tribrid mass spectrometer using a range from 220-1000 m/z, enabling the collection of both targeted and non-targeted features to map alterations in the metabolic pathways, involving P4 and its metabolites. SQUAD allows detection and quantitation of these metabolites while also enabling the retro-mining of non-targeted data that were not part of the original hypothesis, in a single experiment. We here present our set of initial results of elevated P4 levels in four cancerous and two control mice and describe the challenges and opportunities we face moving forward.

35. Abstract: Xinya Su

Deep-phosphoproteomics analysis of intracellular acid stress characterizes pH-dependent phosphorylations and highlights potential pH-sensing kinases

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Acidic microenvironment is a common feature of cancerous tumor cells and emerging evidence suggests that intracellular protons can serve as ‘second messengers’ that regulate specific target proteins involved in signal transduction. We recently discovered a pH-sensitive phosphorylation

site (Serine 3) in the yeast heterotrimeric G protein, Ste18, that behaves as a direct reporter of intracellular pH. Unlike most pH-dependent phosphorylation sites in yeast, acid-dependent phosphorylation of $Ste18^{S3}$ is unique due to its independence from cell wall and plasma membrane stress that is concomitant with intracellular acidification, suggesting the possibility that unknown pH-sensing kinases may exist. To identify similar phosphosites in the yeast phosphoproteome – and elucidate pH-sensing kinases – we conducted a SILAC-based deep-phosphoproteomics analysis in which pH and cell wall stress-dependent phosphoproteomes are distinguished. We identify ~100 pH-specific phosphorylation sites, whose phosphorylation is induced by intracellular acidification independent of cell wall stress. Gene ontology analysis indicates enrichment in a wide variety of cellular processes including ER membrane organization, SNARE complex, positive regulation of glycerol transport, and mitosis. Motif analysis of these phosphosites finds no highly significant motif but a unique +2/3 position aspartic acid (D) enrichment known to be phosphorylated by a small number of kinases. To have a better characterization of this kind of phosphorylation, further analysis of surrounding physiochemical properties reveals a fingerprint of these sites and refines a list of potential pH-sensing kinases. Ongoing work seeks to understand intracellular pH signaling processes by mapping pH-regulated kinase-substrate relationships. Taken together, these data reveal a network of proteins undergoing PTM-based regulation in response to intracellular acidification.

36. Abstract: Hoang Kim Ngan Thai

HILIC-HRIM-MS/MSMS application towards the analysis of complex N-glycans

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N-linked glycosylation is an important post translational modification, and the change of N-glycan structures is known to be associated with numerous human diseases. Tandem mass spectrometry (MS/MS) is a commonly used method in glycomics analysis and allows identification and differentiation between some positional N-glycan isomers. For instance, a tri-antennary N-glycan can be differentiated from its positional isomer consisting of a bi-antennary glycan with a Galβ1-4GlcNac extension on one of the antennae. However, glycans have other types of isomers, particularly ones arising from linkage, anomeric, and stereochemical differences, all of these isomers are difficult/impossible to differentiate using only MS approaches. Hence, challenges remain in glycomics analysis and require the use of additional analytical techniques to provide a more complete picture of complex glycan isomers. The addition of hydrophilic interaction liquid chromatography (HILIC) can allow the separation of linkage isomers, which are difficult to identify by mass spectrometry. Studies have reported the separation between α-2,6 sialylated and α-2,3 sialylated glycan in which α-2,6 sialylated elute later in HILIC compared to α-2,3 sialylated glycans. Glycans differing by linkage to sialic acids give identical MS/MS spectra, and thus cannot be identified by MS/MS. Recently, we also observed unique fingerprints of such complex isomers through high resolution ion mobility (HRIM). These fingerprints can be used to further identify these isomers. Herein, we focus on the synergistic combination of HILIC-HRIM-MS/MS to better enable the analysis of complex N-glycan structures.

37. Abstract: Blaise Williams

Selected Ion Monitoring Based Protease Assay for Peptide Inhibitors against the 3-Chymotrypsin-Like Protease of SARS-CoV-2

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The newly emerged SARS-CoV-2 virus triggered the global pandemic in the December of 2019 and as of now this deadly virus infected over 769 million worldwide and killed over 6.95 million. This is a single-stranded RNA (ssRNA) virus which translated 29 proteins in the host cell. Among these proteins, scientists have quickly identified crucial proteins such as 3-chymotrypsin-like protease (3CLpro) which performs a very critical function in viral replication. Various reliable and effective methods were developed to screen small molecules and peptides inhibitors targeting the 3CLpro. Most methods are used to quantify inhibitor affinity for the 3CLpro by employing fluorescence resonance energy transfer (FRET) based assay due to their affordability and accessibility. However, fluorescent assays present a flaw of providing false positive readings due to the background fluorescence of fluorogenic substrate interfering with the optical signal. Instead of using fluorescent assays, selected ion monitoring (SIM) coupled with liquid chromatography-mass spectroscopy (LC-MS) can be used as a convenient option because it provides exceptional sensitivity and reliability due to its reliance on mass-to-charge ratios of the product ion generated from the protease activity on the substrate. Moreover, as this technique does not require any chromophore which can significantly reduce the cost related with fluorescent substrate. Initially, the selected ion monitoring based LC-MS method was used to compare substrate degradation and product formation to obtain the 50% inhibitory concentrations (IC50) values for the known inhibitor, GC-376, of 3CLPro and the result was compared with FRET assay. The estimated 50% inhibitory concentration of GC-376 obtained in LCMS assay was 116 nM which agreed with the IC50 value measured by FRET assay (180 nM). The developed SIM-LCMS method was applied for various linear and staple peptides of Temporin L which acted as the peptide inhibitor of 3CLpro. The estimated IC50 values of the Temporin L and its stable analogue TLP3S1 obtained from LCMS assay were 18.39 μM and 304.6 nM which are comparable with FRET assay (38.80 μM and 574 nM).

38. Abstract: Xing Xu

Systematic investigation of the trafficking of glycoproteins on the cell surface

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Glycoproteins located on the cell surface play a pivotal role in cellular communication, facilitating the transmission of crucial information between the extracellular environment and the intracellular compartments that govern cell proliferation and fate. N-glycosylation is a fundamental protein

modification in eukaryotic cells, profoundly influencing protein folding and trafficking processes.[1] The glycosylation of cell-surface proteins undergoes meticulous regulation by various enzymes, ensuring their localization on the cell surface.[2] Dysregulation of cell-surface glycoproteins is a hallmark of cancer and autoimmune diseases.[3-5] However, the specific impacts of protein folding status and N-glycan maturity on the trafficking of cell-surface glycoproteins remains largely obscure. In this study, we conducted a comprehensive and site-specific analysis of cell-surface glycoprotein trafficking in human cells. Our approach involved the integration of metabolic labeling, bioorthogonal chemistry, and multiplexed proteomics. We quantified 700 N-glycosylation sites from 400 cell-surface glycoproteins in THP-1 cells, either by inhibiting protein N-glycosylation, disturbing N-glycan maturity, or perturbing protein folding in the endoplasmic reticulum (ER). Our findings elucidate the distinct effects of N-glycan maturity and the protein folding status on the trafficking of surface proteins. Specifically, N-glycan maturity has more substantial influences on proteins with high N-glycosylation site densities, while the protein folding exerts a more pronounced influence on surface glycoproteins characterized by larger sizes. Furthermore, our results demonstrate that N-glycan immaturity tends to have a more significant impact on glycoproteins with N-glycosylation sites located at highly exposed or accessible asparagine positions. Additionally, we identified that changes in protein folding or immature N-glycans can affect N-glycosylation sites differently depending on the specific secondary structure of the protein. This systematic analysis of surface glycoprotein trafficking advances our understanding of the mechanisms underlying protein secretion and surface presentation.

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39. Abstract: Xu Yang

Enhancing N-Glycopeptide Enrichment with Phosphorylcholine ZIC-HILIC Resins as a Complement to iSPE®-HILIC

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Glycoproteomic analysis on complex biological samples has become an essential tool in combating significant health threats. Mass spectrometry-based glycoproteomics has emerged as the primary method for this purpose, largely due to the growing availability of glycopeptide enrichment techniques. One such technique, iSPE®-HILIC, a zwitterionic hydrophilic interaction

chromatography (ZIC-HILIC) resin, has been widely used for glycoproteomic studies, particularly with human serum samples. However, the potential biases of iSPE®-HILIC for broader sample types, such as the HEK293 cell line, have received little attention. Another ZIC-HILIC resin, featuring an exposed phosphorylcholine group, has been extensively used for C-reactive protein (CRP) enrichment. In this study, we have introduced a novel enrichment approach, termed phosphorylcholine enrichment (PCE), that employs this resin to complement iSPE enrichment. We evaluated the enrichment outcomes of various combinations of these two resins, using the already powerful Orbitrap Eclipse mass spectrometer. By applying PCE and iSPE on HEK293 cell samples complementarily, we achieved unbiased glycoproteome coverage. Notably, the iSPE-first approach, scavenged by PCE enrichment, increased unique N-glycopeptide detection in unfractionated HEK293 cell digest by a factor of 5.40 compared to unenriched sample. Our study highlights the importance of selecting an appropriate glycopeptide enrichment strategy tailored to the specific research objectives. This work has been supported by the US National Institutes of Health (R24GM137782 to PA); GlycoMIP, a National Science Foundation Materials Innovation Platform funded through Cooperative Agreement (DMR-1933525).

40. Abstract: Yiqing Zhang

Developing Novel capillary electrophoresis tandem mass spectrometry strategies for the characterization of complex mixture Heparan sulfates

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Glycosaminoglycan (GAG) carbohydrates are linear, acidic polysaccharide chains with alternate *GlcA* / *GlcNAc* repeating unit, containing the modifications of sulfation, acetylation, and epimerization that are linked to extracellular matrix or cell surface protein. Heparan sulfate (HS) with *(-GlcA β1,4 -GlcNAc α1,4-)* repeating unit prevalently existed in mammals and mollusks. The complex pattern and ubiquitous distribution among the animals of GAG chains require a smart approach for the effective characterization. Various diseases shows an increase of heparin sulfate level [1] and modification in heparin sulfate [2]. The capillary zone electrophoresis as a traditional analytical tool had been applied to the GAG separation since 1991[3]. The combination of high-res Orbitrap and conventional CZE provide a power tool to characterize heparan sulfate mixtures[4]. The de novo sequencing of complex mixtures of heparan sulfate oligosaccharides have been done by the RPLC-MS/MS [5]. This time capillary zone electrophoresis – mass spectrometry is applied to separate and profile heparan sulfate oligomer mixtures with mobile phase modifier ammonium formate / formic acid. The quantitively statistic study reveals the signal enhancing performance of new background electrolyte (BGE solutions) in negative mode mass spectrum. By combining signal enhancing and pH mediation from the ammonium formate buffer, we can effectively differentiate trace GAG isoforms within complex biological mixtures and identify the structure of heparan sulfate chain. Additionally, the inaugural application of the 'iRT' calibration method to the capillary zone electrophoresis system has demonstrated comparable performance with HPLC system.

GAG oligosaccharides were prepared by enzymatic depolymerization, purification by strong anion exchange column, and desalting with Amicon ultra 3K filter. Capillary electrophoresis separations

were performed on an Agilent HP3D CE. The inner surface of capillary is covalently modified with N-(6-aminohexyl)aminomethyltriethyoxysilane(AHS) and dimethylsilane (DMS) capping agent. Prior to the CZE separation, AHS capillary was conditioned by flushing background electrolyte (BGE, 25mM ammonium formate, 0.1% formic acid, 70% methanol). ±30kV voltage was applied to the inlet and outlet of capillary. The EMASS -II interface was employed to couple the CZE with the Orbitrap Elite mass spectrometer (Thermo Scientific, Bremen, Germany). NETD fragmentation was performed with fluoranthene radical on a Velos Orbitrap Elite. The m/z, migration time features from the electrochromatogram were extracted by MZmine 3.0 opensource software. All the statistical analysis was done in Rstudio online.

Capillary electrophoresis – Mass Spectrometry has been demonstrated with baseline separation ability of mixtures of tetrasaccharides that vary in the sulfation, acetylation, and epimerization pattern. The background electrolyte 25mM Ammonium acetate was proved for effectively separating the complex mixtures of heparan sulfate in the prior paper[4]. However, the low signal / noise intensity issue from the usage of ammonium acetate BGE in the mass spectrometer poses a challenge on the low concentration GAG solution of various mammalian samples. In the present study, the addition of signal enhancing modifier Ammonium formate / formic acid in BGE boosts the peak intensity of heparan sulfate, which provides the opportunity for the structural characterization of low concentration HS[6] . NETD performed with fluoranthene has been demonstrated to effectively fragment GAG chain, up to dp 10[7]. The comparative analysis of peak capacity and sample loading limit are conducted within heparan sulfate mixture between the prior ammonium acetate BGE and novel ammonium formate / Formic acid (AF/FA). The compatibility of FA/AF in glycosaminoglycan applications is tested with enoxaparin. We have performed the AF/FA signal enhanced modifier for both CS and HS samples. The addition of AF/FA has the significant effect on enhancing the signal ten-fold higher compared to the conventional BGE. With the high accuracy of migration time achieved by the iRT method, it provides the optimal solution for characterizing complex GAG mixtures.

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41. Sponsor Abstract: Affinisep

A new SPE Tips method based on an innovative sorbent for fast and efficient peptide fractionation in proteomic studies

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Peptide fractionation for full proteome characterization is very challenging, especially in the case of complex samples. The objective of this study was to develop a simplified procedure for the efficient and fast fractionation of peptides at basic pH, to contribute to further simplification of peptide separation and analysis.

A new reversed-phase sorbent, based on small sorbent particles tightly embedded in a monolithic membrane packed in SPE StageTips was used for the fractionation of peptides resulting from the enzymatic proteolysis of HEK293 cell lysate, and the results were compared to a reference commercial fractionation kit.

Eight fractions were obtained from each the commercial column and the SPE StageTips, with an acetonitrile gradient. Each fraction then was evaporated to dryness before being re-suspended in an appropriate solvent for nanoLC-MS/MS analysis.

The total number of proteins identified and the percentage of peptides eluting in only one fraction, were similar for both sorbents, with a good distribution of peptides over the eight fractions. Yet, it appeared that the fractionation on the new sorbent presented several advantages compared to the reference kit. For one it can be stored dry at room temperature while the commercial columns have to be stored at 4°C in a storage buffer. Moreover, due to the SPE StageTips format, the time required for the evaporation of each fraction is almost halved compared to the commercial columns.

Thus, the new sorbent appears as a promising solution for the fractionation of complex samples and the generation of spectral libraries, since it leads to an increase of more than 25% in the number of proteins identified, compared to unfractionated samples. Finally, this new sorbent offers

flexibility of format and capacity, since it is also available as spin columns, for high amounts of peptides, or as 96 well plates, for high throughput experiments.

42. Sponsor Abstract: Bruker

Optimizing dia-PASEF isolation window schemes for proteomics measurements on a timsTOF ultra instrument

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Data-Independent Acquisition (DIA) is widely used for proteomics as it usually outperforms Data-Dependent Acquisition (DDA) for protein identification and quantitation, due to its higher ion usage and reproducibility, resulting from a fixed scheme of rather broad isolation windows. This advantage can be further increased by combining it with trapped ion mobility separation (TIMS), as the additional separation dimension reduces complexity and the sequential elution of condensed ion packages from the TIMS device allows for even more efficient ion usage (dia-PASEF). The two-dimensional mass and mobility space enables method creation with extensively different window schemes.

43. Sponsor Abstract: Agilent

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The intelligent reflex protocol is a confidence and intelligence feature within Agilent MassHunter acquisition software that aims to provide reflexive re-injection logic to ensure your results are immediately trustworthy and within operational limits. This application note demonstrates the use of the fast-screening function within intelligent reflex—which can be used to improve analytical speed—to rapidly screen for positive samples using an analytical method approximately 3 minutes in length. Prescreening a large batch of samples for presumptive positives using the fast-screening workflow allows for exclusion of negative samples from quantitative analysis, resulting in improved batch completion time.