Oral Presentation Abstracts: AAMSDG 2023

Abstract 1: Revealing the hidden role of metalloproteins and isomeric post translational modifications in neurodegenerative diseases

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Part of the quest to understand neurodegenerative research requires a detailed accounting and measurement of the biological components in neurological cells, tissue and biofluids. The majority of modern research to understand the function of proteins depends on measuring protein abundance. Protein abundance is certainly one mechanism the cell uses to regulate function but is not the only way protein function is regulated. Another major mechanism of modulating protein function is through post-translational modification (PTM). PTMs and non-covalent cofactors, e.g., metals, are often invisible to targeted assays like ELISA, bottom-up proteomics, or westerns. Here we apply quantitative proteomics, and ion mobility mass spectrometry to investigate the role of peptide isomerization of the amyloid beta peptide from human Alzheimer's disease brain tissue. And we apply hyphenated inductively couple mass spectrometry (ICP-MS) to investigate the role of metalloproteins have in the neurodegenerative process. We show that the implementation of quantitative mass spectrometry, ICP-MS and ion mobility reveal hidden biology and improve our understanding of the role of amyloid beta peptide and metals in Alzheimer's disease.

Abstract 2: Unlocking the Structural Information for Proteins Relevant to Cultural Heritage with Native and Top-Down Mass Spectrometry

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Characterizing the materials creating cultural heritage (CH) objects is critical to their interpretation, understanding degradation, and improving conservation strategies. Often only picomole equivalents of sample are obtainable, creating barriers for the analysis of these ultra-precious materials, and in particularly protein related components, by contemporary analytical methods. Native and top-down mass spectrometry (MS) have found widespread success in extensively characterizing protein structures, and when combined with ion mobility (IM) are capable of measuring changes in collision cross sections (CCS) and stabilities. Next-generation MS technologies are needed to unlock the chemical information from ultra-precious and irreplaceable CH samples. Here, triboelectric nanogenerators (TENG) have been developed to produce robust IM-MS datasets at picoliter volumes and nanomolar concentrations, consuming femtomole quantities, and combined with top-down MS to probe chemical mechanisms relevant to CH preservation.

We performed a robust characterization of the TENG source for native mass spectrometry measurements. Standard proteins ranging between 3-800 kDa generated native-like charge states and presented native CCS measurements within 3.5% of their respective database values under TENG-nESI conditions. Experimental CCS values were obtained for Human Serum Albumin (HSA), Lysozyme (LYS), Ovalbumin (OVA), and Ovotransferrin (OVT), and all measurements were within 3% of their respective theoretical CCS values empirically derived from the CCS database. Furthermore, by implementing sub-micron emitters with an orifice of 279nm we retained fidelity of native charge states and CCS values for OVA over a 10-step serial dilution from concentrations of 10µM to 100nM, and only consuming 3.3nL of sample totaling only 3.3×10^{-14} moles of OVA sample being used.

Our presentation will detail the native TENG-IM-MS applications above, our most recent efforts to miniaturize the TENG ion source and combining native and top-down MS datasets to characterize the structural evolution of milk caseins in exemplary paint binders model systems.

Abstract 3: Microglial Kv1.3 channel regulation of immune response in Alzheimer's Disease models

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Background:
In Alzheimer's Disease (AD) and 5xFAD mouse models, disease associated microglia (DAM) have a distinct pro-inflammatory profile, including increased Kv1.3 potassium channel abundance. Pharmacological blockade of Kv1.3 *in vivo* reduces Aβ pathology and pro-inflammatory function of microglia. To identify Kv1.3 channels regulation of microglia in inflammatory and AD models, we examined the interactome of Kv1.3 channels *in vitro*, and Kv1.3 deletion and inhibition *in vivo.*

Methods:
TurboID, a biotin ligase, fused to N-term or C-term of Kv1.3 was stably transduced in BV2 microglial cells. Mass spectrometry (MS) of biotinylated proteins, under resting and lipopolysaccharide (LPS)-treated conditions, identified N and C-terminal interactomes. *Kcna3*(Kv1.3)-floxed mice were crossed to CMV-Cre mice producing a Kv1.3 KO mouse, which were evaluated via RNA-seq of microglia and brain after an LPS challenge. Three- or six-month-old 5xFAD mice were treated with PAP1, a blockade of Kv1.3, for three months. The nine-month cohort evaluated using fear conditioning. Aβ pathology was evaluated using ELISA and Immunofluorescence (IF).

Results:
Proximity-based proteomics of BV2-Kv1.3-TurboID microglia identified distinct N-term interactors (n=991) associated with plasma membrane proteins (e.g., Cars1, Psma2) and mitochondrial trafficking proteins (Timm23). The C-term interactors (n=849) are modified by LPS-stimulation (C3, STAT1, Oasl1) (n=36), and dependent on a PDZ-binding motif (Snx3, ND3, n=70). Kv1.3 blockade in 9mon 5xFAD mice show a reduction in Aβ pathology and a rescue of fear conditioning.

Discussion and Conclusions:

We identified novel N and C-term domain-specific and context-dependent interactors of Kv1.3 channels in microglia. While the N-terminus regulates protein processing and localization, the C-terminus regulates immune signaling during LPS-stimulation. Some interactions are dependent on the C-term PDZ-binding domain. Kv1.3 channel blockade appears to reduce Aβ pathology and associated behavior. Identifying microglial Kv1.3 influence in AD may provide knowledge on future biomarkers and treatments.

Abstract 4: Global quantification of newly synthesized proteins reveals cell type- and inhibitor-specific effects on protein synthesis inhibition

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Manipulation of protein synthesis is commonly applied to uncover protein functions and cellular activities. Multiple inhibitors with distinct mechanisms have been widely investigated and employed in bio-related research, but it is extraordinarily challenging to measure and evaluate the synthesis inhibition efficiencies of individual proteins by different inhibitors at the proteome level. Newly synthesized proteins are the immediate and direct products of protein synthesis, and thus their comprehensive quantification provides a unique opportunity to study protein inhibition.

In this work, we systematically investigate protein inhibition and evaluate different popular inhibitors, i.e., cycloheximide, puromycin, and anisomycin, through global quantification of newly synthesized proteins in several types of human cells (A549, MCF-7, Jurkat, and THP-1 cells). The inhibition efficiencies of protein synthesis are comprehensively measured by integrating azidohomoalanine-based protein labeling, selective enrichment, a boosting approach, and multiplexed proteomics. The same inhibitor results in dramatic variation of the synthesis inhibition efficiencies for different proteins in the same cells, and each inhibitor exhibits unique preferences. Besides cell type- and inhibitor-specific effects, some universal rules are unraveled. For instance, nucleolar and ribosomal proteins have relatively higher inhibition efficiencies in every type of cells treated with each inhibitor. Moreover, proteins intrinsically resistant or sensitive to the inhibition are identified and found to have distinct functions. Systematic investigation of protein synthesis inhibition in several types of human cells by different inhibitors provides valuable information about the inhibition of protein synthesis, advancing our understanding of inhibiting protein synthesis.

Abstract 5: Improved detection of peptides through inclusion of semi-enzymatic peptide cleavages and off-target tandem mass tag reagent interactions in proteomics searches

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Background:
Tandem Mass Tag Mass Spectrometry (TMT-MS) based detection and quantification is a powerful high-throughput tool used to generate protein and peptide level data. TMT-MS can even identify and quantify pre-existing, endogenous protein cleavages as well as off-target modifications generated by the tandem mass tag reagent on the amino acids serine (S), threonine (T), and histidine (H). We analyzed datasets from multiple sample sources (brain, CSF, and plasma) with a variety of search parameters, to improve detection and examine the complexity of endogenously generated cleavage patterns as well as improve detection of PSMs through off-target modifications.

Methods:
We used TMT-MS2 (TMT16) batches from three different datasets, one each of Brain, CSF and Plasma, to identify quantitative differences at the protein, peptide and peptide spectral match (PSM) level. These datasets were searched with the FragPipe proteomics pipeline with the following variations of parameter settings: enzymatic cleavages without off-target modifications, enzymatic cleavages with off-target modifications, semi-enzymatic cleavages without off-target modifications and semi-enzymatic cleavages with off-target modifications. The number of detected PSM, peptides and proteins were then quantified.

Results:
Upon inclusion of semi-enzymatic cleavages, we found an increase in the number of PSMs detected across all three sample types, though the increase in detections was greater in biofluids (~30%) compared to the increase in brain samples (~18%). This increase in number of spectral matches and diversity of peptides translated into an overall improvement in protein coverage, with the number of proteins detected increasing by a smaller margin than PSMs. In addition, inclusion of off-target modifications also increased detection of PSMs across all datasets, with serine being the most common off-target labelling effect followed by threonine and histidine.

Conclusions:
By including semi-enzymatic cleavages and off-target TMT modifications in search parameters for proteomic searches, we see a significant improvement in the number and diversity of PSMs, specifically in biofluids compared to a more modest improvement in detection in tissue. This biofluid-specific effect may reflect differences in underlying biology, revealing endogenous cleavages that may have significant biological relevance.

Abstract 6: Quantitative LC-MS as a solution to sequence homology challenges in clinical measurements of proteins and peptides

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Aim/Background:
Many protein- and peptide-based biomarkers, including angiotensin (Ang) peptides for hypertension and parathyroid hormone (PTH) fragments for thyroid diseases, can exhibit high degrees of sequence homology that prohibit accurate antibody-based or bottom-up (proteolytic) liquid chromatography-mass spectrometry (LC-MS) measurements (Emdin, M. et al., *Clinica Chimica Acta* **2015**, *443*, 85-93; Ulmer, C. Z. et al., *J Am Soc Nephrol* **2022**, 33 (8), 1448-1458). Clinical laboratories increasingly use top-down (no proteolytic digest) quantitative LC-MS methods to overcome antibody-based assay limitations (Seger, C. et al., *Clinical Biochemistry* **2020**, 82, 2-11). Our group developed two top-down, quantitative LC-MS assays to measure Ang peptides and PTH fragments, overcoming associated sequence homology challenges.

Methods:
The Ang method was developed using plasma samples enriched for Ang-(1-7), Ang-(1-9), Ang II, and Ang I using organic precipitation and solid phase extraction. The PTH method was developed to measure 9 PTH fragments (1-84, 7-84, 28-84, 34-84, 35-84, 37-84, 38-84, 45-84, 48-84) using sourced materials and corresponding stable isotope-labeled standards. Capillary-flow LC achieved baseline separations prior to measurements using a triple quadrupole mass spectrometer.

Results:
Ang peptides were measured in 50 commercial plasma samples. Preliminary limits of detection (LODs) were calculated using a signal to noise ratio of 3. Ang peptides spanned wide concentration ranges (Ang-(1-7) LOD of 2.8 – 2,140 fmol/mL, Ang-(1-9) LOD of 1.5 – 261 fmol/mL, Ang I LOD of 1.9 – 324.0 fmol/mL, Ang II LOD of 1.7 – 10,323 fmol/mL).

LC solvents containing dimethyl sulfoxide produced PTH fragments at coalesced and higher charge states, providing increased sensitivity and consistent MS/MS fragments. Use of a triple quadrupole mass spectrometer increased selectivity. Coupled, PTH fragment measurement sensitivities were increased to consistently ≤10 pg/mL, accommodating physiologic PTH values in human samples, ~15-65 pg/mL.

Conclusion:
LC-MS-based methods allow for accurate quantification of disease-relevant biomarkers that are difficult to measure using conventional clinical laboratory approaches. Application of both methods will generate new data that may provide unique, mechanistic insights into disease pathologies.

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.

Abstract 7: Establishing an analytical base to support large-scale exposome epidemiology

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Over a lifetime, humans experience thousands of chemical exposures from multiple sources. A more complete estimate of environmental exposures across the lifespan would be a transformative research initiative. The use of high-resolution, mass spectrometry (HRMS) provides a key platform for assessing the exposome and provides measures of thousands of chemical signals in a single human sample; however, application of internal exposome profiling in large populations has been limited due to challenges in sample throughput, instrument robustness, and data handling methods. To support large-scale population research for exposome epidemiology, we have established a high-throughput untargeted HRMS analytical framework combining parallel analysis by liquid (LC) and gas (GC) chromatography to enable robust analysis of up to 20,000 blood samples per year. Sample preparation is achieved using low-cost and open-source automated liquid handlers allowing parallel processing of extracts for LC and GC analysis from a single blood aliquot of 150µL, eliminating operator effects and allowing daily preparation of 96 samples in under 3.5 hours. Exposome profiles are measured using five different HRMS analytical configurations that include reverse phase and HILIC chromatography with both positive and negative ionization, and GC-HRMS to provide detection of 60,000-100,000 chemical signals. Resources for annotation include a standard library of over 7,500 compounds covering a wide range of environmental, drug and endogenous metabolites. The resulting exposome profiles are being assembled within a framework built upon SQL databases, providing a cumulative resource for assembling exposome-disease atlases and laying the foundation for the Human Exposome Project. Development of a robust and scalable analytical framework for large-scale exposome epidemiology is a critical first step to provide a robust foundation for exposome research and to facilitate development of a knowledge base of environmental chemicals, their products, distributions, and associated effects.

Abstract 8: TENG for the Masses: A Low-cost Triboelectric Ion Source for Lipid Double Bond Localization and Other Nanoelectrospray Applications

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Triboelectric nanogenerators (TENG) are useful tools for converting mechanical energy into electric current using readily available materials. Though the applications for these devices span across many fields, TENG intersects with mass spectrometry (MS) as an inexpensive and effective power supply for nanoelectrospray ionization (nanoESI). Previous work has shown TENG nanoESI to exceed the sensitivity of traditional nanoESI for select analytes (Li, Y. et al., *Nat Commun.* **2020**, 11(1):5625) and to promote useful gas phase reactions, such as lipid double bond epoxidation (Bouza, M. et al. *J Am Soc Mass Spectrom*. **2020**, 31(3):727-734) when using specific solvent compositions. We present a redesigned TENG ion source with a sub $1000 material cost and improved capabilities. A novel application of TENG nanoESI is demonstrated by localizing double bond position on individual phospholipids in complex mixtures.

A sliding freestanding TENG was assembled using adhesive backed sheets of acrylic, polyurethane foam, PTFE, and copper (McMaster-Carr). An Arduino Uno was used to control a 75W belt drive actuator to produce the sliding motion of the TENG. Measurement with a 50 MΩ multimeter showed voltage outputs between 0.60 and 4.2kV varying with stroke speed and stroke delay, which are controlled in real time by two independent dials. Epoxidated lipid ion species were detected during negative ionization for 6 phospholipid classes when using a solvent system of acetone/water/methanol (75/12.5/12.5) with 25 mM ammonium acetate. This gas phase reaction enabled the localization of double bond position using ion trap MS3 to first isolate the desired lipid species, isolate each chain fragment, and finally yield diagnostic fragments which were cleaved at the location of the double bond. Lastly, isopropanol extracts of human blood serum were analyzed by LC-MS with a switching valve employed to fractionate peaks of interest. 2 fractions were then loaded into glass capillaries for TENG-MS analysis to identify double bond position in these naturally occurring lipids species.

Abstract 9: Lipidome Profile of Human Muscle Tissues with Diabetic Ulcer

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Diabetes mellitus (DM) is a prevalent chronic metabolomic disease with hyperglycemia, which leads to long-term and multiple-organ disorders. Approximately one fourth of diabetic patients will suffer a chronic non-healing wound in their lifetime, and a large proportion of these patients require lower limb amputation. It is imperative to develop more effective diagnostic and treatment strategies for delayed wound healing of diabetic ulcers. Studies had shown that a diabatic ulcer involves multiple pathological mechanisms, including peripheral nerve and blood vessel damage

due to hyperglycemia and oxidative stress, skin barrier disruption and inflammation, etc. Most of these will result or involve lipid alterations, so it is necessary to observe the lipid profile of DM patients with delayed wound healing of diabetic ulcer. Here we collected five muscle samples from patients with diabetic ulcer and 5 control muscle samples from non-diabetic control patients during limb amputation. Lipids were extracted with isopropanol and homogenized, then analyzed with reverse phase chromatography coupled to an orbitrap Exploris 240 MS. Compound Discoverer v3.3 was used to process the datasets. Feature annotation was performed by MS/MS spectral matching and accurate mass matching to publicly available and in-house databases. The lipidomic results showed that 787 of 3189 annotated features were observed from analysis in positive mode and 349 of 1747 annotated features were observed from analysis in negative mode. Both diacylglycerol and triacylglycerol are increased in DM muscle tissues. Cholesterol is slightly higher in DM muscle tissues. Sphingobase and most sphingolipid backbone, such as sphingosine, ceramide, sphingomyelin, hexosylceramide and sulfatide, are elevated in DM muscle tissues. On the contrary, most of phospholipids, lysophosphatidylethanolamine, phosphatidylethanolamine, lysophosphatidylcholine, phosphatidylcholine, phosphatidylglycerol, and phosphoinositide are relatively lower in the DM muscle tissues. As major components of cellular membranes, messengers in cell signaling & membrane trafficking and major units of energy storage, lipids definitely play important roles in pathological mechanisms of diabetic ulcer.

Abstract 10: Development and Comparison of Methods for Identification and Quantification of Glycosphingolipids (GSLs)

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Glycosphingolipids (GSLs) are a group of glycolipids with a glycan head glycosidically linked to the C-1 hydroxyl group of a ceramide tail. While ceramides exhibit variations, the primary structural and functional categorizations are based upon the diverse glycans. GSLs play pivotal roles in various biological processes including cell adhesion, signaling, proliferation, endocytosis, intracellular transport, inflammation, and apoptosis. These functional roles are dependent on their structural features. Thus, precise identification and quantification of GSLs hold significant importance. This study adopts a comparative approach for identification and quantification of GSLs. Therefore, two methods for analyzing GSLs were compared. In the first experiment, isolated GSLs were permethylated and analyzed using nESI-MS/MS. In the second experiment, GSLs were first cleaved with endoglycoceramidase (EGCase) II. The released glycans were labeled with procainamide and analyzed with UPLC-FLR-MS/MS. Notably, chromatography in the second method facilitated the separation of isomeric glycoforms that were indistinguishable through the other method. Also, this method showed higher sensitivity, identifying 2 additional glycoforms. The boost of sensitivity in analysis of GSLs in the second method is a result of removing the ceramide, thus reducing the heterogeneity associated with its ceramide variety. The EGCase II released glycans comprised both neutral and sialylated species with varying sizes. Optimization of the reaction and cleanup improved the coverage and sensitivity of this method. MS/MS was used for structure assignment confirmation in both methods. Since permethylation and procainamide labeling changes the m/z value of GSLs and glycoforms, the limited existing

online databases and tools for lipids and metabolites are of small use for data interpretation. A database of possible m/z values for GSLs and glycoforms was compiled for assistance in data interpretation. Finally, we found that utilization of an internal standard can be helpful for quantification of GSLs.

Abstract 11: Whole-cell MALDI-TOF MS coupled with untargeted metabolomics facilitates investigations of microbial chemical interactions

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The emergence of drug resistant pathogens necessitates development of new countermeasures. In this regard, the introduction of probiotics to modulate microbial community composition presents a useful strategy. Application of this strategy requires an understanding of how a probiotic and the pathogen interact. One of the mechanisms for pathogen-probiotic interaction involves the production of small molecules called natural products, which are often absent when cultured under typical laboratory conditions. Some approaches to inducing expression of these cryptic gene clusters include exposure to known antibiotics like trimethoprim, supplementation of isolated molecules from natural product libraries, and culturing with other bacterial strains. Coculturing bacteria has been an established method for inducing natural products, however, identifying and evaluating the induction of these compounds has been technically challenging. Here, we report the use of whole-cell matrix-assisted laser desorption/ionization (MALDI) time-of-flight mass spectrometry to screen natural product production by bacteria isolated from the airway microbiome and a model pathogen in co-culture. We identified a Bacillus velezensis strain that elicits natural product production by the model pathogen (Burkholderia thailandensis) and results in growth inhibition. We found the induction of several cryptic metabolites from each bacteria species only when grown together in coculture. These compounds include the Burkholderia-produced, lasso peptides, capistruins, and n-acyl-anthranilic acids as well as bacillaenes produced by the Bacillus strain. Dereplication of other known natural products in the metabolome of this Bacillus strain suggests that a yet unknown bioactive compound is responsible for the growth inhibition of the pathogen. Thus, we present the use of whole-cell MALDI as a broadly applicable method for screening of natural product composition of microbial co-cultures, which can be combined with other -omics methods to characterize probiotic candidates and be used as a high-throughput screening method for the discovery of novel natural products.

Abstract 12: LCMS-guided Discovery, Structural Characterization, and Biosynthesis of Ureido Peptidic Natural Products from Marine Microbulbifer spp. Bacteria

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Nonribosomally synthesized peptides are a large group of natural products with an extremely broad range of structural and functional diversities, including more than 20 clinically used drugs, such as antibiotics (penicillin, vancomycin), antitumor pharmaceuticals (bleomycin), and immunosuppressants (cyclosporine). Microbulbifer sp. bacteria represent an untapped reservoir of chemically diverse and biologically active secondary metabolites. Using high resolution mass spectrometry, we detected the presence of peptidic natural products in obligate marine Microbulbifer sp. bacteria isolated from the commensal microbiome of Floridian marine sponges. Herein, we reported the LCMS-guided discovery, isolation, structural characterization, and biosynthesis of two new groups of ureido hexapeptides, termed bulbiferamides and pseudobulbiferamides from different Microbulbifer strains. Their planar structures were established by comprehensive 1D and 2D NMR spectroscopy and MS/MS fragmentation analyses. All the configurations of component amino acids were determined to be L by Marfey's method. Notably, bulbiferamides feature an unprecedented group of ureido peptides cyclized by a rare N-aminoacylated Trp indole linkage. Genome sequencing identifies biosynthetic gene clusters encoding production of the bulbiferamides and pseudobulbiferamides to be positioned on the bacterial chromosome and on a plasmid, respectively. Their biosynthetic pathways were proposed. The amino acid specificity of Phe adenolation domain of bulbiferamide A was genetically and biochemically determined. Using imaging mass spectrometry, we find that the two classes of Microbulbifer-derived ureidopeptides occupy different physical spaces relative to the bacterial colony, perhaps implying different roles for these two compound classes in Microbulbifer physiology and environmental interactions. Currently, we are screening a bunch of Microbulbifer strains by employing LCMS and Global Natural Product Social Molecular Networking (GNPS) to identify and mine more peptidic molecules producing microbes.

Abstract 13: Microcystin Detection and Identification in Algae by High Resolution Mass Spectrometry

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Incidents of harmful algal blooms are occurring more frequently worldwide, posing both environmental and public health concerns. These blooms are often comprised of cyanobacteria which can produce hepatotoxic microcystins. Microcystins are cyclic heptapeptides with variable toxicity based on the specific amino acid composition. While all microcystins contain the unique

Adda moiety, or a modified version, there are still over 300 congeners published in literature to date; however, there are less than 15 standards commercially available for comparison of retention times and product-ion spectra to confirm accurate identification. In this work, known-unknown, semi-known-unknown, and unknown-unknown microcystins in a complex algal extract were identified using liquid chromatography coupled to a Thermo Exploris 480 Orbitrap high-resolution mass spectrometer. Known-unknown microcystins are identified with an accurate mass, retention time, and spectral library match to a standard. The spectra from these known-unknown microcystins were used to identify diagnostic product-ions for microcystins. With these diagnostic product-ions, semi-known- and unknown-unknown microcystins were tentatively identified. Semi-known-unknown microcystins have been previously documented in literature, but lack standards, while unknown-unknown microcystins have never been documented in literature. All putative microcystin identifications were largely supported by additional chemical and mass spectral experiments, including neutral loss analysis, thiol reactivity, and esterification reactions. In total, within the Microcystis aeruginosa extract, 7 known-unknown microcystins were identified, along with 11 semi-known-unknown and 4 unknown-unknown microcystins. Broadly, a workflow was established for identification of novel compounds within a class: first, known-unknown compounds are identified, then those are used to determine diagnostic ions and/or neutral losses, then these diagnostics are used to identify similar compounds in the sample, and lastly additional chemical testing is performed to confirm the identities of these compounds. Future work seeks to apply this workflow to other toxin classes, including gonyautoxins and amanitins, as it may be beneficial to determine biotransformations of understudied toxins.

Abstract 14: Sub-Cellular Resolution Biochemical Imaging Technique Combining Electron Microscopy and Mass Spectrometry

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The development of mass spectrometry imaging (MSI) technologies over the past several decades has led to exceptional growth in the ability to biochemically characterize spatially unique samples. Yet, identifying biologically distinct information at the single cell level is inhibited by the performance of current instrumentation. The novel multi-modal MSI technique, BeamMap, combines electron microscopy imaging with highly focused electrospray (ES)-enabled mass spectrometry to topologically and chemically characterize biological samples for a number of biomedical applications. The 10-fold enhancement in spatial and chemical resolutions of the new instrument allows for improved characterization of sub-cellular biomolecule populations useful for the detection, diagnoses, and treatment of diseases.

Completed entirely under an Environmental scanning electron microscope (ESEM) environment, topological and chemical imaging will reveal colocalization and cellular heterogeneity in complex bio-samples. ESEM imaging is shown to produce details in sample topology of up to 50 nm in resolution. In addition, the electron beam can closely monitor the ES source to ensure the spray produces a steady beam of droplets. Producing a stable electrospray under vacuum is challenged by the propensity of the ES solvent to freeze. Here, we show that a combination of spray solvent composition, environment pressure, and emitter geometry not only allows for a stable, unfrozen electrospray but permits the production of highly-focused beams of droplets (<500 nm in

diameter). These sub-micron droplet beams reduce the chemical imaging spot-size required for single-cell analysis. Careful transportation of biomolecules to the mass spectrometer is critical to identifying sample chemistry. Using a novel electrodynamic guiding system, we show that ions can be effectively transferred between two vacuum chamber environments over a distance of 1 m. The RF-frequency of the ion guide and the pressure differential between the two vacuum chambers highly influence the number of ions that can be transferred through the system.

Abstract 15: Mass spectrometric investigation of the influence of exogenous ligands on the transformation of thiolate-protected gold nanoclusters

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Coming soon!

Abstract 16: In-containment Orbitrap mass spectrometry analysis of proteomic changes during SARS-CoV-2 infection in a Syrian golden hamster (Mesocricetus auratus) model

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Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a zoonotic virus causing coronavirus disease 2019 (COVID-19) with varying disease severity. New variants of SARS-CoV-2 continue to emerge, some of which evade memory immune responses. With the continuing impact of the COVID-19 pandemic worldwide, small animal models are used to recapitulate human disease to study pathogenesis, transmission, treatment, and vaccine options. One such model, the Syrian golden hamster (*Mesocricetus auratus*), experiences self-limiting disease with pathological lesions in their lungs similar to patients with COVID-19. Using a system-wide assessment of glycoproteomics, we mapped protein-specific glycosylation features to better understand glycosylation impacts during COVID-19 pathogenesis. A Syrian golden hamster model was used with animals aged 4-6 weeks and weighing approximately 90 grams. Hamsters were divided into groups inoculated with either the 2019-nCoV/USA-WA1/2020 strain, delta variant, or mock-infected as a negative control. Animals were infected via intranasal inoculation with a dose of 1×10^3 PFU. Subsets of each group were sacrificed for lung collection at 1-, 3-, and 7-days post-infection (DPI) to assess tissues at peak viral titers, mid-infection, and recovery, respectively. Each lung sample underwent peptide extraction and C18 clean-up for untargeted glycoproteomic analysis via LC-MS. LC-MS profiles were compared to determine any glycosylation changes throughout the SARS-CoV-2 infection course and structurally elucidate differentially expressed

glycans. Immune response proteins were adequately documented throughout SARS-CoV-2 infections, including an acute phase protein, type I interferon-inducible proteins, a weight-loss associated protein, and an opsonin. MALDI-MSI was also performed on 3 days post-WA1 infected lungs with variations noted between *N*-glycans on infected and control lung tissue. Additional analyses are needed to correlate the localization of these *N*-glycans to known hamster immune responses via histopathology or immunohistochemistry. Future studies are needed to compare more strains and localize *N*-glycans to hamster immune responses.

Abstract 17: Developing new mass spectrometric strategies for assigning heavy isotope enrichment in glycoproteins

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2D NMR is a principal method for determining protein three-dimensional structure. This relies on the enrichment of selected heavy isotopes such as 13C or 15N. For proteins expressed in E. coli, uniform enrichment is possible using minimal media and a single source of the heavy isotope such as 13C6-glucose or 15N-ammonium salts. However, glycoprotein overexpression must be carried out in higher organisms for which uniform enrichment is far more challenging. In addition to uniform enrichment, sparse labeling is a second approach, in which enrichment is directed at specific amino acids, for 13C-labeling of methyl groups in valine or alanine. Determining the level of enrichment globally and at the amino acid level is important data for researchers preparing proteins for NMR analysis.

We have developed methods to assess the enrichment levels of isotopically labeled proteins using MALDI-FTICR and ESI-FTICR at the peptide and intact protein level. We have developed a modified version of the Rockwood algorithm for simulating isotope patterns that can simulate uniformly enriched and sparsely enriched isotope patterns. Isotope patterns are generated across a range of incorporation levels which are fit to the data in both the m/z and intensity dimensions. The best fitting isotope pattern is chosen as the appropriate incorporation level.

This algorithm works well for 15N labeled proteins and can assess incorporation levels for 15N13C using a 2-dimensional grid search strategy. The test and fit method can correctly determine the natural abundance of 13C (1.1%) and 15N (0.4%) in ubiquitin peptides and at the intact level. To date, we have analyzed uniformly labeled 15N13C ubiquitin expressed in e coli at >95% 15N and >99% 13C. an Fc protein expressed in yeast using commercial 15N and 15N13C media which had 92% 15N and 30% ±10% 13C. Another expression that utilized a homemade heavy yeast extract media contained 98 ±3% 15N and 95 ±4% 13C. This algorithm is currently being extended to analyze sparsely labeled proteins and to utilize isotopic fine structure for precise incorporation level determination.