Oral Presentation Abstracts: AAMSDG 2024

Abstract 1: Asymmetrical glycosylation is a prevalent feature of human IgGs

Tala Azzam[1], Jonathan J. Du[1,2], Maria W. Flowers[1], Eric J. Sundberg[1]

[1]Department of Biochemistry, Emory University School of Medicine, Atlanta, GA, 30322
[2]Present Address: Sydney Pharmacy School, Faculty of Medicine and Health, The University of Sydney, Camperdown, NSW Australia 2050

IgGs have a conserved N-linked glycan attached to residue Asn297 of the homodimeric Fc region which modulates antibody effector functions, a critical aspect of the immune response, by altering binding affinities of Fc receptors and complement. The effects on the function of the immune response are reflected in correlations between IgG glycoforms and the progression of disease states such as multiple sclerosis, rheumatoid arthritis, and various infectious diseases. IgG Fc glycosylation has also emerged as an important modulatory feature of monoclonal antibody (mAb) therapeutics.

Despite the importance of understanding the precise role of IgG Fc glycans and developing methods for glycoengineering, current studies fail to characterize these Asn297-linked glycans comprehensively. These studies rely either on symmetrical glycoengineering of IgGs, in which enzymes are used to add specific glycans onto both Fc chains simultaneously, or on analytical methods that require cleavage of the Asn-297 glycan from the Fcs.

We developed an intact LC/MS method to glycoprofile plasma samples from healthy individuals and patients suffering from COVID and Dengue. Our data show that at least one-third of all the tested IgG glycoforms are asymmetric, i.e. the glycans on each of the Fc chains are not identical. This suggests that asymmetric glycosylation may play an important and unique role in the immune response and can provide more accurate and comprehensive correlations between antibody glycosylation and disease states.

Abstract 2: Halogenation of Aminothiazoles and new Biosynthetic pathways

Paul Branham, Nirmal Saha, Vinayak Agarwal
Georgia Institute of Technology, School of Chemistry and Biochemistry, Renewable Bioproducts Institute, Atlanta, GA, USA

This study delves into the unique chemistry of deep-sea red algae, focusing on vanadium-dependent haloperoxidases (VHPOs). These enzymes, capable of regioselective halogenation, offer a solution to the challenges of traditional halogenation methods in organic chemistry. Here, we isolate and utilize the ccVHPO1 enzyme from Chondrus crispus to brominate 2-aminothiazole, which is a key structural component in many bioactive molecules. The optimized reaction conditions yielded high conversion rates and regioselectivity. Subsequent Suzuki coupling reactions expanded the molecular diversity, demonstrating the scalability and applicability of this biosynthetic pathway. These findings highlight new potential of VHPOs in biocatalysis, paving the way for innovative drug modifications and the creation of new chemical entities.

Abstract 3: Determining the Absolute Stereochemical Configuration of Biopolymer Building Blocks by using Ion Mobility-Mass Spectrometry

Mya Brown, University of Georgia, mya.brown@uga.edu
Ron Orlando, University of Georgia, orlando@ccrc.uga.edu

All amino acids except for glycine (achiral) and carbohydrates can exist in two forms, D and L isomers. Amino acids and carbohydrates are involved in many biological processes such as cell-cell recognition, cellular adhesion, protein folding and solubility, metabolism, and immune/host pathogen response. These building blocks can have multiple structures that can make their characterization complex. For example, aldohexoses have four chiral centers and, thus, 24 = 16 isomers. There will be eight d-isomers and eight l-isomers. Isomers create challenges when determining unknown biopolymers by mass spectrometry alone. We have developed a method that allows us to identify the absolute configuration of these biopolymer building blocks. In this method, the analyte is derivatized with an optically pure compound containing an additional stereochemical center, the labeled species can be resolved by ion mobility mass spectrometry. This presentation will describe a method using reductive amination and ion mobility mass spectrometry to determine the absolute configuration of both carbohydrates and amino acids without the use of acid hydrolysis**.**

Abstract 4: Triboelectric Nanogenerator Plasma Ionization for Mobile VOC Analysis

Joseph L. Corstvet[1], Wesley D. Robertson[2], Facundo M. Fernández[1]

[1]School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA
[2] Georgia Tech Research Institute, Georgia Institute of Technology, Atlanta, GA

Volatile organic compounds (VOC) are pervasive in ambient air, both indoors and outdoors. Some may be characterized by pleasant fragrances, many are detrimental to human health, and others have been observed as potential biomarkers of disease. However, due to their high vapor pressure and low water solubility, storing VOC samples may lead to chemical reactivity and sample loss, hampering analysis. To overcome this issue, a mobile triboelectric nanogenerator plasma ionization mass spectrometry (TENG-PI-MS) system was created to analyze VOC samples in situ. This was developed by mounting a Waters QDa mass spectrometer, a plasma ionization source, and computer on a mobile platform. All components were powered by an EcoFlow Delta 2 Max power station, which allows the entire system to operate for six hours on battery power. Plasma ionization was achieved by a pulsed corona discharge sustained between a tungsten needle (point radius ~10 micron) and ground in the presence of helium using the TENG ion source. The miniaturized TENG source offers lower power consumption than a traditional DC power supply, granting extended mobile analysis time. Three VOC standards were used to characterize the system; limonene, 3-heptanone, and 1,3-butanediol. All standards have been successfully ionized using the TENG-PI-MS system, however limonene is inconsistently observed using the QDa MS. Varying helium flow rates were also tested, with higher He flow (up to 3.5L/min) leading to improved analyte ionization. The current configuration is capable of analyzing VOC standards at

concentrations down to 2 μM. Interestingly, VOC measured in ambient lab air were not detected after moving the system to a different lab. This was determined to originate from Elegoo 3D printer resin, demonstrating the functionality of this mobile system for air quality testing. To further improve the sensitivity of analysis, pulsing of helium gas is being tested for each TENG actuation.

Abstract 5: Effects of Deoxygenation and Acidification on Acropora Cervicornis of Different Genotypes

Alex Doty, Neha Garg
Georgia Institute of Technology, Atlanta, GA

Globally, coral reefs are threatened by deoxygenation events that are predicted to happen more frequently as a result of climate change and costal eutrophication. Worldwide, 10% of corals are predicted to be at risk for hypoxia, and approximately half of all tropical dead zones are known to contain coral reefs. Furthermore, in natural settings deoxygenation is accompanied by increased acidification which further exacerbates the stress imposed on these at-risk corals. Metabolomic analysis aids in the discovery of small molecule biomarkers and allows for the generation of hypotheses to examine coral response to hypoxic and acidic treated phenotypes.

In this study 6 different genotypes were subjected to either acidic, hypoxic, or acidic and hypoxic conditions in triplicate over 10 days then sacrificed and extracted for metabolomic analysis. Data were acquired on extracts using untargeted liquid chromatography-mass spectrometry. A suite of chemometric and statistical tools were employed to extract metabolite features variably detected between treatments and genotypes. Non-metric multidimensional scaling of genotype and treatment reveals clustering by genotype, which suggests that genotypes of A. Cervicornis used in this study was resistant to stress conditions. The top 50 features driving group differences between genotypes and treatments ranked by ANOVA were visualized with heat maps. The treatment heat map reveals that several acylcarnitines were detected in lower abundance in corals subjected to one of the stress treatments. Several classes of lipids were identified as drivers of genotypic differences, and most of these lipids were detected in highest abundance in specific genotypes. Finally, to identify metabolite features that were only produced in certain treatment conditions an UpSet plot was generated. The two treatments with the highest number of independently detected features were the hypoxic and hypoxic and acidic combination treatment, which suggests that metabolites are being produced in response to stress treatments.

Abstract 6: Method for Detection of Naturally Occurring Toxins in Human Urine Using High Resolution Mass Spectrometry

Bryan E Hettick, Logan C Krajewski, Anisha Saddy, Elizabeth I Hamelin
Centers for Disease Control and Prevention (CDC), Atlanta, GA, USA

Alkaloids and other non-protein toxins constitute a broad group of naturally occurring hazardous compounds. These natural toxins, originating from plants, animals, and fungi, possess diverse structures and chemical mechanisms which can result in an extensive overlap in exposure symptomology. The continued risk of accidental exposure from foraging worldwide, food

contamination, and natural product use accentuates the need to identify natural toxins in clinical specimens to support poisoning investigations.

A qualitative method was developed to detect natural toxins in biological samples. A total of 179 toxins were spiked into urine samples to be tested with the method; ten of these were selected to serve as quality control (QC) standards. Samples were analyzed with reverse-phase liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS). Retention time, chromatography signal-to-noise, mass accuracy and fragmentation library matching were used as parameters to confidently identify natural toxins in urine.

Validation experiments demonstrated a robust method capable of generating highly reproducible data. Of the 179 tested compounds, 117 were fully validated and successfully added to the method. This method can be expanded to include toxins as they become of interest. This will be useful for future emergency responses to rapidly identify potential natural toxins involved in an unknown exposure event.

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. Use of trade names is for identification only and does not imply endorsement by the Centers for Disease Control and prevention, or the US department of Health and Human Services.

Abstract 7: Using non-targeted metabolomics to characterize the protective effects of airway probiotics against *Burkholderia Thailandensis* infection

Daniel Hicks, Neha Garg
Georgia Institute of Technology, Atlanta, GA

Burkholderia pseudomallei is a highly virulent, Tier 1 HHS/USDA Select Agent bacterial pathogen that has recent been declared endemic to the southern United States. There is an urgent need to develop alternative treatments, and airway probiotics show potential. Preliminary data shows that the airway probiotic *Brevibacillus borstelensis* can protect mice from *Burkholderia thailandensis* lung infection (a convenient surrogate for *B. pseudomallei*). Non-targeted LCMS metabolomics was used on mice lung samples to characterize the effects of *B. borstelensis* and *B. thailandensis* on the lung metabolome both alone and when inoculated together.

Both bacteria significantly altered the lung metabolome. Metabolites associated with stress and immune response such as corticosterone and 12-epi-LTB4 were significantly higher in the *B. thailandensis* infected lungs. However, protection with *B. borstelensis* brought the abundance of these metabolites down to near-healthy levels during infection. This suggests that *B. borstelensis* is successfully helping the host manage *B. thailandensis* and decreasing stress due to infection.

Many LCFA (long chain fatty acid) derivatives such as HpDHA and Protectin D were significantly higher in lungs inoculated with *B. borstelensis*. These are host signaling molecules that have a role in a variety of pathways including inflammation, immune signaling, and cell differentiation. Our data supports the hypothesis that *B. borstelensis* protects against *B. thailandensis* infection in mice through "priming" the host immune system to be better prepared to deal with a lung infection.

This work highlights the potential of airway probiotics to protect against bacterial infections. We also provide insight into the effects of the lung microbiome on the host metabolome both in during infection and health.

Abstract 8: VOC Analysis of Spaceflight-Relevant Microorganisms

Sarah Kingsley[1], Hang N. Nguyen[2], Aaron N. Knopp[3], Thomas Orlando[1], William T. Wallace[4], and Sarah L. Castro-Wallace[4]

[1]Georgia Institute of Technology, School of Chemistry and Biochemistry, 901 Atlantic Dr. Atlanta GA 30318
[2] JES Tech, 16870 Royal Crest, Houston, TX 77058
[3] KBR, 2400 NASA Pkwy, Houston, TX 77058
[4] NASA Lyndon B. Johnson Space Center, Houston, TX 77058

NASA's Moon to Mars initiative will involve spacecraft that are intermittently inhabited, necessitating methods for microbial monitoring during uncrewed (dormant) periods [1, 2]. This study investigates the potential of using gas chromatography-mass spectrometry (GC-MS) to detect and differentiate microorganisms on spacecraft via analysis of microbial volatile organic compounds (VOCs). Five microorganisms previously identified on the International Space Station (ISS) were analyzed: the Gram-positive pathogens *Staphylococcus aureus* and *Enterococcus faecalis* [3, 4], and the Gram-negative waterborne bacteria *Ralstonia pickettii*, *Burkholderia contaminans*, and *Klebsiella aerogenes* [5-7].

The pathogens were cultured in Tryptic Soy Broth (TSB), a rich media. GC-MS analysis of their headspace VOCs allowed for clear detection. Principal Component Analysis (PCA) effectively differentiated between the VOC profiles of the two pathogens. The Gram-negative bacteria were grown in both R3A, a minimal medium, and a simulant of spacecraft wastewater. VOC analysis showed that all three bacteria were detectable and distinguishable in R3A through PCA. However, in the wastewater simulant, only *R. pickettii* and *B. contaminans* survived, with *K. aerogenes* failing to thrive. Notably, two VOCs, tentatively identified as isoprene and dimethyl disulfide, were detected in *B. contaminans*, with dimethyl disulfide also present in *R. pickettii*. These VOCs enabled differentiation between the microbial data and the wastewater simulant control.

This study demonstrates the feasibility of using GC-MS for microbial detection and identification in a spacecraft-relevant environment. While further exploration of VOC analysis techniques is needed to evaluate the effects of spacecraft conditions on emitted VOCs and to achieve the sensitivity needed to apply a VOC-based method on spacecraft, this investigation helps to lay the foundation for future work and answer questions such as what microbial VOCs may be present on spacecraft.

References:

1. Sargusingh, M. J.; Perry, J. L. In *Considering Intermittent Dormancy in an Advanced Life Support Systems Architecture*, AIAA SPACE and Astronautics Forum and Exposition, 2017; p 5216.

2. Broyan, J.; McKinley, M.; Stambaugh, I.; Ruff, G.; Owens, A. In *NASA Environmental Control and Life Support Technology Development for Exploration: 2021 to 2022 Overview*, 51st International Conference on Environmental Systems: 2022.
3. Castro, S. L.; Nelman-Gonzalez, M.; Nickerson, C. A.; Ott, C. M., Induction of attachment-independent biofilm formation and repression of Hfq expression by low-fluid-shear culture of Staphylococcus aureus. *Applied and Environmental Microbiology* **2011,** *77* (18), 6368-6378.
4. Bryan, N. C.; Lebreton, F.; Gilmore, M.; Ruvkun, G.; Zuber, M. T.; Carr, C. E., Genomic and functional characterization of Enterococcus faecalis isolates recovered from the International Space Station and their potential for pathogenicity. *Frontiers in Microbiology* **2021,** *11*, 515319.
5. Nguyen, H. N.; Sharp, G. M.; Stahl-Rommel, S.; Justiniano, Y.-A. V.; Castro, C. L.; Nelman-Gonzalez, M.; O'Rourke, A.; Lee, M. D.; Williamson, J.; McCool, C., Microbial isolation and characterization from two flex lines from the urine processor assembly onboard the international space station. *Biofilm* **2023,** *5*, 100108.
6. Bruce, R. J.; Ott, C. M.; Skuratov, V. M.; Pierson, D. L., Microbial surveillance of potable water sources of the International Space Station. *SAE transactions* **2005**, 283-292.
7. Yang, J.; Barrila, J.; Mark Ott, C.; King, O.; Bruce, R.; McLean, R. J.; Nickerson, C. A., Longitudinal characterization of multispecies microbial populations recovered from spaceflight potable water. *npj Biofilms and Microbiomes* **2021,** *7* (1), 70.

Abstract 9: Minimizing Presence of Salt in Sample Preparation Reduces Complexity in Polar Feature Detection in Non-Targeted LC-MS Experiment

Ying Liu, Samuel Moore, David A. Gaul
School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA 30332

Reduction of technical variables is necessary for non-targeted metabolomic experimental design. When working with cells and tissues, residual buffered solution in the samples is difficult to remove completely resulting in non-consistent salt content. We investigated the role of salt in sample preparation for a non-targeted LC-MS experiment analyzing polar metabolites and studied whether replacement of salt by mass spectrometry friendly ammonium salt may reduce the complexity of polar feature detection.

Rat liver tissue was homogenization and extracted by methanol:aqueous solvent (80:20), where the aqueous solvent was water, PBS, or concentrated PBS. Both liver and Vero cells were prepared by exchanging PBS salts for Mass Spectrometry friendly ammonium salts (ammonium formate, ammonium acetate, and ammonium bicarbonate). Cell viability was assessed following salt exchange. Polar metabolites were analyzed by a Waters BEH Amide column coupled with an Orbitrap IDX mass spectrometer. Data was processing by Compound Discoverer v3.3 software.

Results show that sodium chloride, 0.137M in PBS, interacts with the mobile phase additive NH4HCO2 to create salt clusters (NaOC2H and NH4Cl) which elute over 4-6 min of the 12 min gradient. Of the 3331 detected features, 24% of these features had a 2x fold change with the use of PBS in the extraction. The salt addition was observed to enhance or to suppresses ion detection dependent on the analyte. Removal of salt with solid phase extraction technique yields increased complexity. PBS replacement with mass spectrometry friendly additives was shown to reduce the suppression and the enhancement effects observed with PBS present. These suggest it

is important to control the salt content during sample preparation to minimize unwanted technical variation which could lead to misinterpretation of the data.

Abstract 10: Developing a high-throughput workflow for large-scale exposome epidemiology

Catherine E. Mullins[1], Mattie E. Braselton [1], Ronald D. Smith [1], Karthikeyan B. Shanmugam [1], Sarah Timbie [1], Justin J. Byun [1], Brismar Pinto-Pacheo [2], Douglas I. Walker [1]

[1]Emory University, Rollins School of Public Health, Gangarosa Department of Environmental Health, Atlanta, GA, United States
[2]Mount Sinai, Icahn School of Medicine, New York, NY, United States

The "exposome" refers to the integrated compilation of all physical, chemical, biological, and psychosocial influences that impact biology. As genetics alone fails to explain most health outcomes, identification and quantitation of exposures is a critical frontier in understanding human health. Untargeted high resolution mass spectrometry is ideal for this task, enabling identification of thousands of chemical signals within biological samples. Optimal methods must involve the following: 1) easy sample preparation to reduce human error and variability, 2) high-throughput analysis to increase study power and decrease assay costs, and 3) broad coverage to capture as many different features as possible. To this end, the Comprehensive Laboratory for Untargeted Exposome Science (CLUES) at Emory University has developed an untargeted analytical framework for preparation and analysis of biological samples using liquid chromatography with high resolution mass spectrometry (LC-HRMS) supporting large scale analysis of the human exposome. Sample preparation protocols were optimized using Opentrons OT2 automated liquid handlers, enabling semi-automated preparation of separate 96 well-plates for HILIC and C18 chromatography in <2 hours by a single operator. Samples are analyzed using a pair of Thermo Exploris 120 Orbitraps coupled to Vanquish Duo liquid chromatography systems, allowing simultaneous analysis of samples in HILIC and C18 chromatography modes. By multiplexing on each instrument, each study sample is analyzed in four modes (HILIC+, HILIC-, C18+, C18-) in ~16 minutes total, allowing detection of >50,000 chemical signals in ~500 samples/ week. To assess the quality of sample preparation and instrument performance within and across batches in all modes, internal standard mixtures of C-13 and N-15 isotopically-labeled compounds were developed, and QAQC reports generated for each batch using R. Combined with a comprehensive data extraction and chemical annotation workflow, this method fills a critical need for high-coverage, efficient analysis of the exposome in 10,000+ samples/ year.

Abstract 11: Sphingolipid Dysregulation in Juvenile ALS

Devesh Pant, PhD
Emory University School of Medicine, Atlanta, GA, 30322

Juvenile amyotrophic lateral sclerosis (jALS) is a rare condition affecting motor neurons, marked by their gradual deterioration. Recent findings have linked mutations in the human SPTLC1 gene

to jALS. This gene encodes Serine Palmitoyltransferase, a vital enzyme in sphingolipid production. Studies have revealed that missense and deletion mutations in SPTLC1 are linked to ALS onset in childhood. Notably, these mutations occur within the gene's transmembrane domain, leading to an overproduction of sphingolipids. However, the precise mechanism by which changes in sphingolipid levels contribute to the disease remains unclear. We analyzed SPTLC1-JALS mutation using both in vitro and in vivo models. We identified alterations in sphingolipid profiles in the SPTLC1-jALS mutant through targeted lipidomics studies. These findings suggest that mutations in SPTLC1 disrupt sphingolipid metabolism, causing cellular abnormalities that might result in neuronal degeneration in jALS. Our goal is to delve deeper into the role and regulation of sphingolipid signaling and biology in ALS.

Abstract 12: Rover-like Biosignature Detection in Hypersaline Mars Analogs with TMAH Thermochemolysis

Chad Pozarycki[1], Lydia Kivrak[2], Amy J Williams[2], Karina Bergstrom[1], Tatiana Gibson[3], Jeff S Bowman[4], Sanjoy M Som[5], Emily R Paris[6], Steffen Buessecker[6], Luke Fisher[4], Margaret M Weng[7], Arthur Odenheimer[8], Enrica Quartini[9], Cathryn Sephus[4], Amina T Schartup[4], Benjamin Klempay[4], Ellery D Ingall[3], Jessica M Weber[10], Taylor Plattner[3], Margaret Birmingham[11], Peter T Doran[8], Britney Schmidt[9], Amanda M Stockton[1], and the Esperance Tjaltraak Native Title Aboriginal Corporation

[1]School of Chemistry and Biochemistry, Georgia Institute of Tech.
[2]Department of Geological Sciences, U. of Florida
[3]School of Earth and Atmospheric Sciences, Georgia Institute of Tech.
[4]Scripps Institution of Oceanography, U. of California San Diego
[5]Blue Marble Space Institute of Science,
[6]Doerr School of Sustainability, Stanford U.
[7]Department of Biology, Georgetown U.
[8]Department of Geology & Geophysics, Louisiana State U.
[9]Astronomy and Earth & Atmospheric Sciences, Cornell U
[10]NASA Jet Propulsion Laboratory, California Institute of Tech.
[11]Department of Geology, U of Kansas

The Western Australia Transient Lakes (WATL) are hypersaline systems that host evaporite crusts which can serve as analogs for ancient Martian environments. Our study investigates the capability of mass spectrometric techniques to detect organic biosignatures, particularly fatty acids and amino acids, within salt crusts, shoreline clays, and brines from these lakes. We also include samples from Chile's Atacama Desert for comparison as an analog for modern Mars.

Using techniques relevant to planetary exploration missions, we employed capillary electrophoresis-mass spectrometry (CE-MS) for brine analysis along with pyroGCMS with TMAH thermochemolysis, similar to that used by the *Curiosity* rover and the upcoming *Rosalind Franklin* rover to analyze solid samples. We found fatty acid methyl esters (FAMEs) ranging from C8-C18 in sulfate-rich evaporite crusts, with an even-over-odd chain-length distribution suggestive of biological origin, while halite and smaller grain-sized clays presented lower

concentrations of these compounds and no clear pattern. We discuss how mineralogical differences play a role in biosignature detectability.

Our findings highlight the potential and the limitations of mass spectrometry in identifying biosignatures in hypersaline environments, relevant for future planetary science missions.

Abstract 13: Simultaneous Quantitation and Discovery (SQUAD) Liquid Chromatography Mass Spectrometry of Progesterone Steroids Relevant to Ovarian Cancer

Elisabeth Schwiebert[1], Samuel G. Moore[1,2], Jaeyeon Kim[3], David A. Gaul[1,2], Facundo M. Fernández[1,2]

[1]School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA 30332
[2]Petit Institute of Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA 30332
[3]Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indiana University, Melvin and Bren Simon Comprehensive Cancer Center, Indianapolis, IN 46202

High-grade serous carcinoma (HGSC), the most common and lethal subtype of ovarian cancer (OC), is an aggressive subtype, often going undiagnosed until advanced stages. The risk of HGSC and other subtypes of OC 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 often undergo prophylactic removal of the ovaries and fallopian tubes, reducing their lifetime risk, yet most of them will never develop OC. These invasive surgeries could be avoided in lower-risk individuals with alternative assessments that evaluate metabolomic factors. Based on previous work demonstrating that progesterone (P4) treatment induced tumorigenesis and metastasis of HGSC in ovariectomized BRCA1 mice, our hypothesis is ovarian P4 is an essential early factor in OC development in BRCA1/2-mutation carriers and affects the organism's broader metabolome.

To test this hypothesis, serum from 60 double knock-out (DKO) mice was processed and analyzed via our SQUAD LC-MS method on a tribrid mass spectrometer, permitting the simultaneous collection of both targeted data and non-targeted data that was not initially of interest. Our targeted data, collected in both ion modes, focused on nine steroids that contribute to the steroid metabolic pathway. While many of these steroids of interest fell below our nanomolar limits of detection (LOD), P4 and corticosterone were the only targeted steroids that yielded detectable signals across the mice samples where elevated levels of both steroids were apparent in the P4-treated mice in relation to other treatment groups. Of the thousands of features detected from both ion modes, initial results of the non-targeted data suggest significantly elevated and depleted levels of numerous metabolites that contribute to unique phenotypes associated with different HGSC progression rates among the four mice treatment groups as seen by the added time-point layer of this study.

Abstract 14: Targeted proteomic analysis of 3R and 4R tau in marmoset and human brain tissue

Sarah M. Shapley[1+], Hasi Huhe[2+], Duc M. Duong[1], Fang Wu[1], Seung-Kwon Ha[3], Sang-Ho Choi[3], Julia Kofler[4], Yongshan Mou[3], Thais Rafael Guimaraes[3], Amantha Thathiah[3], Caroline M. Watson[1], Lauren K.H. Schaeffer[3], Gregory W. Carter[5], Nicholas T. Seyfried[1], Afonso C. Silva[3], Stacey J. Sukoff Rizzo[2,3]

[1]Emory University School of Medicine, Department of Biochemistry, Atlanta, GA, USA, 30322
[2]Aging Institute, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA, 15219
[3]University of Pittsburgh School of Medicine, Department of Neurobiology, Pittsburgh, Pennsylvania, USA, 15213
[4]Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA, 15213
[5]The Jackson Laboratory, Bar Harbor, Maine, USA, 04609
+ Co-first authorship

The microtubule-associated protein Tau becomes pathological in a family of neurodegenerative disorders termed Tauopathies. The predominant Tau isoform in aggregates characterizes these conditions. Tau isoforms are labeled by the exclusion (3R) or inclusion (4R) of one of the four microtubule repeat domains. Alzheimer's Disease (AD) represents a mixed tauopathy, where both 3R and 4R isoforms co-aggregate in neurofibrillary tangles. The discrimination of Tau proteotypes relies on a distinctive peptide located within the junction of the included repeat domain. 3R-Tau contains a lysine (K) and 4R-Tau contains a serine (S) residue at this site. A targeted parallel reaction monitoring mass spectrometry (PRM-MS) assay was developed to assess the naturally occurring Tau isoform in marmosets, a promising new model organism for AD research. Brain lysates from marmosets, human AD patients, human non-demented controls, and mice underwent a dual parallelized complementary digestion approach. Recombinant 3R and 4R (rTau) proteins were used to construct peptide spectral libraries. Library-free relative quantitation of these peptides was conducted by spiking in heavy-labeled peptides. Tau peptides were identified by analyzing the resulting product ion retention time and co-elution. The marmoset product ions replicated the human peaks and closely resembled the 3R and 4R peptide library spectra, providing mass spectrometry evidence for the expression of both isoforms in marmoset brains. Human samples exhibited a majority of 3R-Tau isoform. Meanwhile, adult marmosets displayed 42.6% 3R-Tau. Mouse lysate contained a majority 4R at approximately 90%. Additionally, Tau ratios were significantly correlated ($R^2 = 0.87$) across digestion approaches, providing confident orthogonal quantitation. Overall, we found marmosets to be a promising translational model of AD that overcomes the limitations of mice concerning the expression of both 3R and 4R isoforms. Additionally, proteotyping of other tauopathies may lend insight into novel biomarkers and personalized therapeutics.

Abstract 15: Using High-Resolution Ion Mobility for the Separation and Identification of α-Gal containing glycans from their non-α-Gal isomers

Hoang Kim Ngan Thai, University of Georgia, nht95736@uga.edu
Ron Orlando, University of Georgia, orlando@ccrc.uga.edu

The administration of biotherapeutics containing non-human α-Gal (galactose-α-1,3-galactose) glycans can lead to severe allergic reactions. The detection of α-Gal glycans remains a challenging task due to the presence of their isomeric species. For instance, A2G1(α-Gal)1F has an identical mass to A2G2F, one of the most common N-glycans found in antibodies, making the detection of A2G1(α-Gal)1F difficult. This study evaluates the ability of different analytical approaches to detect □-Gal glycans by testing their ability to detect A2G1(α-Gal)1F in the presence of A2G2F. Cetuximab is known to have N-glycans containing α-Gal, including A2G1(α-Gal)1F, and was chosen for the study. The released N-glycans from Cetuximab were first run on a HILIC-MS/MS system. While HILIC-MS/MS can separate α-Gal species when the number of Galactose is greater than the antenna number, i.e., A2G3F, A3G4F, this combination struggles to identify α-Gal species when the number of Galactose is equal or less than the antenna number, i.e., A2G2F vs A2G1(α-Gal)1F. An Ion Mobility Spectrometer (IMS) was added to the system to improve resolving power. The mobiligram of A2G2F (m/z 1003.9) from Cetuximab contained two peaks whose collision cross section (CCS) values were $440 \pm 2 Å^2$ and $445 \pm 2 Å^2$ which suggested the existence of a secondary species. Exoglycosidase digestions were performed to confirm the Gal linkages in these two species. It was found that A2G2F gave rise to the peak at $440 Å^2$, while the $445 Å^2$ peak is A2G1(α-Gal)1F, containing an □-Gal. The study reveals HILIC-IMS-MS as a powerful analytical technique that allows the separation and detection of α-Gal from their non-α-Gal isomers.

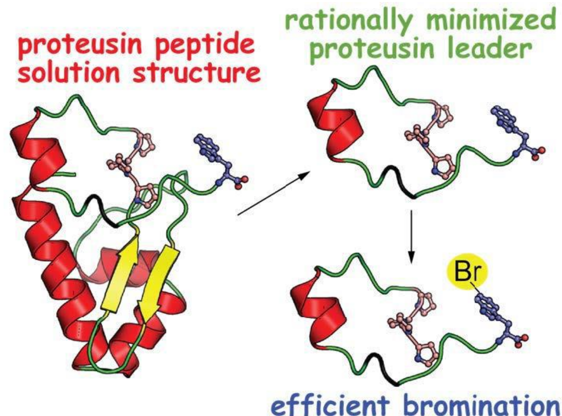
Abstract 16: A genomic-guided approach for enzymatic construction of post-translationally modified peptidic natural product

Fnu Vidya, Vinayak Agarwal
Georgia Institute of Technology, Atlanta, GA 30332

The pharmaceutical importance, and the enzymological ingenuity that underlies the biosynthesis of ribosomally synthesized and post-translationally modified peptides (RiPPs) cannot be overstated. RiPPs are initially produced as precursor peptides by the ribosomes, and then undergo post-translational modifications by dedicated peptide tailoring enzymes known as maturases. RiPP precursor peptides typically consist of the N-terminal leader and the C-terminal core regions. Our current understanding dictates that RiPP leaders are short and largely unstructured. However, the proteusin RiPP precursor peptides challenge this characterization by possessing unusually long leaders. Despite being widely encoded in bacterial genomes, proteusin peptides have not been structurally characterized. This knowledge gap leaves us with limited insight into how these atypical leaders interact with their corresponding modifying enzymes.

Figure presented herein demonstrate that that unlike other RiPP leaders, proteusin leaders are preorganized into a rigidly structured region and a smaller intrinsically disordered region. With residue level resolution gained from NMR titration experiments, the intermolecular peptide-protein interactions between proteusin leaders and a brominase enzyme are mapped onto the

disordered region, leaving the rigidly structured region of the proteusin leader to be functionally dispensable. The structural findings are corroborated using biochemical experiments.



Armed with these insights, we now aim to investigate whether other maturases exhibit a similar lack of dependence on the structured region of the proteusin leader peptides, or not. This line of inquiry promises to deepen our understanding of RiPP biosynthesis and could uncover novel mechanisms underlying peptide modification processes.

Efficient Bromination of Proteusin Peptide Following the Removal of the Rigidly Structured Region of the Proteusin Leader.

Abstract 17: Systematic Investigation of the Trafficking of Glycoproteins on the Cell Surface

Xing Xu, Kejun Yin, Ronghu Wu
School of Chemistry and Biochemistry and the Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, Georgia 30332, USA

Glycoproteins located on the cell surface play a pivotal role in nearly every extracellular activity. N-glycosylation is one of the most common and important protein modifications in eukaryotic cells, and it often regulates protein folding and trafficking. Glycosylation of cell-surface proteins undergoes meticulous regulation by various enzymes in the endoplasmic reticulum (ER) and the Golgi, ensuring their proper folding and trafficking to the cell surface. However, the impacts of protein N-glycosylation, N-glycan maturity, and protein folding status on the trafficking of cell-surface glycoproteins remain to be explored. In this work, we comprehensively and site-specifically studied the trafficking of cell-surface glycoproteins in human cells. Integrating metabolic labeling, bioorthogonal chemistry, and multiplexed proteomics, we investigated 706 N-glycosylation sites on 396 cell-surface glycoproteins in monocytes, either by inhibiting protein N-glycosylation, disturbing N-glycan maturation, or perturbing protein folding in the ER. The current results reveal their distinct impacts on the trafficking of surface glycoproteins. The inhibition of protein N-glycosylation dramatically suppresses the trafficking of many cell-surface glycoproteins. The N-glycan immaturity has more substantial effects on proteins with high N-glycosylation site densities, while the perturbation of protein folding in the ER exerts a more pronounced impact on surface glycoproteins with larger sizes. Furthermore, for N-glycosylated proteins, their trafficking to the cell surface is related to the secondary structures and adjacent amino acid residues of glycosylation sites. Systematic analysis of surface glycoprotein trafficking advances our understanding of the mechanisms underlying protein secretion and surface presentation.