Poster Presentation Abstracts: AAMSDG 2024-25

1. Abstract: Trevor Adams

Site-specific cross-clade N-glycopeptide analysis of HIV-Env NFL trimers

Trevor Adams, Mukaddes Sena Cinar, Fikri Avci

The densely glycosylated Env trimer is the sole antigenic target of broadly neutralizing antibodies (bnAbs) during HIV infection. However, the glycan shield, predominantly composed of N-glycans, provides the virus with a robust defense by protecting conserved regions of the underlying protein. In previous work, our group identified a novel glycosylated epitope capable of being presented to a T cell receptor (TCR) and stimulating an immune response. This finding highlights the underappreciated potential of glycosylated peptides as novel T cell epitopes. It is therefore useful to develop an understanding of the conservation of site-specific glycoforms across clades rather than just the underlying peptide. Here, we expressed three variants of HIV-Env in Exp293 cells as native flexibly linked (NFL) trimers that represent three major clades of virus: A (BG505), B (JR-FL), and C (16055). The site-specific glycosylation of these trimers was characterized using both glycomics and glycopeptide analysis. The glycopeptide analyses utilized high-field asymmetric waveform ion mobility spectrometry (FAIMS) to enrich glycopeptides in a non-biased manner. By utilizing a variety of protease conditions, we achieved broad coverage across most sites of N-glycosylation. This work advances our understanding of the conservation of glycoforms across clades and has potential applications in the development of novel vaccines and therapeutics.

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: David Brewer

Revealing the alteration of *S. aureus* and tissue-derived lipids *in vitro* using lipid-enhanced media

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Staphylococcus aureus can alter its native membrane composition by using exogenous fatty acids that are liberated from intact lipids by its lipase, glycerol ester hydrolase (*Geh)*. The resulting free fatty acids can then enter lipid biosynthesis through the fatty acid kinase pathway (*FakA/B1/B2*). While most research has studied the incorporation of specific mammalian fatty acids, such as oleic acid, *S. aureus* has access to many different types of lipid substrates and fatty acids in the environment of a deep-tissue infection. In this study, we investigate this interplay using broth that is enriched with tissue-specific lipid extracts. *S. aureus* strain JE2 was incubated for 16 hours in tryptic soy broth (TSB) supplemented with 50 µg/mL of commercial bovine heart, brain, or liver polar lipid extracts. Lipids were extracted from both bacterial pellets and broth and analyzed by reversed-phase liquid chromatography and mass spectrometry (RPLC-MS). *S. aureus* pellets collected after growth in lipid-enriched broth contained phosphatidylglycerols (PGs) with fatty acyl tails that had one to six double bonds, indicating incorporation of bovine lipid extract-derived mono- and polyunsaturated fatty acids. Analysis of the spent broths revealed an increase in lyso-phospholipids after incubation with *S. aureus*. The majority of these lyso-lipids had the remaining acyl tail at the *sn-2* position, indicating a preference for *Geh* to act on the acyl tail at the *sn-1* position of the glycerol backbone. Our results reveal that lipids of both the bacterium and its local environment are significantly altered through the combined actions of *Geh* and *FakA/B1/B2*.

4. Abstract: Justin Byun

Optimizing solid phase extraction to prepare wastewater samples for untargeted exposome screening

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Increasing evidence suggests that environmental exposures drive risk of a number of non-communicable diseases; however, current methods to evaluate environmental factors within a population do not account

for the complex chemical exposures that likely occur. With the need for a more complete environmental exposure assessment to understand the association between environmental influences and their biological response, wastewater-based epidemiology holds potential to identify the chemical exposome without requiring individual samples from a study population, avoiding the costly and logistical constraints of human biomonitoring of biofluids. The purpose of this study was to optimize and validate a solid phase extraction (SPE) method to prepare wastewater samples for untargeted screening using liquid chromatography-high resolution mass spectrometry (LC-HRMS). We evaluated how SPE can enhance determination of the composition of analytes in wastewater to potentially identify previously unrecognized exposures for a more comprehensive chemical exposome profile. To evaluate the potential of using SPE to increase detection of exposures in wastewater, samples from 27 different sites in Louisville, Kentucky were collected, including a range of industrial and residential areas and analyzed using SPE with LC-HRMS. SPE significantly increased the intensity of detected features in wastewater and the total number of detected features in wastewater compared to traditional sample preparation methods. Each site exhibited a larger number of detected features in wastewater prepared through SPE compared to wastewater prepared with traditional methods. Future steps include analyzing LC-HRMS data to determine if concentrations of certain detected chemicals differ significantly between wastewater sites with high and low median income levels.

5. Abstract: Ryan Coyle

Increased levels of Erythrocytic α-Synuclein using targeted mass spectrometry

Idiopathic Parkinson's disease (iPD) is the second most common neurodegenerative disease after Alzheimer's disease (AD). Mutations in the SCNA gene, which encodes the protein alpha-synuclein (α-syn), are associated with familial forms of Parkinson's disease (PD). Additionally, Lewy bodies (LBs) rich in α-synuclein are a hallmark of idiopathic Parkinson's disease (iPD) pathology. Unlike AD, there are no effective blood-based diagnostic assays for iPD. Recent studies show that misfolded α-syn can aid in diagnosing PD via cerebrospinal fluid (CSF) and skin biopsies, suggesting that the altered cellular processes in the brain may also occur in the periphery. However, CSF and skin biopsies are invasive, highlighting the need for a blood-based diagnostic assay. Erythrocytes (red blood cells) are the richest source of α-syn in the body, and we hypothesized that peripheral α-syn changes could be detected in erythrocytes in iPD. To test this hypothesis, we used a targeted liquid chromatography-mass spectrometry (LC-MS) assay, employing 15N-enriched recombinant α-syn as an internal standard, to compare α-syn levels in erythrocytes from iPD patients, AD patients, and healthy controls. The results showed α-syn concentrations (standard deviation) of 42.6 µg/mL (27.8) in healthy controls, 36.7 µg/mL (19.0) in AD, and 50.3 µg/mL (29.2) in iPD. Although α-syn levels were significantly elevated in iPD, the receiver operating characteristic (ROC) analysis yielded an area under the curve (AUC) of 0.63, indicating that elevated α-syn levels alone are not sufficient for diagnostic purposes.

6. Abstract: Jessica Eyram Kugblenu

Histidine^{421} mutation alters catalysis and metal content of *Pseudomonas aeruginosa* D-2-Hydroxyglutarate Dehydrogenase

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P. aeruginosa is an opportunistic gram-negative, aerobic, and non-spore-forming bacterium found in soil, water, and skin flora[1–4]. It causes infections in immunocompromised individuals with ailments such as cystic fibrosis, cancer, AIDS, and lung diseases[5,6]. In 2017 alone, *pseudomonas* infections caused 3000 deaths and 30,000 nosocomial infections[5]. *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[1,2,7]. Knockout of the *Pa*D2HGDH gene inhibits *P. aeruginosa* growth, making *Pa*D2HGDH a therapeutic target against *P. aeruginosa*[2,8]. Upon recombinant expression of *Pa*D2HGDH, there was only a 15% flavin reduction. Previous studies on closely related FAD-dependent dehydrogenases showed increased enzyme activity upon exogenous metal addition[6]. When *Pa*D2HGDH was analyzed using ICP-MS, significant but non-stoichiometric amounts of Mg^{2+} and Zn^{2+} were bound to *Pa*D2HGDH; however, only zinc could increase *Pa*D2HGDH activity[1]. 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[1,7]. 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[9]. 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[2]. However, the role of H^{421} in *Pa*D2HGDH has not been established.

In this study, site-directed mutagenesis was employed 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 and biophysical properties. ICP-MS was used to detect the metal content of the variant enzymes. The ICP-MS data showed that all variants retained significant amounts of the zinc metal while Mg^{2+}, Cd^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+}, and Fe^{2+} occurred in trace amounts. The kinetic data for the variant enzymes showed a 400-fold decrease in activities in comparison to the turnover rate of the wild type suggesting that though the zinc metal was present in the variants, the fully conserved H^{421} residue is important for *Pa*D2HGDH catalysis.

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7. Abstract: Anna Ivanova

EThcD-MS/MS method application towards the analysis of complex N-glycans in biological samples

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Glycosylation is one of the major protein post-translational modifications which covalently attaches a carbohydrate to the biomolecules. It plays a critical role in protein structure, stability, and functions. Numerous studies have shown the key role of glycosylation in various disorders, including cancer, liver, cardiovascular, and Alzheimer's diseases. Nevertheless, a detailed analysis of protein glycosylation is still required to establish molecular linkage between glycosylation and human disease states and promote new diagnostic biomarkers. To address this need, we examined the N-glycosylation status of the human plasma proteins using a bottom-up mass spectrometry that implements the electron-transfer/high-energy collision (EthCD) method. This study utilized the commercially available recombinant human purified proteins and a non-disease human plasma pool. The samples were subjected to reduction by dithiothreitol and alkylation by iodoacetic acid in the presence of 0.05% RapiGest (Waters), followed by digestion with various enzymes. Then, Liquid Chromatography–Mass Spectrometry (LC-MS/MS) analysis was performed on a Thermo Fisher Orbitrap Eclipse Tribrid mass spectrometer. Collected MS/MS data were processed using the Byonic node (Protein Metrics) within the Proteome Discoverer 3.0 (Thermo) software. To ensure the most complete coverage of detected N-glycosylation sites, we analyzed various combinations of different digesting enzymes. This method not only applies to purified protein but also to complex biological fluid samples, such as human plasma. For example, the glycosylation status of the proteins was monitored in the plasma pool sample. Our results demonstrate the method's effectiveness in mapping protein glycosylation, providing critical insights into protein function and interactions in metabolism.

Disclaimer. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention. The use of trade names is for identification only and does not imply endorsement by the CDC.

8. Abstract: Jiangpeiyun Jin

Deciphering the Mechanism of Antibiotic Response in Cystic Fibrosis Bacteria and Their Crosstalk with Fungal Pathogen Using Metabolomics

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Burkholderia cenocepacia can cause chronic infections in immunocompromised individuals, such as cystic fibrosis (CF) 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 natural products, some of which serve as virulence factors during infections, providing a fitness benefit to survive and adapt to the environmental changes. Production of natural products is dependent on the expression of their biosynthetic gene clusters (BGCs), which are typically silent under normal growth conditions. Antibiotics, as an important constituent of the CF lung environment, can act as external stimuli to activate the production of natural products in *B. cenocepacia* via an unknown mechanism. In addition, the presence of several different pathogen species in CF lungs provides a great opportunity for polymicrobial communication. We utilized mass spectrometry based untargeted metabolomics to investigate the response of *B. cenocepacia* to clinically used antibiotics at

sublethal concentration and leveraged mutations in quorum sensing genes to mechanistically characterize such response. Using the metabolomics approach, we linked QS-controlled gene regulation to the antibiotic mediated metabolic response in *B. cenocepacia*. Furthermore, we revealed the chemical interactions between *Burkholderia cenocepacia* and *Aspergillus fumigatus*. This work emphasized that antibiotics can act as signaling molecules at sublethal concentration to mediate microbial BGCs expression that can involve in cell-to-cell communication. This work enabled us to improve our understanding of pathogenic physiology of *B. cenocepacia* under clinically relevant conditions.

9. Abstract: Katherine J. Kenney

Lipid Profiles of Pre-Breast Cancer Women using Simultaneous Quantitation and Discovery (SQUAD) Liquid Chromatography Mass Spectrometry

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Breast cancer is the most prevalent cancer globally, accounting for 12.5% of new annual cases worldwide. Emerging evidence suggests that the steroid hormone progesterone may be a critical intrinsic factor for breast cancer risk in BRCA1/2 carriers. Using metabolomics, we are examining the association between metabolomic alterations and progesterone levels in women who develop breast cancer later in their lifetime. We aim to identify metabolite changes linked to progesterone levels and signaling and to determine whether these steroidomic alterations are associated with breast cancer risk.

We studied serum samples from 450 breast cancer and control patients from the Komen Tissue Bank at Indiana University. Using a SQUAD liquid chromatography mass spectrometry (LC-MS) approach we collected both targeted and non-targeted metabolomics data in a single experiment. SQUAD is an innovative acquisition scheme that interleaves targeted and non-targeted scans in mass spectrometry platforms. Non-targeted experiments enable the generation of hypothesis associated with progesterone and breast cancer based on BRCA status. Targeted experiments included progesterone, beta-estradiol, estrone, cortisone, hydrocortisone, corticosterone, testosterone, androstenedione, and 17alpha-hyroprogesterone, producing data that can be compared across batches and over time. These targets were selected to cover a variety of enzymatic steps along the progesterone pathway.

Both targeted and non-targeted SQUAD LC-MS experiments yielded informative preliminary data, highlighting the potential differences among steroid pathways. Targeted data focused on species of interest that contribute to the steroid metabolic pathway such as progesterone, cortisone, beta-estradiol, hydrocortisone, corticosterone, estrone, testosterone, androstenedione, and 17alpha-hydroxyprogesterone. All steroids except beta-estradiol were analyzed in the positive ion mode, while beta-estradiol analysis was conducted in the negative ion mode. Additionally, MS/MS was collected for the untargeted data, to help identify several of the steroids of interest when confirmation was needed or to annotate unknowns with significant fold changes. MS2 data was compared against an in-house mzVault database along with accurate mass measurements. Annotation of known and unknown metabolites presents both an opportunity to better understand breast cancer biology but also a significant challenge.

The SQUAD LC-MS approach ensures that both qualitative and quantitative differences in the steroid and metabolome profiles are thoroughly investigated, providing deeper insights into breast cancer biology.

10. Abstract: Yi Lasanajak

Advanced LC-MS Capabilities for Protein, Glycan, and Oligonucleotide Analysis at the Emory EGMIC Core

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Emory Glycomics and Molecular Interactions Core (EGMIC) is equipped with top-of-the-line LC-MS instruments. Our core provides extensive support to researchers through a broad range of mass spectrometry tools and techniques. This includes the options to learn to conduct LC-MS analysis for trainees or to have our dedicated staff run the samples. We are unique in the world by offering ion mobility mass spectrometry with electron capture dissociation. These techniques allow characterization of the shape and quaternary structure of proteins, and resolve glycan, and lipid isomers. In addition, we conduct validation of reagents especially recombinant proteins and synthetic peptides. This allows the user to confirm they received what they ordered or made the correct protein. We find 10% or more of synthetic peptides from reputable suppliers are incorrect and recombinant proteins contain surprising additions or truncations ~25% of the time. LC-MS offers precise molecular mass measurements and comprehensive analyses of proteins, peptides, glycans, and oligonucleotides. For antibodies, proteins and peptides, LC-MS provides insights into purity, folding stages, dimer configurations, protein interactions, post-translational modifications, and top-down sequencing. In glycan analysis, LC-MS enables the study of various glycoforms and glycosylation in monoclonal antibodies, utilizing workflows such as Agilent InstantPC tagging for rapid and high-throughput N-glycan composition analysis. Validation of synthetic oligonucleotides, LC-MS delivers critical data on sequence confirmation, purity, and structural modifications. Our team is dedicated to helping you leverage LC-MS to gain deeper insights into your samples, ensuring high-quality data and impactful scientific outcomes.

11. Abstract: Bilkis Mehrin Moni

Assessment of Alternative Metal Binding to D2-Hydroxyglutarate Dehydrogenase Using Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

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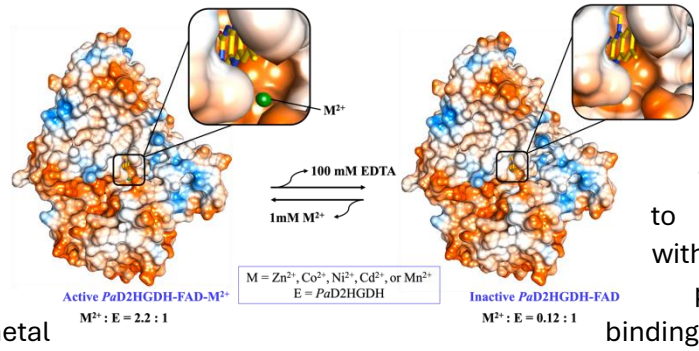
The enzyme D-2-hydroxyglutarate dehydrogenase (*Pa*D2HGDH) is a metallo-flavoenzyme, primarily relying on Zn^{2+} to catalyze the oxidation of D-2-hydroxyglutarate to a-ketoglutarate. (1,2) The enzyme can also function with Co^{2+}, Ni^{2+}, Mn^{2+}, and Cd^{2+} as alternative metal cofactors, (2) though their roles in the catalytic mechanism are less explored. In this study, Zn^{2+} was substituted with Co^{2+}, Ni^{2+}, Cd^{2+}, or Mn^{2+} to investigate the significance of alternative metals on the rate of flavin reduction and catalysis of *Pa*D2HGDH. Recombinant His-tagged *Pa*D2HGDH was treated with 100 mM EDTA to create the metallo-apo-enzyme (E-FAD) with 1 mM chloride salts of various metals. The M^{2+} to protein ratio was determined by ICP-MS to assess metal binding to the enzyme. The mol M^{2+}:protein stoichiometry for E-Zn^{2+} was 2.2:1, E-Co^{2+} was 0.6:1, E-Ni^{2+} was 2:1, E-Cd^{2+} was 1.2:1 and E-Mn^{2+} 0.3:1. The anaerobic reductive half-reaction showed a ≤ 2-fold increase in k_{red} values (85-160 s^{-1}) for Co^{2+}, Ni^{2+}, and Mn^{2+} compared to Zn^{2+} (70 s^{-1}), indicating that alternative metals modulate the rate of flavin reduction in *Pa*D2HGDH. K_d values for D-malate were similar for Co^{2+} and Mn^{2+} (~10 mM) compared to Zn^{2+} (8 mM) but decreased by ~2-fold with Ni^{2+} (4 mM), suggesting a higher binding affinity of E-Ni^{2+} for the substrate. Steady-state kinetics of *Pa*D2HGDH loaded with different metal ions at varying D-malate revealed a ≤6-fold variation in k_{cat}/K_m (950 - 40,000 $M^{-1}s^{-1}$) and ≤4-fold in k_{cat} values (10-45 s^{-1}), indicating that alternative metals alter substrate capture and the slowest step of catalysis. Notably, the k_{cat} for E-FAD with 400 µM Zn^{2+} was 16 s^{-1}, increasing to 34 s^{-1} with 800 µM Zn^{2+}. For Co^{2+}, k_{cat} was 20 s^{-1} at 250 µM Co^{2+}, rising to 50 and 60 s^{-1} at 450 and 800 µM Co^{2+}, respectively, suggesting different enzyme conformations at different metal concentrations. Steady-state kinetics of E-FAD (0.20 µM-25 µM) with 800 µM Co^{2+} at varying D-malate concentrations showed a sigmoidal increase in k_{cat} and k_{cat}/K_m with increasing the ratios of Co^{2+} to E-FAD enzyme, suggesting that the binding of Co^{2+} to the active site cooperatively influences the binding of additional Co^{2+} ions to the other sites, enhancing the enzymatic activity in a cooperative manner. Collectively, these findings indicate that the alternative metal ions alter the rate of flavin reduction and catalysis of *Pa*D2HGDH.



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12. Abstract: Mónica Monge-Loría

Wielding untargeted metabolomics to explore marine bacteria community interactions

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The ocean covers the majority of Earth's surface and hosts an immense diversity of organisms. Particularly, coral reefs harbor 25% of the ocean's biodiversity[1] rendering them an ecosystem of interest. This diversity translates into a wide variety of compounds with unique molecular scaffolds, functions, and bioactivities.

Such variation stems, in part, from the intricate relationships formed in the reef, as some of these compounds are useful for intra- and inter-species communication, pathogenesis, nutrient acquisition, and defense[2]. However, this full array of molecules is often not detectable under normal laboratory conditions, as the genes that encode for their production are commonly silent[3]. In this work, we aim to induce these biosynthetic gene clusters through bacterial cocultivation and leverage the untargeted metabolomics workflow to survey the resulting interactions. We specifically cocultured marine bacteria with the coral pathogen *Vibrio coralliilyticus* Cn52-H1, as it produces the secondary metabolite elicitor andrimid[4]. Using this approach, along with NMR characterization, we detected the degradation of amphibactin siderophores from *V. coralliilyticus* Cn52-H1 by *Microbulbifer* sp. CN-SA-002. Further experiments have revealed this to be an enzymatic process, leading to protein isolation and analysis. Determining the enzyme responsible for this degradation will allow us to parse out the interactions at play between these bacteria and gain insight into iron acquisition by *Microbulbifer*.

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13. 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), a common neurodegenerative disorder, is characterized by the loss of dopaminergic neurons and the formation of Lewy bodies. While motor symptoms are typically used for diagnosis, early detection is critical. Lipids, essential for various cellular functions, are implicated in PD pathogenesis. Genetic mutations in lipid-producing enzymes and genes related to lipid metabolism have been associated with sporadic PD and interactions between α-synuclein and lipids are believed to influence disease progression.

This study employed LC-MS/MS to analyze lipid profiles in red blood cells (RBCs) and plasma to discover potential lipid biomarkers for PD and explore disrupted lipid regulatory pathways. A total of 286 patient samples (140 PD patients and 146 control subjects in total) from the Victorian Parkinson's Disease Registry were analyzed using butanol/methanol extraction. The samples were processed using a Bruker timsTOF flex mass spectrometer with a data-dependent method. Feature extraction was performed using MS Dial, followed by verification and relative quantification using Skyline.

Multivariate analysis with the least absolute shrinkage and selection operator (LASSO) identified 12 lipid biomarkers from RBCs with a receiver operating characteristic (ROC) of 0.84. In plasma, 10 lipid biomarkers were identified with an ROC of 0.85. A combined panel of RBC and plasma lipids achieved a higher ROC of 0.89 with 12 lipids. The WGCNA co-expression network revealed specific modules enriched in pathways such as ferroptosis, sphingolipid metabolism, glycerophospholipid metabolism, and sphingolipid signaling.

14. Abstract: Susan O. Kim

Identification of butyrylcholinesterase-derived small molecule peptides indicative of Novichok nerve agent exposures

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Novichok nerve agents, including A-230, A-232, and A-234, were classified as Schedule 1 chemicals by the Organisation for the Prohibition of Chemical Weapons (OPCW) following poisoning incidents in 2018.[1–7] As a result, production, storage, and use of these chemicals are strictly prohibited. The identification of biomarkers indicating Novichok exposure in humans is crucial for prompt detection and response to potential incidents involving these banned chemical weapons. In this study, BChE was isolated from human serum samples exposed to Novichok nerve agents *in vitro* using immunomagnetic capture, followed by enzymatic digestion with pronase or proteinase K to generate new peptide biomarkers indicative of exposure. We identified nine new Novichok-adducted peptides generated through enzymatic digestion with proteinase K and pronase using liquid chromatography high-resolution mass spectrometry. Two peptides, [Agent]-serine-alanine for proteinase K digestion and [Agent]-serine-alanine-glycine for pronase digestion, were selected for optimization due to their abundance. The analysis was subsequently transferred to an LC-triple quadrupole system to enhance throughput and detect these new biomarkers at the limits of detection corresponding to BChE inhibition levels of 3.90% or less. These additional biomarkers can improve laboratory preparedness for OPCW-designated biomedical testing laboratories as well as other clinical and investigative laboratories tasked with responding to emergencies involving these highly toxic chemicals.

15. Abstract: Madison E. Platt

MALDI-IMS imaging of lipids in the fixed-frozen human brain

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Matrix-assisted laser desorption ionization imaging mass spectrometry (MALDI-IMS) is a powerful technique for visualizing lipid distribution in tissues. Traditionally, MALDI-IMS is conducted on fresh-frozen tissues, particularly for lipid analysis. Fresh tissue is not always available from tissue banks particularly for smaller regions of the brain like the substantia nigra (SN) or locus coerleous (LC). However, formal fixed samples, which are commonly used for long-term storage in clinical settings, are often available. For lipid analysis, fixed tissues present challenges such as signal suppression and lipid class elimination. Recent advancements in sample preparation, including the application of ammonium formate (AF) pre-treatment and optimization of a variety of matrices, have shown promise in overcoming these limitations by enhancing lipid signal sensitivity and intensity. Despite these developments, there remains a gap in understanding the efficacy of these methods for formal fixed frozen human brain tissue. This study introduces a method to preserve lipid signal and classes in formalin-fixed human brain tissue and compares lipid identifications and intensities between AF pre-treated fresh-frozen and fixed-frozen samples. Our findings aim to assess the feasibility of utilizing formalin-fixed tissues for MALDI-IMS lipidomics, potentially broadening the scope of tissue analysis in clinical research.

16. Abstract: Alexandria Van Grouw

Automated Single Cell Lipidomics for Mapping MSC Heterogeneity

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Mesenchymal stromal cells (MSCs) are multipotent stem cells with anti-inflammatory properties that show promise in treating various immune disorders and injuries. However, their therapeutic potential has been difficult to harness due to individual cell heterogeneity and relatively rapid progression to senescence compared to other cell types. A novel single cell selection method paired with ultra-sensitive high resolution mass spectrometry permits metabolomic analysis at the single cell level.

In order to obtain an initial sensitivity assessment, cell lysate from HEK cells at a concentration of 10,000 cells/µL that was diluted 10x, 100x, 1000x, and 10,000x so that the lowest calibration level was at a concentration of 1 cell/µL. These samples were analyzed using multiple techniques in order to compare sensitivity between multiple mass spectrometry methods. They were analyzed using microflow reverse phase liquid chromatography (LC) at 400 µL/min and 50 µL/min, as well as using nanoflow reverse phase LC at a flow rate of 500nL/min. Samples were also analyzed on multiple mass spectrometers including a Thermo Orbitrap Astral and Thermo Orbitrap Exploris 240. All samples were run in positive ion mode with DDA for MS2 spectra collection. Data was processed and analyzed using Thermo Compound Discoverer 3.3 and Lipid Search.

Nanoflow LC separation combined with analysis on a Thermo Orbitrap Astral utilizing MS1 data collection in the Orbitrap with simultaneous MS2 scans in the Astral mass analyzer achieved the greatest sensitivity with 172 lipid annotations at the single cell level. With such appropriate sensitivity achieved, we

will employ a single cell selection method using a robotic automatic single cell patch clamping setup that leverages a machine vision algorithm for cell detection detection. Once the single cell sample preparation has been fully optimized, profiles of single cell lipidomes of MSCs will be measured and used to map colony heterogeneity.

17. Abstract: Jose Villalobos

Comparison of colostrum and colostrum products utilizing glycomic techniques

Jose Villalobos, Stephanie Archer, Parastoo Azadi

Colostrum is a milk-like, nutrient-dense substance produced during the first few days after birth. It is crucial for developing the newborn's immunity and contains a wealth of immune factors, growth and tissue repair elements, immunoglobulins, and natural antimicrobial agents such as milk oligosaccharides (MOs). In recent years, several companies have started marketing bovine colostrum—a byproduct of the dairy industry—as a supplement for adults, claiming benefits for digestion, skin, hair, nails, and post-exercise recovery. While much attention has been given to the immune factors in bovine colostrum, there has been less focus on comparing the MOs present.

We have recently developed methodologies to profile and perform detail characterization of milk oligosaccharide by a process of isolation, purification, and permethylation (Porfirio et al (2020) Glycobiology 30:10, 774-786). Prior to permethylation, proteins are precipitated out and lipids are removed by solid phase extraction. Reducing ends of purified MOs are then reduced yielding a more stable product. Our improved permethylation method stabilizes the structure of the oligosaccharides, enhances sensitivity, and enables detailed sequential structure analysis to identify potential isomers, providing a level of analysis deeper than that of most underivatized protocols. This study aims to provide detailed comparisons of milk oligosaccharides in human and bovine milk, including comparisons of MOs in colostrum versus mature breast milk, human versus bovine MOs, and fresh bovine colostrum MOs versus those in three commercial supplement products.

18. Abstract: Mujeeb A. Wakeel

Investigating Enzyme-Substrate Interactions in Proteusin Ribosomally Synthesized and Post-translationally Modified Peptides Biosynthesis

Ribosomally synthesized and post-translationally modified peptides (RiPPs) are natural products with varied chemical structures and biological activities, making them valuable for drug discovery. RiPP precursor peptides consist of an N-terminal leader peptide that binds to RiPP precursor peptide recognition elements (RRE) of modifying enzymes and a C-terminal core peptide that undergoes enzymatic modifications. After modifications, a protease cleaves the mature C-terminal core peptide from the N-terminal leader peptide.

The RiPPs group of natural products consists of various sub-families often defined based on the nature of the chemical modifications afforded by the modifying enzymes on the mature natural products (e.g., linear azol(in)e-containing peptides). Among the well-characterized family of enzymes that modify RiPPs are the YcaO-like cyclodehydratases that catalyze (methyl)oxazoline/thiazoline heterocycle formation by cyclodehydrating Thr/Ser/Cys residues in the core peptide. Most RiPPs, such as cyanobactins, derive from precursor peptides with a short, unstructured leader peptide (about 30 amino acids).

Cyanobactins have been reported to use a conserved C-terminus LXXXXL motif in the unstructured leader peptide for binding YcaO-like cyclodehydratases.

However, proteusins, another RiPP family, feature a much longer (about 75 amino acids) leader peptide with a highly structured N-terminus. The relevance of this structured region in the post-translational modification of proteusins is yet to be described. Our research involves mining biosynthetic gene clusters (BGCs) containing putative proteusin precursor peptide(s) and using biophysical and biochemical tools to investigate protein-protein interactions between the precursor peptide and the RRE of modifying enzymes.

Our results suggest that proteusin precursor peptides require their structured N-terminus region and the conserved LXXXXL motif of the unstructured region in binding YcaO-like cyclodehydratases for efficient processing of the core region. These findings indicate another distinct binding site/mode for proteusin precursor peptides and offer new insights into the protein-protein interactions essential for proteusin biosynthesis, potentially aiding in developing innovative combinatorial biocatalysis approaches.

19. Sponsor Abstract: Agilent

Systematic Evaluation of Hydrophilic Interaction Liquid Chromatography Stationary Phases for Oligonucleotide Characterization by LC/MS

Ion-pairing reversed-phase chromatography (IP-RPLC) coupled to MS represents the most common analytical method for oligonucleotide analysis. However, alternative separation methods are desired as alkylamine ion-pair reagents force users to have dedicated instruments. While ion-exchange chromatography (IEX) represents a viable alternative technique due to its excellent selectivity for oligos based on their length, it is not preferred due to mobile phase incompatibility with MS detection. Hydrophilic interaction chromatographic (HILIC) is a valuable alternative to IP-RPLC and IEX as HILIC mobile phases are compatible with MS and provides flexibility in instrument-use. This work highlights the utility of HILIC for oligonucleotide analysis and critical parameters that need to be considered to optimize LC/MS performance.

20. Sponsor Abstract: Andson Biotech

Rapid Online Buffer Exchange with the DynaChip Platform for Complex Biological Analysis

Top-down and native analysis of complex biomolecules by mass spectrometry (MS) offers new insights into their higher-order structures. However, samples must be stored in specialized buffer solutions to preserve structure, and these solutions are rarely compatible with MS due to the required high concentrations of non-volatile salts. Buffer exchange is therefore necessary to transfer analytes into MS-compatible solutions. This transfer is commonly accomplished with manual offline spin-column washing protocols or online with lengthy size-exclusion chromatography methods. In contrast, the DynaChip platform uses tangential flow filtration/microdialysis to enable rapid online buffer exchange with sample-to-sample analysis time under 15 minutes.

The DynaChip features a microfluidic chip containing a biochemically inert membrane with tunable pore size, allowing for retention and analysis of diverse analytes while effectively removing various interferents. The conditioner flow can be customized to incorporate MS-compatible solutions (e.g., ammonium acetate), supercharging molecules (e.g., mNBA), or protonation sources (e.g., formic acid) based on analytical requirements. This vendor-agnostic platform, currently optimized for nanoESI

applications, seamlessly integrates with existing workflows for push-button operation by non-experts, significantly enhancing sample preparation efficiency and throughput.

DynaChip's rapid online buffer exchange has streamlined MS workflows across diverse protein types and complex biological samples while preserving structural integrity. This versatility spans multiple analytical approaches, including native MS and charge detection MS (CD-MS). For native MS, we've successfully characterized mAb glycoforms, protein-ligand complexes, and membrane proteins, while also enabling specialized techniques like collision-induced unfolding. With CD-MS, the DynaChip has facilitated rapid analysis of larger biomolecular assemblies including AAVs, DNA plasmids, and viral complexes. Beyond these applications, the platform has also been used for challenging sample preparations, from surfactant removal to cell culture media secretome analysis. The DynaChip's broad compatibility with various samples, buffers, and analytical techniques significantly enhances both the accessibility and efficiency of complex biological analysis by MS.

21. Sponsor Abstract: Bruker

Ultra-high Sensitive Single Cell Proteomics on the timsTOF Ultra

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For single cell proteome analysis, ultra-high sensitivity mass spectrometry is key to reach proteome coverages necessary for understanding the cellular heterogeneity on a cell-by-cell level. Latest enhancements in ion transfer with a larger transfer capillary, an additional higher-pressure segment for more effective ion collection, two orthogonal deflections, to maintain robustness, and high-capacity TIMS pushes the limits of detection to single cell level.

K562 cell digest (Promega) dilution series from 16 ng to 15 pg, were prepared. One, five, ten and twenty HeLa cells and FACS pre-sorted T-Cells (CD4+, CD8+) B-Cells (CD19+) and monocytes (CD14+) from peripheral blood mononuclear cells (PBMCs) were isolated and prepared with a cellenONE (Cellenion). Peptides were loaded onto an Aurora Elite column (IonOpticks) separated with a 22 min active gradient (32SPD) or an Aurora Rapid column (IonOpticks) with a 10 min active gradient (80SPD) using a nanoElute2, eluting peptide detection on a timsTOF Ultra in dia-PASEF, and analysis with Spectronaut 18.

Processing of K562 dilution series acquired in dia-PASEF mode identified >1,000 protein groups out of 15 pg, with 80SPD, and 32SPD and > 6,000 protein groups (80SPD) and >7,000 protein groups (32SPD) for 16 ng. Quantitative accuracy at 250 pg was around 8 - 9% and about 4 - 6% at loads of 4, 8 and 16 ng. Protein groups identified for peptide loads < 500 pg were comparable between 80SPD and 32SPD. Analysis of HeLa cells resulted in about 4,000 protein groups from single cells and up to 6,000 protein groups for 20 cells in 80SPD and 32SPD. For FACS pre-sorted PBMCs, the 80SPD workflow identified in total 1,713 protein groups, with distinct proteomic phenotypes for the four cell types.

Deep proteome coverage and high reproducibility using the timsTOF Ultra combined with automated single cell isolation and sample preparation on the cellenONE® platform.

22. Sponsor Abstract: Opentrons

Automating protein sample preparation on Opentrons Flex

Automating protein sample preparation streamlines complex workflows, increases throughput, and enhances reproducibility. In protein quantification, normalization, digestion, and cleanup ahead of LC-MS analysis, automation also minimizes manual intervention, reducing human errors and maintaining efficiency in sample handling. Here, we present a series of protocols developed to perform an end-to-end, automated workflow for protein sample preparation on the Opentrons Flex, a robotic liquid handling platform.