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Poster #1

AKT Isoform Specific Regulation of Hepatic Steatosis Induced by Bioactive Lipids

Mario Alba, Ielyzaveta Slarve, Qi Tang, Dante Dikemen, Jared Khan, Yiren Zhou, Yunyi Jia, Aditi Datta, Elizabeth Elton, Brandon Ebright, Zixin Zong, Whitaker Cohn, Handan Hong, Pranav Pammidi, Phillip Nguyen, Lina He, Guo Zhang, Karam Ashouri, Anastasia Martynova, Christina Nakhoul, Jonathan Katz, Anthony El-Khoul, Julian Whitelegge, Stan Louie, Bangyan Stiles

University of Southern California Alfred E Mann School of Pharmacy, Ellison Institute of Technology, UCLA

Background Despite the clinical advancement of immune checkpoint inhibitors, hepatocellular carcinoma (HCC) median survival continues to be less than twenty months and most patients develop resistance. The rate of liver cancer continues to rise, which is coupled to the high prevalence of steatosis and steatohepatitis in the general population. Pathologically, 80% of liver cancer occurs in patients with an underlying liver disease that displays liver steatosis. Thus, it is paramount to better characterize the transition from steatosis to HCC. Results The PTEN regulated PI3K/AKT signal is induced in 54% of all liver cancers and represents the dominant signaling pathway regulating liver cancer progression. Utilizing a PTEN genetic knockout model that recapitulates HCC progression, we performed longitudinal differential proteomic analysis to understand the molecular signatures altered during liver disease. Our analysis revealed significant dysregulation in eicosanoid metabolism among the top enriched pathways when the phenotype progressed from steatosis to HCC. Analysis of patient data using the Proteomic Data Commons also showed similar correlations. Our analysis using targeted lipidomics further validated significant increases in prostaglandin synthesis and decreases in resolving metabolism during disease progression. Phosphoproteomic analysis of AKT isoform specific genetic knockout hepatocytes revealed distinct signaling cascades regulating eicosanoid metabolism. Proteomic and lipidomic analysis of double knockout PTEN-AKT isoform specific mice further validated isoform specific regulatory roles in eicosanoid metabolism and steatosis. Analysis of immunotherapy treated recurrent HCC patient serum also revealed correlations between resolving eicosanoid metabolite levels and stable disease duration. Conclusion Multiomic analysis reveals significant dysregulation of eicosanoid metabolism in liver disease progression as the balance between pro-resolving and pro-inflammatory eicosanoid synthesis is lost. Hepatic eicosanoid biosynthesis is AKT isoform specific in regulation. Higher levels of resolving eicosanoid metabolites are associated with increased duration of stable disease in immunotherapy treatment, demonstrating the clinical significance of these bioactive lipids.

Mario Alba

My name is Mario Alba, and I am a 4th year graduate student finishing up my dissertation work at USC. I originally started off my career in science back in 2014 as an analytical chemist where I spent several years in industry before joining The Ellison Institute as a mass spectrometry focused cancer researcher for another few years. In this role, I realized how much I enjoyed research and so I eventually transitioned towards joining my PhD program in 2020. Since then, I have dived into liver disease and in particular liver cancer where my work has focused on the metabolic dysregulation happening during disease progression. More specifically, I like to study eicosanoid metabolism and the loss of balance between pro-resolving and pro-inflammatory eicosanoid synthesis during HCC progression. To drive this research, I primarily use proteomics and targeted lipidomic panels to validate my findings along with integrating data from the proteomic data commons. In parallel to this research, I also continue to build out mass-spec based collaborations at USC where I bridge researchers together with our newly established mass spec core at the school of pharmacy and as well as with other mass spec groups to find ways to integrate proteomics, metabolomics, and public data from resources like CPTAC to assist in driving various research projects. As a Hispanic minority scientist, I also like to try to find ways to help enable other young minority researchers and regularly mentor and teach students within the Los Angeles area.
Plasma lipids as predictors of metabolic health in people with Type 1 Diabetes

Jessica W. Davidson1,2, Lauren W. Y. McLester-Davis3,4,5, Dominique A. Baldwin1, Isabella James1,2, Andrew Bersh6, Amy Shultz6, Dawn Davis7, and Judith Simcox1†

1 Howard Hughes Medical Institute, Department of Biochemistry, University of Wisconsin-Madison, Madison, WI, USA 2 Integrative Program in Biochemistry, University of Wisconsin-Madison, Madison, WI, USA 53706 3 Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin - Madison 4 Native American Center for Health Professions, School of Medicine and Public Health, University of Wisconsin – Madison 5 Department of Medicine, School of Medicine and Public Health, University of Wisconsin - Madison 6 Department of Population Health Sciences, University of Wisconsin-Madison, Madison, WI, USA 53706 7 Department of Medicine, Division of Endocrinology, Diabetes, and Metabolism, University of Wisconsin-Madison, Madison, WI 53705, USA; William S. Middleton Memorial Veterans Hospital, Madison, WI 53705, USA

People with type 1 diabetes (T1D) have increased morbidity and mortality from cardiovascular disease (CVD). One of the leading drivers of this increased mortality is the distinct etiology of CVD in T1D. Unlike the general population where CVD is predicted by increased levels of low density lipoprotein cholesterol (LDL), LDL is not elevated in T1D associated CVD and other markers of diabetic dyslipidemia are unchanged. This is challenging because LDL is known to directly contribute to atherosclerotic plaque formation, and lowering LDL with statins or cholesterol absorption inhibitors are effective therapeutic interventions for CVD. Better understanding the plasma lipids and other molecular signaling that contribute to T1D associated CVD will identify diagnostic and therapeutic markers. We sought to identify lipids that predict CVD risk and explore the role of oxylipids in CVD pathogenesis by evaluating the plasma lipidome in participants without diabetes, participants with T1D, and participants with type 2 diabetes from the Survey of the Health of Wisconsin (n=126). Participants were stratified by blood pressure, since high blood pressure predicts and contributes to CVD risk. We found that glycerolipids with 18:2- and 18:3-acyl chains were associated with blood pressure in participants with T1D. Additionally, omega-3 fatty acid and CYP450-derived epoxide levels were lower in those with high blood pressure and T1D. Our findings suggest that dysregulation of oxylipids contributes to higher risk of CVD in those with T1D.

Dominique Baldwin

I completed my undergraduate degree in biochemistry at the University of Michigan-Flint. I then pursued graduate training at Indiana University School of Medicine under the mentorship of Dr. Amber Mosley. My thesis work utilized mass spectrometry to characterize protein-protein interactions surrounding RNA Polymerase II and developed techniques for accurately measuring protein phosphorylation changes in biological systems, focusing on transcription elongation. After earning a PhD in biochemistry and molecular biology in the summer of 2023, I joined Judi Simcox's lab as a postdoctoral associate in Fall 2023. Currently, my research involves utilizing mass spectrometry based lipidomics in collaboration with Agilent Technologies to develop an automated pipeline for measuring plasma levels of oxylipids in human patients for clinical diagnostics and assessing exogenous acylcarnitine uptake and processing in cells.
The adipose tissue serves as the major energy reservoir in the body and stores extensive amounts of lipids that can be mobilized for energy production or used as signaling molecules to regulate ligand-activated transcription factors. To activate these transcription factors, lipids must be transported to the nucleus. However, free lipids are inherently toxic within cells, which implies the existence of protein chaperones that bind and transport lipids across various organelles safely. Lipid-binding proteins (LBPs) are a critical class of proteins that bind to and ensure safe mobilization of lipids. Among the LBPs, perilipins (PLINs) and fatty acid-binding proteins (FABPs) are highly expressed in adipose tissue. To understand the mechanisms by which PLINs and FABPs orchestrate intracellular lipid trafficking, we performed proteomics analysis on subcellular fractions (nuclei, cytosol, mitochondria, and whole cell lysates) of primary brown adipocytes. Proteomics data reveal extensive relocation of proteins in response to adrenergic stimulation (norepinephrine or CL 316,243), including LBPs and multiple enzymes moonlighting in the nucleus. Notably, PLINs showed significant nuclear enrichment, indicating a potential role in nuclear lipid signaling and transcriptional regulation. In contrast, FABPs were predominantly retained in the cytosol, suggesting their primary function in cytoplasmic lipid buffering and transport. This differential behavior underscores the distinct functions of these LBP classes within the cellular lipid trafficking network. Future research will focus on elucidating the precise mechanisms underlying perilipin-mediated nuclear translocation and its impact on gene expression and metabolic regulation. Investigating the interaction networks of PLINs in the nucleus will be crucial for understanding their role in transcriptional control. Understanding the interplay between perilipins, FABPs, and other lipid-interacting proteins will provide a more comprehensive picture of cellular lipid homeostasis. Ultimately, these insights could pave the way for developing targeted therapeutic strategies aimed at modulating LBP functions to treat metabolic diseases characterized by lipid dysregulation.

Yutong Bao

Yutong Bao received her B.S. in human biology from the University of California, San Diego in 2022. In the same year, she joined the graduate program of Cellular and Molecular Biology at UW-Madison. Since starting her graduate program, she joined Dr. Andrea Galmozzi's lab which focuses on the research of adipose tissue biology.
Extensive Multi-Omics of Blood Plasma for Molecular Characterization of Acute- and Long-COVID

William F Beimers1, Salma Abou Elhassan1, Joseph Balnis3,4, Katherine A Overmyer2, Joshua J Coon1,2, Ariel Jaitovich3,4

1. UW-Madison Department of Biomolecular Chemistry, Madison WI 53706, USA; 2. Morgridge Institute for Research, Madison WI 53715, USA; 3. Division of Pulmonary and Critical Care Medicine, Albany Medical Center, Albany NY 12208, USA; 4. Department of Medicine and Molecular and Cellular Physiology, Albany Medical College, Albany NY 12208, USA

Long COVID, also known as Post-Acute Sequelae of SARS-CoV-2 infection (PASC), is a multi-system condition characterized by persistent symptoms such as fatigue, brain fog, shortness of breath, and autonomic dysfunction lasting weeks or months after the initial SARS-CoV-2 infection. Between 10% and 30% of patients who recover from acute COVID-19 experience PASC. The molecular mechanisms underlying PASC remain largely unknown, hampering the development of effective treatments. This research aims to elucidate the multi-omic landscape of PASC by leveraging a unique cohort of 400 plasma samples from individuals with PASC, acute COVID-19 infection, non-COVID-19 acute infections, follow-ups, and healthy controls. Our study is employing state-of-the-art mass spectrometry approaches such as Seer Proteograph XT nanoparticle enrichment method for plasma proteins coupled to DIA analysis with the Orbitrap Astral. Using these technologies, we are conducting in-depth profiling of plasma proteins, lipids, and metabolites to identify differential expression associated with PASC and progression of symptoms. Our integrative multi-omics analysis combines proteomic, lipidomic, and metabolomic data with clinical metadata, including symptom severity scores, immune profiling, age, gender, and comorbidities to gain a comprehensive understanding of the molecular pathways dysregulated in PASC. We expect findings to reveal distinct signatures in PASC patients, with alterations in immune response pathways and metabolic processes. Based on previous work assessing COVID-19 severity via multi-omic analysis, lipid, metabolite, and protein levels are all shifted based on severity of COVID-19 infection, which may persist in PASC. Using Proteograph XT and Orbitrap Astral, the level of proteome depth and throughput is greatly improved from previous studies, allowing integration of low-abundance proteins and an expanded sample set. These discoveries will provide critical insights into the pathophysiology of PASC and may inform the development of targeted therapeutic interventions and patient stratification strategies.

William Beimers

I am entering my 3rd year as a PhD student in the Coon Lab at UW-Madison. I graduated with a BA in Chemistry and Music from St. Olaf College in Northfield, MN in 2020. From 2020-2022 I worked in the Maher Lab studying cancer metabolism in paraganglioma as a part of the post-baccalaureate Graduate Research Education Program at Mayo Clinic Rochester. In Fall 2022 I began my graduate studies in the Integrated Program in Biochemistry here at University of Wisconsin-Madison and joined the Coon Lab. As a member of the Coon Lab, I have focused on innovative methods in plasma proteomics, primarily leveraging Seer’s Proteograph XT technology in combination with nanoLC and Thermo Scientific Orbitrap Trybrid and Orbitrap Astral mass spectrometers. We have collaborated with the Wisconsin Alzheimer’s Disease Research Center to evaluate differential expression in pilot cohorts of healthy and AD study participants using these technologies. Most recently, I have led a study of Acute- and Long-COVID plasma proteins with a parallel analysis of lipids and metabolites for a multi-omic characterization of Long-COVID. My overall interests lie in the integration of different types of -omics data to study overall biological systems, the study of metabolism at a molecular and organismal level, and incorporating new technologies to research under-studied proteomes like microbial communities and plants.
In-Depth Characterization of Protein Kinase Complexes by Hybrid Top-Down Mass Spectrometry

Hsin-Ju Chan (1), Boris Krichel (2,3,4), Liam J. Bandura (1), Emily A. Chapman (1), Man-Di Wang (1), Emily A. Reasoner (1), Ying Ge (1,2,3)

(1) Department of Chemistry; (2) Department of Cell and Regenerative Biology; (3) Human Proteomics Program, University of Wisconsin-Madison, Madison, Wisconsin, USA; (4) University of Siegen, Siegen, Germany

Protein kinases play crucial roles in cell signaling cascades and are often regulated through sophisticated molecular events, including non-covalent interactions and post-translational modifications. Regulatory events, such as phosphorylation and allosteric binding, can significantly alter their conformations and functions. However, comprehensive analysis of kinase complexes remains challenging due to proteoform complexity. Top-down mass spectrometry (TDMS) offers unique opportunities to provide a “bird’s-eye” view of the proteoform landscape. Hence, we aim to develop a robust hybrid top-down method, integrating native and denatured TDMS, to better understand kinase proteoforms, their structures, and regulatory mechanisms. In this study, we demonstrated our hybrid top-down approach, using AMP-activated protein kinase (AMPK) as an example. First, we investigated the phosphorylation kinetics of AMPK αβγ complex by conducting a time-course phosphorylation reaction. Denatured TDMS revealed an unreported wealth of AMPK proteoforms at different time points (0 to 200 min). For the regulatory subunit β1, the most abundant proteoform at 200 min was bis-phosphorylated. By fragmenting the bis-phosphorylated precursor through electron-capture dissociation (ECD), we identified and localized five phosphorylation sites on β1 Ser24, Ser25, Thr28, Thr80, and Ser108. Second, native TDMS provides insights into the non-covalent interactions of the heterotrimeric complex. The complex-up analysis showed that AMPK complex dissociated into monomeric (β and γ) and dimeric (αγ and αβ) subunits. The results indicated that the interaction between β and γ subunits was easier to disrupt compared to αγ and αβ. Moreover, the dissociated αγ dimer was observed to bind with 0 to 2 AMP molecules, supporting literature that AMP binds to the nucleotide-binding domains on the γ subunit. In summary, this method enables us to resolve multiple proteoforms and capture their non-covalent interactions, thereby deciphering the molecular complexity of the essential kinases.
Exploring D-amino Acids as Biomarkers in Diabetes

Shuangshuang Chen1, Cindy J. Lee1, Tian A. Qiu2, Stanislav S. Rubakhin1, Jonathan V. Sweedler1
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Diabetes is a complex disease involving issues with canonical islet hormones. While the islet peptide hormones can be informative biomarkers as can protein adducts such as glycation, recent data suggests that D-amino acids are intricately involved in islet physiology and inter-islet communication. Here we explore D-amino acids as biomarkers to characterize diabetes progression and compare these enigmatic molecules to more traditional protein biomarkers. D-amino acids (D-AAs) are involved in cell-to-cell signaling and neuromodulation, acting as co-agonists of the N-methyl-D-aspartate receptor (NMDAR) with significant implications for metabolic disorders such as diabetes. We report several methodological developments for more robust quantitation of the D-AAs and apply them to both serum and tissue samples to track changes in D-AAs in samples from healthy and T1 and T2 diabetics. Chiral liquid chromatography-tandem mass spectrometry (LC-MS/MS) is used in the first project to quantify D/L-alanine, serine, aspartate, glutamate, and proline levels in T1D-affected human serum samples. Our results demonstrated significantly lower D-aspartate, D-glutamate, D-alanine percentages, and a higher D-aspartate percentage in serum samples with no clinically detected C-peptide (used as our marker of T1D) compared to samples exhibiting C-peptide levels exceeding 0.2 nmol/L. Additionally, a negative correlation between disease duration and C-peptide levels was observed, alongside positive correlations between D-alanine, D-aspartate, and D-serine with C-peptide levels. These results suggest that serum D-AA levels are important indicators of T1D progression. Our group has previously characterized D-serine and D-aspartate in T2D-affected human islets via capillary zone electrophoresis with laser induced fluorescence (CZE-LIF). We have increased the number of samples analyzed and find that both D-serine percentage and D-aspartate show significant decreases in pre-diabetic and T2D groups compared to healthy islets, further highlighting the physiological functions of these D-AAs in diabetes.
Extended Characterization and Multiplexed Quantification via a Chemical Proteomics Platform Using High-field Asymmetric Waveform Ion Mobility Spectrometry (FAIMS)

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The advancement of chemical proteomics provides powerful approaches to investigating small-molecule protein interactions. Utilizing the isobaric labeling strategy in chemical proteomics has enabled high-throughput target screening and protein profiling. Recently, our newly developed 12-plex Cleavable DiLeu-Biotin-Azide (cDBA) tag demonstrates the capability to achieve both high-throughput and efficient enrichment simultaneously, for cysteine-containing peptides. To alleviate the co-isolation of tag-containing peptides, here, we employed the online high-field asymmetric waveform ion mobility spectrometry (FAIMS) to extend the characterization depth of the profiled proteome as well as to improve the quantification accuracy using our new cDBA tag. Upon comparing cDBA-labeled cysteine-containing peptides with the DADPS-biotin-labeled peptide, we observed that following cDBA tag labeling, there was an increase in peptide charge. This phenomenon persisted regardless of whether the samples were run on Exploris 480 orbitrap system. The dominant charge changed from 2 to 3, after cDBA labeling. Precursors were separated based on mass and charge through the application of differential ion mobility prior to mass isolation. For this purpose, we employed a non-isotopic cDBA (NI-cDBA) tag for the initial stage of differential ion mobility separation. To assess the effectiveness of precursor separation via differential ion mobility, we compared the charge states of MS1 features at compensation voltages ranging from -50 to -80. The lower compensation voltages (CVs) exhibited a predominance of 3+ ions, while higher CVs demonstrated a prominent increase in the number of 2+ and charge states greater than 3+. Remarkably, the profile of cysteine-containing peptides closely resembled that of non-FAIMS methods when utilizing only one compensation voltage (CV). Subsequently, we compared the peptide counts for cysteine-containing species between experiments employing two CVs and three CVs. In line with previous findings, the use of three CVs resulted in a coverage increase of more than twofold compared to the use of a single CV.
Multi-omic Characterization of Human Pluripotent Stem Cell-derived Cardiomyocyte Maturation during Long-term 2D Culture

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Human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) offer the ability to enhance drug discovery and restore damaged heart tissue through cellular therapy. However, generating hPSC-CMs with a mature phenotype remains a challenge. Although several methods to enhance hPSC-CM maturation have previously been investigated, hPSC-CM immaturity continues to limit in vitro and in vivo utility. Interestingly, few studies have sought to characterize hPSC-CM maturation at the systems biology level. To gain a better understanding of the molecular dynamics which occur during maturation, we performed an integrative, multi-omic profiling of hPSC-CMs matured via extended culture through 190 days. We identified significant changes in 305/948 intracellular metabolites, 1,158/3,556 proteins, and 12,813/23,309 transcripts. Additionally, we have characterized the dynamic sarcomeric proteoform and extracellular metabolite profiles. Interestingly, we identified different timescales in which various aspects of CM maturation occur. For example, substantial sarcomeric maturation occurs within 60 days and plateaus by 100 days (evidenced by transcript and proteoform switches). However, metabolic maturation continually increases throughout the entire 190 days, with glucose utilization and lactate secretion declining while transcripts related to oxidative metabolism continually increase. Moreover, metabolic pathway analysis demonstrates an increase in fatty acid oxidation from day 30 to 190. In addition to temporal waves of maturation, we have identified putative novel maturation markers and compared them to other studies of hPSC-CM and in vivo maturation. These findings will serve as a benchmark for future studies accelerating hPSC-CM maturation to unlock their full potential for drug discovery, disease modeling, and heart repair.
Unlocking the Crustacean Neuropeptidome through EndoGenius and Data-Independent Acquisition Mass Spectrometry

Lauren Fields¹, Tina C. Dang², Mitchell Gray¹, Wenxin Wu¹, Angel Ibarra¹, Lingjun Li¹,²*

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Neuropeptides represent a unique class of signaling molecules that have garnered much attention but require special consideration when gleaning identifications from mass spectra. With highly variable sequence lengths and susceptibility to rapid degradation, neuropeptides must be analyzed in their endogenous state. Further, neuropeptides share great homology within families, differing by as little as a single amino acid residue, complicating routine analyses and necessitating optimized computational strategies. We present EndoGenius, a database searching strategy designed specifically for elucidating neuropeptide identifications from mass spectra by leveraging optimized peptide-spectrum matching approaches, an expansive motif database, and a novel scoring algorithm to achieve broader representation of the neuropeptidome and minimize re-identification.

We implement capabilities for EndoGenius to search data-dependent acquisition (DDA) spectra, from which a spectral library can be curated for analysis of mass spectra collected in a data-independent acquisition (DIA) manner. With respect to DDA analyses, we report more identifications at 1% false discovery rate than other software packages in five neuronal tissue types in the model organism, blue crab, Callinectes sapidus, ideal for integration in neuropeptide discovery pipelines, such as biomarker or physiological state induced molecular signature identification. We sought to leverage the power of EndoGenius’ DDA algorithm to build a comprehensive spectral library for analysis of DIA mass spectra. Using high-pH offline fractionation to improve sample coverage, amplified through use of high-field asymmetric waveform ion-mobility spectrometry, we built the most extensive spectral library documented for crustacean neuropeptides, encompassing more than 10,000 spectra corresponding to 1,217 unique peptides. Utilizing this library to search DIA spectra through neural-network-based DIA-NN programming, we were able to conduct the deepest characterization of the crustacean neuropeptidome to date. EndoGenius is freely available with a user-interface for improved accessibility and is the first standalone platform for optimized neuropeptide identification from mass spectrometry data, achieved through novel scoring algorithms.

Lauren Fields

Lauren Fields received a BS in chemistry from the University of North Carolina Asheville in 2020 where she developed analytical methods to assess the mechanism of action of antibiotics. She is pursuing a PhD in Analytical Chemistry under the supervision of Professor Lingjun Li at the University of Wisconsin-Madison and her present work involves improving the identification of neuropeptides. She is currently developing data-independent acquisition mass spectrometry and bioinformatics strategies to enable high-throughput, optimized identification and quantification of neuropeptides.
A Multidimensional Native Separation for the Online Analysis of Endogenous Protein Complexes from Human Heart Tissue

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Native top-down mass spectrometry (nTDMS) can capture the highest levels of protein structural complexity, including post-translational modifications and non-covalent interactions. However, the requirements of moderate temperatures, aqueous mobile phases, and near-neutral pH restrict the use of online liquid chromatography in nTDMS experiments, limiting analyses to simple samples such as purified recombinant proteins. Here, we develop a novel multidimensional liquid chromatography (MDLC) strategy to enable online nTDMS analysis of complex mixtures. A combination of size-exclusion chromatography (SEC) and online mixed-bed ion-exchange (IEC) provided a higher-resolution, multidimensional, nondenaturing separation. The use of volatile salts makes SEC and IEC directly compatible with downstream nTDMS analysis. SEC is selected as a separation dimension to enable detection of larger protein complexes by avoiding coelution with smaller proteins. Since SEC has low resolution and causes sample dilution, we utilized online, mixed-bed IEC as a second dimension. First, we optimized and characterized the online IEC method using standard proteins. Because nMS analyses must be conducted near neutral pH, a mixed-bed column containing both weak anion and cation exchange material was selected, enabling retention over a large range of electrostatic properties. Non-covalent interactions including metal binding and oligomerization were preserved throughout IEC-nMS analyses. We verified that the mechanism of this separation is related to surface charge rather than solely isoelectric point by comparing the mixed-bed phase with its isolated cationic and anionic components. Next, we applied a 2DLC approach to analyze human heart protein extract, combining SEC prefractionation with online IEC. These results were compared with a 1D online IEC separation. The multidimensional separation allowed for the detection of more high-MW proteins and protein complexes (> 146 kDa). Various metal-binding proteins were also identified. We envision that improved online, nondenaturing separations will provide flexibility to nTDMS analyses and allow the study of more complex systems.
Respiratory syncytial virus (RSV) is a human orthopneumovirus that infects the airways, exacerbating existing morbidities and potentially causing chronic lung damage in immunocompromised individuals. RSV primarily targets the airway epithelium wherein pattern recognition receptors activate interferon regulatory factors (IRFs) and NF-κB, promoting nuclear translocation and association with co-activators on genes related to the secretion of cytokines, interferons, and damage-associated patterns. IRF1 is a member of the IRF family with inducible transcriptional activity in response to viral infection. We have recently demonstrated IRF1 is an important factor in mediating RSV resistance in airway basal cells via constitutive expression of innate immune response (IIR) genes, suggesting its broader role in host viral defense. Preliminary immunofluorescence images have identified a cytoplasmic subpopulation of IRF1 during infection, potentially indicating functionality in addition to gene transcription. Herein, we utilize a mass spectrometry-based bottom-up proteomics approach coupled with cell-fraction and affinity enrichment of FLAG-tagged IRF1 from RSV-treated adenocarcinoma cells. Differential enrichment analysis indicates that RSV induces IRF1 protein-protein interactions with RSV proteins, viral protein degraders such as HERC5, and ribosomal proteins. Gene ontology analysis demonstrates an enrichment in pathways related to inhibited viral genome replication and protein degradation through K48-linked ubiquitination and ISGylation. Other enriched pathways include type I/III interferon signaling and the IIR, aligning with previously reported functions of IRF1. These findings suggest a novel function of the transcriptional regulator IRF1 as an active participant in the anti-viral response to RSV, reiterating the complementarity of high-throughput interactomics to immunology.
Optimization of isolating synaptic vesicles for comprehensive glycoproteomic and neuropeptidomic analysis in neurodevelopmental disorders.

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Synaptic plasticity is essential in the neurophysiological process within the central nervous system. As valued information and memories are presumably stored within the brain, studying the nerve terminals containing isolated synaptic vesicles (SVs) provides a valuable approach to investigate the synaptosome’s overall functionality. Studying these SVs is prevalent as when these synaptic connections become damaged, there is substantial evidence that several neurological disorders occur including fragile X syndrome and Alzheimer’s disease. Therefore, optimization of these isolated SVs is crucial for elucidating the unknown ambiguity of cognitive development as disruptions in the synaptic plasticity are tied to the causation of neurodevelopmental disorders. We performed an Orbitrap Fusion Lumos MS analysis and Proteome Discover search to identify glycopeptides and to determine whether the SVs were successfully extracted from the homogenized mouse brain tissue sample. Out of around 6000 peptides examined from the Syn-PER SDS synaptic supernatant, 178 peptide groups were identified with high confidence. To further gain insight into the molecular function and validate that these peptide groups originated from the isolated SVs, pathway and process enrichment analysis was conducted using Metascape, in which several expected peptides were confirmed to be present within the isolated SVs. Thus, we were able to confirm that using the Izon automatic fraction collector (AFC) in our methodology was successful in isolating the SVs from the homogenized tissue sample for glycopeptide analysis. However, although peptides such as synaptophysin and PSD-95 extracted from the AFC were quantified using Western blotting, the collected ratio of PSD-95/HDAC-2 (1.4) was non-ideal. This was a 5.8-fold enrichment from whole brain extraction, which yielded a ratio of 0.24. Hence, by researching and further testing, optimization of this current methodology would improve the ability to separate and distinguish a larger variety of individual neuropeptides and glycopeptides confidentially present within the synaptosome.
Biomineral skeletons are common in various organisms, such as sea urchins, crustaceans, corals, mollusk shells, and leaf-cutting ants like Acromyrmex echinatior. Unlike the usual calcium carbonate, Ac. echinatior has unique high-magnesium calcite armor, which enhances survival in ant conflicts and reduces fungal infections. A protein-rich epicuticle precedes the biomineral layer formation, and its absence prevents biomineral layer development, highlighting its crucial role. Proteome analysis of this protein layer can reveal interactions and underlying mechanisms. Leaf-cutting ants, Acromyrmex echinatior, were collected from Panama at different ages (two, four, seven, and eleven days post-eclosion) with four biological replicates per age group. Ants were homogenized in a buffer with protease inhibitors, centrifuged, and treated with DTT and IAA before trypsin digestion. Peptides were desalted, purified, and analyzed via dia-PASEF on TimsToF fleX, with results processed using DIA-NN against the Uniprot database (taxonomy ID: 103372). The biomineralization in Acromyrmex echinatior involves a protective metal armor formed through an intricate process. Age-dependent proteomic analyses were conducted on various ant stages using dia-PASEF on TimsToF for global proteome analysis and label-free quantification (LFQ). ANOVA analysis identified 181 proteins with significant differences across age groups, including Troponin, Calponin, and metal-binding proteins. Changes in 36 chitin-related and cuticle proteins indicate the involvement of surface proteins in biomineralization. Gene ontology enrichment analysis highlighted functions related to metal-binding, actin-binding, metal transportation, and ef-hand domains. Dissolving the protein layer with hydrochloric acid and conducting dda-PASEF analysis revealed around 20 candidate proteins essential for biomineralization. MALDI mass spectrometry imaging (MSI) confirmed these proteins’ presence on the ants’ bodies. Identifying these proteins has provided valuable insights into the biomineralization process, establishing potential gene targets for future knockdown experiments to further understand the underlying mechanisms.
Modulation of hepatic TFEB activity during cold exposure uncovers direct regulation of bis(monoacylglycerol)phosphate lipids by Pla2g15

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Cold exposure is an environmental stress that elicits a rapid metabolic shift in endotherms and is required for survival. The liver provides metabolic flexibility through its ability to rewire lipid metabolism to respond to an increased demand in energy for thermogenesis. We leveraged cold exposure to identify novel lipids contributing to energy homeostasis and found that lysosomal bis(monoacylglycerol)phosphate (BMP) lipids were significantly increased in the liver during acute cold exposure. BMP lipid changes occurred independently of lysosomal abundance but were dependent on the lysosomal transcriptional regulator transcription factor EB (TFEB). Knockdown of TFEB in hepatocytes decreased BMP lipid levels. Through molecular biology and biochemical assays, we found that TFEB regulates lipid catabolism during cold exposure and that TFEB knockdown mice were cold intolerant. To identify how TFEB regulates BMP lipid levels, we used a combinatorial approach to identify TFEB target Pla2g15, a lysosomal phospholipase, as capable of degrading BMP lipids in in vitro liposome assays. Knockdown of Pla2g15 in hepatocytes led to an increase in BMP lipids. Together, our studies uncover a required role of TFEB in mediating lipid liver remodeling during cold exposure and identified Pla2g15 as an enzyme that regulates BMP lipid catabolism.
DeAla structure was designed based on amino acid structure, utilizing diethylated alanine and beta-alanine amino acids whose heavy-isotopic reagents are commercially available and cost-effective. DeAla has been synthesized using acetaldehyde, followed by coupling with beta-alanine, achieving a yield of 75% through three steps. Our initial experiments revealed that the 4-plex tags exhibited superior stability and labeling efficacy compared to the current DMTMM-activated tags in our laboratory. The new synthetic route enables the purification of tags prior to sample labeling, effectively eliminating activation reagents that might lead to undesirable byproducts. With the optimized collision energies, the labeled peptide generates decent relative abundances between reporter ions and peptide fragment ions. This improvement facilitates precise multiplexed quantification without sacrificing the number of identifications. To enhance the multiplexing capacity to a 13-plex version, deuterium atoms have been strategically positioned adjacent to nitrogen atoms, thereby effectively mitigating chromatographic shifts among different isotopologues. In 13-plex DeAla configuration, the reporters consist of five atoms of stable heavy isotopes with an m/z range of 100 to 104, which enables the simultaneous analysis of fourteen samples in a single-shot LC-MS/MS experiment. To assess quantitative performance, a 13-plex analysis was conducted as a proof-of-principle by labeling the identical amounts of fourteen PNAC-1 tryptic peptides respectively at 10:1 tag:peptide ratio by weight. Subsequently, the labeled peptides were pooled in the molar ratios of 1:1:1:1:1:1:1 and 1:2:4:6:8:10:12 prior to desalting. In the LC-MS/MS analysis, the abundance of reporter ions exhibited an expected ratio, suggesting that our method showed a promising quantification performance. Additionally, the labeled peptides had negligible shifts in LC retention time across different channels. In conclusion, the 13-plex DeAla reagents provide a highly effective tool for large-scale, high-throughput quantitative proteomics research due to their improved quantitative efficiency, cost-effectiveness, and broad multiplexing capacity, presenting a compelling alternative to commercial options.
Development and application of highly efficient LC-MS/MS methodology for investigating collagen crosslinking in idiopathic pulmonary fibrosis

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Idiopathic Pulmonary Fibrosis (IPF) is a progressive, fatal disease with minimal treatment options. Accumulation of extracellular matrix (ECM) proteins like collagen drives pathogenesis and reduces lung function through elevated expression and altered post-translational crosslinking modifications. Thus, evaluating collagen crosslink dynamics could broaden our understanding of fibrotic diseases like IPF, and crosslinking pathways could be used as therapeutic targets. One challenge, however, has been the complexity of existing methods for crosslink analysis. Here, we aimed to maximize efficiency of this methodology. We eliminated the need for specialized equipment by using tissue homogenization rather than lyophilization and removed the standard solid phase extraction (SPE) column purification step. These alterations reduced hands-on sample preparation time significantly while increasing feasibility for labs without access to or experience with lyophilization and SPE column purification. Beyond modifications to sample preparation, we reduced total run time on liquid chromatography tandem mass spectrometry (LC-MS/MS) instrumentation and increased throughput. While published methods typically require at least ten minutes per sample, our method only requires two minutes per sample. We then implemented the adapted method to evaluate collagen crosslinking in fibrotic lung tissue. As has been published with other methods, we showed an increase in DHLNL in human IPF lungs and in fibrotic mouse lung models. Furthermore, we demonstrated the anti-fibrotic compound SM16 reduced DHLNL in a fibrotic mouse model suggesting this assay could be used to assess compound efficacy. Finally, we evaluated crosslinking in a longitudinal fibrotic mouse lung model and found differences between aged and young mice. Overall, we developed a highly efficient, accessible method for assessing collagen crosslinking and successfully applied this to measure changes in crosslinking dynamics in preclinical models of lung fibrosis.
Unveiling Novel L-to-D Amino Acid Residue Isomerization in Neuropeptides via Multi-faceted Single-Cell Mass Spectrometry

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Introduction: Endogenous L-to-D amino acid residue isomerization, a critical yet understudied post-translational modification, plays a pivotal role in intercellular peptide signaling across diverse animal phyla. Despite the importance of D-amino acid-containing peptides (DAACPs) in physiological processes, identifying and characterizing them and their L-stereoisomers is challenging due to their similar physicochemical properties. Using ion mobility mass spectrometry, we discovered and localized the isomerization site of three novel DAACPs from the lobster neuropeptide signaling system. Mass spectrometry imaging profiled the spatial distribution of novel DAACPs in the neural tissue. Additionally, DAACPs were found in single neurons within the lobster’s cardiac ganglion, highlighting the critical role of neuropeptide stereochemistry in neuromodulation.

Methods: Neural tissues, including the brain, sinus gland, and single neurons from the cardiac ganglion, were dissected from the American lobster, Homarus americanus. DAACP isomerization residues were localized using a Waters Cyclic ion mobility (IM) mass spectrometer. Mass spectrometry imaging of DAACPs and their L-stereoisomers in the sinus gland was performed using a Bruker timsTOF fleX mass spectrometer. Single neuron analysis was conducted using the Thermo Orbitrap Exploris 480 Mass Spectrometer. Preliminary Data: The extracted ion chromatograms of three mature neuropeptides (FDAFTTGFGHN, VYGPRDIANLY, GDYDVYPE) showed two distinct high-intensity peaks with different retention times, despite similar MS/MS fragmentation spectra, indicating endogenous L-to-D post-translational isomerization. Using ion mobility spectrometry (IMS) to compare the arrival time of fragment ions of peptide epimers, we resolved the isomerization sites to be [D-Phe4] in FDAFTTGFGHN, [D-Tyr2] in VYGPRDIANLY, and [D-Asp2] in GDYDVYPE. Optimizing ion mobility parameters enabled baseline separation of DAACPs and their L-stereoisomers, leading to the first IMS-based mass spectrometry imaging (MSI) of DAACPs. DAACPs were also found in single neurons from the cardiac ganglion, maintaining a roughly 1:1 abundance ratio with their L-stereoisomers, suggesting a significant role of peptide stereochemistry in neuromodulation and cardiac function.
FAIMS-DIA-MS-based structural proteomics and N-glycoproteomics to discover Alzheimer’s disease staging biomarkers

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Alzheimer’s disease (AD) is the most prevalent form of neurodegenerative disease. The discovery of reliable biomarkers proves advantageous for diagnosing and monitoring its progression. Given its association with the misfolding of certain proteins, we hypothesized that analysis of protein structures could offer valuable insights into AD, potentially leading to the identification of conformational biomarker. Alongside protein structure abnormalities, glycosylation also plays a significant role in the pathological processes of AD, with several key proteins affected in AD that are either glycosylated themselves or influence the glycosylation of other proteins. Despite emerging mass spectrometry (MS)-based proteomics and N-glycoproteomics studies of cerebrospinal fluid (CSF) and serum have been reported, staging-based conformational biomarkers, N-glycopeptides, N-glycoproteins, and N-glycosylation site occupancy in paired CSF and serum remain comprehensively uncharacterized. To systemically identify staging biomarkers of AD, we perform structural proteomics enabled by limited proteolysis-mass spectrometry (LiP-MS) integrating high-field asymmetric waveform ion mobility spectrometer with data-independent acquisition mass spectrometry (FAIMS-DIA-MS). Furthermore, N-glycoproteomics is conducted via enrichment by hydrophilic interaction liquid chromatography (HILIC) in paired CSF and serum. Collectively, we aim to reveal conformational biomarkers and N-glycosylation aberrations during AD progression.

Haiyan Lu

Haiyan Lu is a postdoctoral research associate in Prof. Lingjun Li’s lab at UW-Madison since Sep. 2021. She received her Ph.D. from Jilin University in Jul. 2020 under the supervision of Prof. Huanwen Chen, where she focused on the development of novel ambient ionization mass spectrometry approaches for cancer research. With her new postdoc career in UW-Madison, she continues to expand her academic training in the interdisciplinary field of bioanalytical mass spectrometry through collaboration with Prof. Lingjun Li. Her current research focuses on employing mass spectrometry-based structural proteomics strategies for the discovery of novel conformational biomarkers related to Alzheimer’s disease (AD), aiming at discovery of novel conformational biomarkers for AD and exploring their underlying molecular mechanism during the AD pathological process. Concurrently, she is engaged in research investigating the interplay between alterations in protein conformation and post-translational modifications within various model systems. She was one of the awardees of 2023 ASMS Postdoc Career Development Award. To date, she has published more than 30 peer-reviewed papers in refereed journals, including 16 first-author publications, and four first-author manuscripts.
Integrating MALDI-MSI and LC-MS/MS for Spatial Localization of Disease Markers in a Model of Benign Prostatic Hyperplasia

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Mass spectrometry imaging (MSI) allows for spatial mapping of multiple analyte classes, ranging from peptides, metabolites and lipids to post-translational modifications. However, MS/MS confirmation can only be targeted with MALDI-MSI, unlike DDA- and DIA-based untargeted approaches utilized in LC-MS/MS, forcing users to do additional LC-MS/MS extractions post-imaging to obtain MS/MS confirmation. While software exists that allows for data integration of MSI and LC-MS/MS datasets, many are not open-source, requiring researchers to manually merge results from both datasets. This hinders analyses of disease states, such as benign prostatic hyperplasia (BPH), where vast cellular heterogeneity makes it difficult to determine cell populations contributing to disease progression. Here, we focused on integrating MALDI-MSI and LC-MS/MS data for on-tissue localization of known markers contributing to BPH disease progression in mouse models. Bladder tissues collected from mice were formalin fixed and paraffin embedded (FFPE) prior to sectioning onto ITO-coated slides. Tissues were dewaxed and underwent antigen retrieval followed by tryptic digestion and CHCA matrix application for MALDI-MSI imaging analysis using a Bruker RapifleX at a spatial resolution of 25 μm. A serial section was prepared similarly until matrix application, at which point digestion was quenched and peptides were extracted, desalted and resuspended for LC-MS/MS confirmation using a Thermo Q-Exactive HF mass spectrometer. For preliminary work, accurate mass matching of MALDI-MSI and MS2 confirmation via LC-MS/MS showed increases in Col1a1 versus control mice, which has shown to be significantly increased in our mouse model and contributes to prostatic fibrosis. We then developed IMSight, an open-source platform designed to automate the merging of MALDI-MSI and LC-MS/MS datasets for confident localization of peptides in an untargeted manner. IMSight further increases the confidence and throughput of MSI workflows through application of automated tissue segmentation and merging with IHC images, providing a detailed understanding of regulatory underpinnings involved in BPH.

Hannah N. Miles

Hannah N. Miles completed her Bachelor of Arts in Chemistry at Carthage College in Kenosha, Wisconsin. She did a year of research within the lab of Dr. John L. Nitiss located at the University of Illinois Chicago College of Pharmacy in Rockford. There, her work focused on characterizing novel human topoisomerase II mutants using yeast as a model organism. She became a graduate student at the University of Wisconsin-Madison in 2019 and is currently an NRSA fellow and PhD candidate performing research in Dr. Lingjun Li and Dr. Will Ricke’s research groups within the School of Pharmacy and Department of Urology within the School of Medicine and Public Health. Her research focuses on integrating mass spectrometric approaches into prostate disease research to uncover molecular mechanisms driving disease pathogenesis as well as identifying novel biomarkers for earlier detection and therapeutic intervention.
Heart disease is the leading cause of hospitalization for older adults in the United States. Over 70% of older adults with heart failure present with preserved ejection fraction (HFpEF), and the prevalence of HFpEF is increasing, with most pharmacological treatments ineffective. Senescence, a process in which cells are in a state of cell-cycle arrest, plays a role in HFpEF pathophysiology. In collaboration with the Mayo Clinic, we studied the effects of rapamycin on patients with HFpEF; rapamycin modifies the senescence-related secretory phenotype (SASP) by inhibiting the mammalian Target of Rapamycin (mTOR). We performed metabolomics analyses on collected plasma using a newly developed HILIC method. 24 patients aged 60+ diagnosed with coronary artery disease were included; 12 received a placebo, and 12 received rapamycin treatment. Cohorts were demographically matched and returned at zero, three, and six months for sample collection and physicals. Plasma was extracted and analyzed using a HILIC LC method coupled to Agilent 6495C triple quadrupole. Rapamycin treatment for 6 months led to significant physiological changes, including an increase in VO2 max, suggesting enhanced cardiovascular fitness. Systolic blood pressure decreased slightly in the treated cohort but was not significant. 137 metabolites were quantified; notably, valine was significantly different in rapamycin-treated patients versus control. Creatinine and N-acetylputrescine, markers of general health, were significantly changed, indicating improved renal function and decreased disease state in rapamycin-treated patients. Overall, this project demonstrated the effectiveness of the HILIC method in clinical research by quantifying metabolites in human plasma samples.

Jericha Mill

Jericha is a postdoctoral associate in the Judith Simcox lab in the UW-Madison Department of Chemistry. She received her bachelor’s degree from Butler University in Indianapolis, IN, where she studied volatile organic compounds in the conservation science lab at the Indianapolis Museum of Art. After graduation, she spent a year working for Corteva Agrisciences as an analyst in the Environmental Fate and Metabolism lab. Both of her research experiences fueled her interest in small molecule mass spectrometry, which brought her to UW-Madison where she joined the Lingjun Li lab; there, she focused her efforts on method development for the study of aging and age-related diseases. She received her PhD in 2023 and transitioned to her current position, where she applies mass spectrometry-based metabolomics and lipidomics to a variety of biological questions. In her spare time, she applies her personal and research experience with age-related diseases as a volunteer Ambassador for the Alzheimer’s Association.
Achieving In-Depth Multi-omic Coverage of Extracellular Vesicles/Exosomes through Systematic Comparison of Multiple Extractions and High-Resolution Mass Spectrometry

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Extracellular Vesicles (EV)/exosomes play key roles in intercellular signaling. In cancer, EV/exosomes are integral to angiogenesis, immune suppression, and metastasis. The functions of these vesicles are governed by their protein, lipids, metabolite composition and cargos, and EV/exosomes have emerged as a cornerstone in cancer liquid biopsies. Mass-spectrometry (MS-based) multi-omics is a powerful approach to analyze proteins, lipids, and metabolites comprising EV/exosomes. However, challenges remain in sample preparation for multi-omics to achieve comprehensive assessment of the chemical composition and cargo of EV/exosomes. Herein, we have taken a systematic approach to evaluate multiple extraction methods and MS-platforms for in-depth metabolome, lipidome, and proteome coverage and high reproducibility. Methanolic, Folch, and BAW (n-butanol, acetonitrile, water) extractions were performed on EV/exosomes isolated from HeLa (cervical cancer) cell culture media. Bottom-up proteomics, enabled by Azo was performed on precipitated proteins. Lipids were separated using reverse-phase liquid chromatography (RPLC) interfaced with an Agilent 6545XT QTOF mass spectrometer. Peptides were separated using nano-flow RPLC interfaced with a timsTOF mass spectrometer and analyzed in PASEF mode. Metabolites have been isolated from extracts and will be analyzed using hydrophilic interaction liquid chromatography (HILIC) interfaced with an Agilent 6545XT QTOF mass spectrometer. All extractions were performed in triplicates. Lipids were analyzed in positive and negative ion modes, and 1904 metabolic features were identified from the lipid extractions, with the Folch extraction provided the most features. A total of 1689 proteins were identified, and of those, 617 had gene ontology (GO) annotations related to extracellular exosomes (FDR = 4.90*10^{-176}) and 619 proteins had GO annotations related to extracellular vesicles (FDR = 1.64*10^{-175}). Ongoing work is targeted towards evaluating reproducibility provided by each extraction.
High-throughput LC-MS/MS enables protein Quantitative Trait Loci mapping in the progression of Metabolic Dysfunction-Associated Steatotic Liver Disease

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Using nanoflow separations coupled to an Orbitrap Astral MS, we achieve quantitation of over 9,500 liver proteins following a 30 minute gradient, single-shot experiment. This high throughput methodology is adaptable to studies of all kinds relying on deep proteomic profiling and precise quantitation. By additionally coupling this method with mapping of protein Quantitative Trait Loci (pQTL), we can begin to understand how genetic polymorphisms affect the abundance of certain proteins that give rise to disease states like Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD). We leveraged this high-throughput method to acquire deep proteomes of the livers of 541 mice from a four-way cross of mouse strains both susceptible and resistant to MASLD. These 541 mice are genetically unique and phenotypically diverse, modeling varying degrees of liver disease severity. This dataset, which was collected in under 4 weeks, is now being leveraged to precisely map differences in protein expression to genetic polymorphisms using pQTL analysis. In total, we detected 12,410 unique protein groups (12,343 genes), of which 5781 were detected in all runs. pQTL studies have previously been greatly limited by sample size and proteomic depth. With this methodology, we can expand the power of these studies to utilize large cohorts, opening the door to study patient samples. And thus, we can begin to determine the phenotypic outcomes of population-wide genetic variation.

Margaret Robinson

I am really excited about coupling cutting edge technology and innovative experimental design to drive discovery in biological systems. As a graduate student in the Coon Lab, I harness our group’s mass spectrometry expertise to tackle pressing questions in human health with discovery proteomics, focusing on creating novel high-throughput methodology. After graduating from Bates College in 2020, I spent two years as a Research Associate at the Broad Institute under Steve Carr learning the basics of proteomics research, particularly how to harness leading experimental approaches to best study complex problems in human health, like cancer resistance mechanisms and exercise physiology. In graduate school, I have tried to take this knowledge to inform the other side; how do we improve technology to meet research needs? In this, I am also always working to improve my ability to demonstrate the capabilities of our technology, as well as its gaps, to a wide audience so we can best work with biologists to advance the field. I am passionate about not only making proteomics technology accessible, but also making our field more welcoming to historically excluded groups. I hope to pursue a teaching career, and one day help my future students get a foot in the door and continue to push the bounds of proteomics research.
Dilated cardiomyopathy (DCM) is the most common form of heart disease, characterized by patients developing ventricular dilation, contractile dysfunction and ventricular arrhythmias, ultimately leading to heart failure. A pathogenic mutation in the phospholamban (PLN) gene, resulting in the deletion of Arg14 (PLN-R14del), has been associated with the onset of DCM. PLN is a transmembrane protein in the sarcoplasmic reticulum (SR) that is dynamically regulated by its post-translational modifications (PTMs) and plays a crucial role in calcium-handling and heart contractility. The dysregulation of PLN's PTM state when mutated impacts its ability to regulate SR calcium-ATPase (SERCA2a) and the translocation of calcium. However, the molecular mechanism of pathogenesis remains unclear as a notable subset of carriers remain asymptomatic in later age, contributing to high phenotypic variability. Current treatments for PLN-R14del patients focus on symptom management rather than preventing disease progression, emphasizing the need for a deeper understanding of PLN-R14del, its downstream consequences, and clinical implications. We have previously developed a surfactant-enabled extraction method that facilitates the characterization of human cardiac PLN from heart tissue. Herein, we leveraged mass spectrometry (MS)-based top-down proteomics and human clinical samples carrying the mutation to enable a deeper characterization of PLN-R14del cardiomyopathy. For the first time, we show the individualized proteoform landscape of PLN-R14del patients where we observed no phosphorylated PLN proteoforms with the mutation, indicating that both the Ser16 and Thr17 sites are affected. This contrasts previous literature, which stated calcium/calmodulin-dependent protein kinase II is able to phosphorylate Thr17. Moreover, key sarcomere proteins (cardiac troponin I and myosin light chain 2v) exhibited decreased phosphorylation in PLN-R14del patients. These preliminary findings reveal novel insights into the phosphorylation potential of mutant PLN and the cardiac proteome, while highlighting the method's ability to provide individualized proteoform information to bridge the gap between genotype and phenotype.

Holden Rogers
Holden Rogers is a third-year Chemistry Graduate Student in Professor Ying Ge’s lab at the University of Wisconsin-Madison. His thesis work aims to develop and apply mass spectrometry (MS)-based top-down proteomics methods to characterize proteins critical to cardiac function. Thus far, he has developed novel strategies that address significant challenges in top-down proteomics, resulting in the acquisition of high-value biological results. In 2023, Holden was first-author on a manuscript where he developed a method to extract and characterize phospholamban, a transmembrane protein in the sarcoplasmic reticulum critical to cardiac contraction. The method enabled site-specific localization of multiple post-translational modifications on PLN and showed a significant decrease in phosphorylation levels of human patients with ischemic cardiomyopathy. His work on PLN has led to multiple collaborations with worldwide leaders in the cardiovascular research community as he is now focused on elucidating the role of cardiac proteoform alterations in the pathogenesis of PLN-R14del cardiomyopathy. In 2024, Holden's second first-author paper focused on the analysis of high molecular weight proteins from minimal sample amounts. By developing a small-scale serial size exclusion chromatography workflow, he was able to increase the detection of high molecular weight species while maintaining the ability to characterize low molecular weight proteoforms, using as little as 1 mg of human cardiac tissue. With a focus on membrane and high molecular weight proteins, which are traditionally difficult to study with MS, his work has and will continue to provide new opportunities and avenues of research to better understand cardiac disease etiology.
Integrated Proteomics Analysis of In Vitro Hypertrophic Cardiomyopathy Models

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Hypertrophic cardiomyopathy (HCM) is the leading cause of sudden cardiac death in young adults. Nearly 1,500 HCM-related mutations in sarcomeric genes have been identified, but the molecular events leading to the disease phenotype remain largely unknown. While previous studies have focused on transcriptional changes in HCM-specific human induced pluripotent stem cell-derived cardiomyocyte (hiPSC-CMs) models, comprehensive characterization of proteome changes in regards to functional phenotype has yet to be explored. Herein, we have generated 3D engineered cardiac tissues (ECTs) hiPSC-CM models from patient lines containing an HCM-causing (MYH7 R663H) mutation to be evaluated by twitch force kinetics and mass spectrometry (MS)-based proteomics. Functionally, the HCM ECTs displayed a prolonged time to contraction and time to relaxation compared to control ECTs. Top-down protein analyses revealed differential expression of myosin light chain 2 (MLC-2) isoforms in HCM ECTs, mirroring the upregulation of the ventricular isoform observed for HCM manifestation in vivo. Furthermore, our HCM ECTs exhibited significant decreases in phosphorylation for several contractile proteins. Global bottom-up proteomics data determined that extracellular matrix (ECM) proteins were downregulated in our disease models. Dysfunction of the mitochondria in our HCM ECTs was indicated by differential expression of metabolic and fission/fusion proteins. Our integrated methodology suggests a correlation between the measured contractile parameters and proteoform landscape. The decrease in phosphorylation of key sarcomeric proteins mimics an immature contractile state, indicating HCM pathogenesis may include compensatory physiological mechanisms underlying the disease phenotype. The decrease in ECM proteins may suggest fibrosis (upregulation of ECM) in mature HCM as a secondary outcome in response to an altered metabolic state. Indeed, our work to integrate functional and molecular parameters in “early stage” HCM constructs provides mechanistic insight into the progression of cardiac disease.

Kalina Rossler

Kalina Rossler is a 4th year PhD candidate and NSF-GFRP fellow in Molecular and Cellular Pharmacology at University of Wisconsin-Madison. She received her BSc in Biochemistry and BA in Vocal Music from Calvin University in 2020. Her research interests center around combining mass spectrometry-based ‘omics and functional assays to more accurately characterize and understand human biology. She is a self-proclaimed “biochem-physiologist”, aspiring to bridge the gap between analytical methodology, clinical research, and medical practice in her future career. When she is not in lab, she enjoys training for 10Ks with her husband, hiking with her Pembroke Welsh Corgi, exploring new restaurants and trying to recreate dishes at home, singing the latest Broadway hit musicals, or scuba diving in the cloudiest lakes of the Midwest.
Diffuse midline glioma, H3 K27-altered (DMG-Alt) are highly aggressive malignancies of the central nervous system (CNS) that primarily affect the pediatric population. Large scale spatial transcriptomic studies have implicated that tumor microenvironmental landscape plays an important role in determining the phenotypic differences in tumor presentation and clinical course, however, data connecting overall transcriptomic changes to the protein level is lacking. The NanoString GeoMxTM Digital Spatial Profiler platform was used to determine the spatial transcriptomic and proteomic landscape in a cohort of both pediatric and adult H3 K27-altered DMG biopsy samples. Three fluorescently labeled antibodies targeting immune cells (CD45), epithelial cells (PanCK), tumor cells (H3 K27M) and a nucleic acid stain (SYTO-13) were used to establish regions of interest (ROI) for genomic and proteomic analysis. We found genetic alterations within the tumor which can be delineated across patient age and spatial location. We show that the H3 K27M mutation itself has a profound impact on tumor cells transcriptomics and interestingly we found limited fidelity between overall transcriptome and proteome. Our data also validate the previously described OPC like precursor signature at the proteomic level and reveal a special shift in the signature based on the local TME composition.

Jack Shireman

My name is Jack Shireman, and I am a 4th year graduate student in the Dey Laboratory at UW-Madison within the department of Neurological surgery. I aspire to complete both my MD and PhD and become a clinician scientist with the overall goal of being able to translate discoveries from the bench to the bedside. My thesis aims to bridge molecular and computational comparisons of high grade brain tumors such as Glioblastoma or Diffuse Intrinsic Pontine Glioma in order to understand an individual’s immune system response to the tumors. Since beginning my research career I have authored over 20 publications and been selected for various awards such as the American Brain Tumor Association Scholar in Training Award. I also serve as the student representative on the Neuroscience Training Program Steering Committee as well as on the leadership team of the Carbone Cancer Cancer Trainee Network. In my free time I enjoy spending time with my partner and our two dogs, going on runs around Madison, and playing golf.
High-throughput platelet proteomics from small amounts of previously frozen samples adds another layer to plasma-based immunophenotyping

Dylan Nicholas T Tabang; Kathrin Korff; Kaitlin Brannon; Komal Mandal; Julia Tilburg; Patrick Van Zalm; Arthur Viodé; Arjun Ravishankar; Jin Gyu Cheong; Steven Z Josefowicz; Joseph E Italiano; Kinga K Smolen; Hanno Steen

Boston Children’s Hospital

Platelets, a key blood component, are most known for their role in hemostasis and wound healing. They also play an important part of the inflammatory response and immune regulation. Platelet proteins remaining in standard plasma preparations are overshadowed by more abundant soluble proteins in the current paradigm for molecular immunophenotyping. Current platelet isolation procedures require larger volumes of plasma than needed for typical plasma proteomics. Here, we show results using banked, previously frozen platelet-rich plasma samples, a novel approach, using down to one microliter, demonstrating that sample sparing can enable accessible, high-throughput mapping of the platelet proteome. Previously frozen EDTA platelet-rich plasma was used for bottom-up platelet proteomics using Opentrons OT-2 automated liquid handlers. Proteins were digested on magnetic beads. LC-MS was performed using an Eevosep ONE system connected to a timsTOF Pro 2 using a 60 SPD gradient and both DDA- and DIA-PASEF modes. Spectral libraries were generated from DDA files using FragPipe, followed by DIA-NN analysis. Due to heterogenous centrifugation conditions between labs and protocols, we evaluated various speeds and durations to determine more concretely optimal “platelet-rich” plasma preparations for platelet proteomics. A key aspect of this work was implementing sample sparing and examining protein detection rates from increasingly small volumes of plasma. Using >25 µL we identified >2000 proteins. Unsurprisingly, reducing the volume ten-fold significantly reduces protein count, though three-fourths of these proteins can be identified using as little as 5 µL of plasma. Optimized methods were applied on a cohort to study effects of COVID-19 severity on platelet proteomes, identifying dysregulated proteins related to platelet activation and the phagosome. This data underscores that sample sparing can be implemented while ensuring accessible platelet proteomics despite low individual sample amounts and that platelet proteomics can be performed on existing frozen plasma sample cohorts if platelets were not completely removed.
Poster #27

Azo-Enabled Extracellular Matrix Proteomics for High-throughput Quantitative Analysis of Lung Tissue

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University of Wisconsin-Madison

The pulmonary extracellular matrix (ECM) proteome ("matrisome") provides structural support for cells and is critical in the regulation of cellular function and homeostasis. The ECM is an attractive target for biomarker discovery for its role in the development and pathogenesis of fibrosis, including structural remodeling and protein composition turnover. Our lab has previously developed an efficient and reproducible dual-step protocol for the enrichment of insoluble ECM proteins from human lung tissue enabled by the anionic surfactant Azo for bottom-up proteomics. However, a dual-step extraction method is not efficient for experiments necessitating large biological cohorts due to the time required for sample processing and data analysis of two fractions per sample.

Here, we aimed to apply a single-step Azo-enabled extraction method to effectively enrich matrisome proteins from lung tissue for high throughput quantitative analysis of the ECM from a single protein extract. We compared matrisome protein coverage between the dual-step and single-step Azo extraction methods to assess the efficacy of a single-step Azo extraction for ECM proteomics applications. Reduced total unique peptide and matrisome peptide identifications are observed with single-step Azo extractions compared with dual-step Azo fractions. Despite reduced peptide coverage, the total number of matrisome protein identifications were the same between single-step and dual-step Azo extracts. A total of 336 unique matrisome proteins were identified in the dual-step decellularized and Azo fractions, and 318 matrisome proteins were identified in the single-step Azo extracts, indicating 18 additional matrisome proteins were identified with the dual-step method. For experiments requiring large biological cohorts, the benefits of identifying a few additional matrisome proteins with a dual-step method are outweighed by the efficiency of a single-step Azo extraction. These results demonstrate a single-step Azo extraction effectively and reproducibly enriches the ECM for bottom-up proteomics applications.
Neuropeptides are key modulators of multiple physiological processes, including stress response, pain modulation, and appetite control. Recognized for their therapeutic potential, they are increasingly targeted for the treatment of neurological conditions such as Alzheimer’s disease, Parkinson's disease, cerebellar ataxias, and autism, as well as metabolic disorders like diabetes. With simpler and accessible nervous systems and diverse neuropeptide expressions homologous to humans, crustaceans have served as essential models in neuroscience. In crustaceans, these signaling molecules are secreted into the circulating fluid (hemolymph) by endocrine organs, exerting long-lasting downstream effects. Microdialysis facilitates the continuous, real-time, in vivo collection of secreted neuropeptides in behavioral experiments without disturbing or sacrificing the animal, reducing the complexity of hemolymph composition, and minimizing neuropeptide degradation in the dialysate. By leveraging mass spectrometry and the powerful microdialysis sampling technique, this study aims to investigate secreted neuropeptides in the American lobster Homarus americanus hemolymph in response to feeding stimuli. A 20-kDa MWCO microdialysis probe was implanted in the heart chamber of the lobster and perfused with physiological saline at 0.5 µL/min. Dialysates were periodically collected at 30-min intervals upon feeding and spiked to a final concentration of 0.2 µM isotope-labeled bradykinin and 5% FA. Furthermore, several buffer systems for optimal extraction of endogenous neuropeptides directly from crude hemolymph were evaluated and compared for efficiency with the microdialysis method. All samples were desalted, analyzed with a Thermo Orbitrap Exploris 480 mass spectrometer, and searched against the Uniprot American lobster proteome database using PEAKS Studio (v10.6). Overall, our findings identified 40 mature neuropeptides across 14 families, likely acting as hormonal regulators that alter physiological states post-feeding. This study not only advances our knowledge of the neuroendocrine control in crustaceans but also highlights the translational potential of invertebrate models in clinical proteomics, offering novel insights into neuropeptide-based therapies for appetite-related disorders.
RNA-protein interactions are critical to normal cellular function mediating activities including transcription, translation, splicing, and localization. Disruption of cellular RNA-protein interactions is characteristic of many disease states. In virally infected cells, these interactions are critical to understanding viral pathogenesis and host-virus interplay during infection. Characterization of the protein interactome of specific RNAs is both essential to understanding its biology and may aid in the discovery of therapeutic targets. In recent years, multiple technologies have emerged to better characterize RNA-protein interactomes. One such technology is hybridization purification of RNA-protein complexes followed by mass spectrometry (HyPR-MS), a multiplexable strategy to probe the protein interactomes of specific RNA targets in parallel by combining sequence-specific RNA capture with mass spectrometry-based proteomics. This technology has been applied to a variety of systems including tumors, cells infected with SARS-CoV-2, and cells transfected with engineered hepatitis B virus constructs.
High-throughput simultaneous quantification of glycopeptides and phosphopeptides enabled by 12-plex DiLeu isobaric tags and dual-functional Ti(IV)-IMAC material

Feixuan Wu, Danqing Wang, Dylan Nicholas Tabang, Haiyan Lu, Peng-Kai Liu, Zicong Wang, Yuan Liu, Lingjun Li
University of Wisconsin-Madison

Glycosylation and phosphorylation are the most common, and important PTMs. Alteration of glycosylation correlates with the progression of cancer and many other disease states. Phosphorylation regulates various cellular signaling pathways, especially in tumors. However, without proper fractionation, substantial phosphopeptides that coelute in the enrichment step can cause suppression of the glycopeptide signals in subsequent liquid chromatography tandem mass spectrometry (LC−MS/MS) analysis. Here, glycosylated and phosphorylated peptides were simultaneously enriched using a dual-functional Ti(IV)-immobilized metal affinity chromatography (IMAC) approach. The strategy was first adapted to enrich PTM peptides from PANC-1 cell line. Six fractions were collected, including four fractions under acidic conditions and two in basic conditions. The result shows that a good separation of glycopeptides and phosphopeptides was achieved. Specifically, in 1:1:1:1 ratio, 62, 291, 751, 405 glycopeptides were found in the acidic fractions and 986 and 360 phosphopeptides were identified in the basic conditions, respectively. Similar results were obtained in 1:2:5:10 ratio. Upon demonstration of proof-of-concept results, the strategy was employed for GDAC knockout mouse brain sample analysis. The work was found to be a promising tool for simultaneous quantification of glycopeptides and phosphopeptides, with minimal interference between the fractions. Its considerable hydrophilicity and metal ion affinity make it an excellent platform for analyzing phosphorylation and N-glycosylation, and their potential crosstalk.

Feixuan Wu

I am a third-year graduate student in Lingjun Li research group at University of Wisconsin-Madison. The most enjoyable thing for me is collaborating with others with diverse research interests in answering biological questions using analytical chemistry techniques. Currently I am working on analysis of post-translational modifications (PTMs) in pancreatic cancer and Alzheimer’s disease using mass spectrometry, especially glycosylation.
Lipids are crucial for cellular homeostasis, with their functions closely linked to their structures and concentrations. Accurate lipid identification and quantification are key in studying their roles, but their diverse structures make comprehensive quantification challenging. This study introduces a novel method for sensitive, high-throughput quantification of multiple lipid subclasses using 12-plex isobaric dual-reactive dimethylaminopyridine-activated N,N-dimethyl leucine- (DiLeu-DMAP) tags and nanoLC-MS/MS analysis. DiLeu-DMAP labeling boosts detection of nonpolar subclasses by up to 200-fold, enhancing annotation accuracy. The excellent performance in labeling efficiency, quantification accuracy and range are validated. Utilizing this method, we analyzed brain tissue lipid changes in Alzheimer's disease mice, quantifying 243 lipid species across 11 subclasses. This revealed potential disease biomarkers, marking a significant step in biomarker discovery.
Poster #32

Multi-Omic Application of MALDI-MSI and MALDI-IHC for Biomolecular Profiling of Immune Cells in Tissues and Single Cells

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Immune desert tumors, characterized by their lack of immune cell infiltration and response, pose significant challenges in the treatment. These tumor types exhibit resistance to immunotherapy, underscoring the necessity for advanced diagnostic techniques to better understand their microenvironment and cellular composition. N-glycosylation and glycogen reservoirs are indispensable features of both innate and adaptive immune cells for proper development, activation, and trafficking throughout the body. To gain a better understanding of immune cell metabolic status and cellular composition, we employed Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry Imaging (MALDI-MSI) and MALDI-immunohistochemistry (IHC) for the spatially resolved analysis of biomolecular and cellular distributions at both the tumor tissue section and single-cell levels. We obtained serial clinical tissues sections of the immune cold tumors, colon, prostates and pancreatic cancers, and employed the high-throughput single cell array-based platform with antibodies for CD4, CD8, CD19, and CD14 to profile human PBMC isolates. For both tissues sections and single cells, N-glycans were released by PNGase F PRIME, glycogen accumulations were digested by isoamylase and cellular compositions were determined by established photocleavable-tagged antibodies from Ambergen Inc. We identified distinct immune N-glycan signatures, glycogen exhaustion, and a unique cellular composition of each tumor tissue section for CD20+ B cells and CD3/CD44+ T cells. In addition, PBMC analysis revealed over 40 N-glycans of distinct N-glycan structural classes present in each immune cell subtype that cleanly differentiate CD4 and CD8 T-cells, and CD19 B-cells, each also with variable glycogen levels. This multimodal interrogation enhances our knowledge of tumor biology and offers potential biomarkers for identifying patients who might benefit from specific therapeutic interventions. These insights pave the way for novel therapeutic strategies aimed at converting immune desert tumors to immune-responsive states, potentially improving the efficacy of immunotherapies and patient outcomes.

Lyndsay Young

Currently, I am a postdoctoral fellow in Dr. Richard Drake’s laboratory in the Department of Cell and Molecular Pharmacology and Experimental Therapeutics. I recently received my Ph.D. in Biochemistry in Dr. Matthew Gentry’s lab from the University of Kentucky in 2022. My current research focuses on the implementation of spatial mass spectrometry imaging for disease profiling and mechanistic investigations. The Drake Lab has pioneered MALDI-MSI to access N-glycosylation from the tissue microenvironment from FFPE and frozen sections. These N-glycan tissue maps serve as guides to target tumor-localized glycoprotein targets for proteomic analysis, as well as providing molecular determinants for histopathology applications. Previous work in the lab has revealed the importance of glycans in cancer progression. My postdoctoral work has focused on defining the N-glycome across colorectal carcinoma progression. We detected a shift in N-glycome profiles from normal to cancerous tissues, marked by a decrease in high mannose N-glycans. Further analysis of the tumor microenvironment by MALDI-MSI and MALDI-IHC identified a unique N-glycan signature indiscriminate of the cancer stage that correlates with populations of a B-cell nucleus and T-cell halo of the tertiary lymphoid structures which has been published in Frontiers in Immunology. This work will provide necessary information in spatial N-glycome, proteome, and extracellular matrix composition understanding of the tumor and tumor microenvironment in disease progression. Ultimately, I aspire to be a successful postdoctoral fellow in the field of tumor metabolism and attain a tenure-track faculty position at an R1 institution.
Poster #33

**Boosting Quantification of N-Glycans by Enhanced Isobaric Multiplex Reagents for Carbonyl-Containing Compound (SUGAR) Tagging Strategy**

**Jingwei Zhang, Zicong Wang, Lingjun Li**

University of Wisconsin-Madison

Glycans are intricate molecules composed of numerous subunits, featuring varying levels of branched polymer structures. There has been significant research focused on mass spectrometry (MS)-based qualitative and quantitative studies of glycans due to their vital roles. Nevertheless, the conventional data-dependent acquisition approach in MS analysis only targets a limited range of abundant ions during the MS1 scans for fragmentation in the following MS2 stages. Here, we have developed an enhanced isobaric labeling technique, incorporating an additional boosting channel. This innovation boosts the effectiveness of isobaric multiplex reagents for carbonyl-containing compound (SUGAR) tagging in quantitative glycomics studies. The approach is particularly beneficial for the characterization of low-abundance N-glycans and proves effective in discerning minor quantitative variations in N-glycan profiling. With the integration of FAIMS, more than 150 quantifiable N-glycans were identified from 1 μg human serum released N-glycans with reliable quantification performance. Furthermore, this strategy was applied to human serum samples to differentiate N-glycan expression between Alzheimer’s disease (AD) patients and non-AD donors. Our results demonstrated the feasibility of using this strategy for glycomic analysis of size-limited samples. Taken together, we developed and optimized a strategy for the enhanced quantitative N-glycan characterization with SUGAR labeling, showing significant promise for identifying novel therapeutic targets or biomarkers in biological systems with a limited sample quantity.
Sequestration of RNA binding proteins in cytoplasmic granules disrupts posttranscriptional process in RBM20 cardiomyopathy

Yanghai Zhang, Zachery R. Gregorich, Eli J. Larson, Ying Ge, Wei Guo

University of Wisconsin-Madison

Objective: RNA binding motif protein 20 (RBM20) is a muscle-specific splicing factor located in the nucleus of cardiomyocytes (CMs). Localization of RBM20 in the nucleus promotes nuclear granules that regulate normal splicing, while cytoplasmic localization of RBM20 facilitated by genetic variants promotes cytoplasmic granules that cause severe dilated cardiomyopathy (DCM). Herein, we aimed to determine the protein components in nuclear and cytoplasmic granules and investigate whether they contribute to severe DCM in RBM20 cardiomyopathy. Methods and Results: To identify the protein components of nuclear and cytoplasmic RBM20 granules, we utilized proximity labeling proteomics analysis. Constructs for proximity labeling were generated by fusing WT or RBM20S640G to the promiscuous biotin ligase TurboID and transduced in H9c2 cells. Biotinylated proteins were pulled-down using streptavidin and analyzed by mass spectrometry. Totaling 388 proteins was identified with high confidence, in which 27 and 14 proteins are positively enriched in WT and RBM20S640G versus controls, respectively. Five RNA binding proteins (RBPs), including CELF1 and MBNL2, are present in both nuclear and cytoplasmic RBM20 granules, indicating sequestration of them in cytoplasmic RBM20 granules, thus impeding their splicing functions in the nucleus. We then confirmed CELF1 and MBNL2 in cytoplasmic granules in H9c2 and adult mouse CMs by Western blot and immunocytochemical staining. We also revealed the mis-splicing of their target genes in WT and RBM20S639G mice. Conclusion: These findings suggest cytoplasmic granules sequestered RBPs are important for splicing. Sequestration of these RBPs disrupt splicing which could partially contribute to severe DCM in patients carrying RBM20 genetic variants.
Rising Star Finalists

Zhan Gao, Postdoc, University of Wisconsin – Madison

Isabella James, Predoc, University of Wisconsin – Madison

Morgan Mann, Postdoc, Stanford University

Cory Matsumoto, Predoc, University of Illinois - Chicago

Pei Su, Postdoc, Northwestern University

Yanqi Tan, Predoc, University of Illinois Urbana-Champaign

Lyndsay Young, Postdoc, Medical University of South Carolina

Zhijun Zhu, Predoc, University of Wisconsin - Madison
Global Proteoform Analysis in Human Hypertrophic Cardiomyopathy through Top-Down Proteomics

Zhan Gao, Kalina J. Reese, Holden T. Rogers, Timothy J. Aballo, Emily A. Chapman, Matthew S. Fisher, Boris Krichel, Yanlong Zhu, Ying Ge
University of Wisconsin-Madison, Madison, Wisconsin

HCM has classically been characterized as “a disease of the sarcomere”, with over 1,400 HCM-related mutations identified in at least 11 sarcomeric proteins. Nonetheless, sarcomeric proteoforms alone may not fully account for the complex clinical manifestations observed in late-stage HCM. Here, we approached a global top-down proteomics approach based on a novel Azo-enabled sequential extraction platform that further expands protein coverage to gain insights into proteoform changes in HCM. Combining three extracts, yielding around 2000 proteoforms detected in each sample. We successfully identified many sarcomeric proteins, including myosin heavy chain (223 kDa), as well as proteins from the nucleus, mitochondria, and ribosome. Consistent with our previous data, we found dysregulated phosphorylation in key sarcomeric proteins. Additionally, our data revealed significant changes in the phosphorylation of cofilin 1 and 2, members of the ADF/cofilin family of actin-binding proteins. This is the first time the altered phosphorylation of cofilins has been detected in HCM, suggesting the phosphorylation of these proteins may play a crucial role in HCM. Moreover, we observed a significant decrease in the phosphorylation of phospholamban, a critical Ca2+ handling protein, which exhibited a positive correlation with troponin I phosphorylation. Lastly, a newly identified PTM, succinylation, was detected on a variety of mitochondrial proteins. Succinylation provides a mechanism for coordinating metabolism and signaling. We discovered for the first time from human HCM tissues that several ATP synthase subunits and enzymes involved in the citric acid cycle showed a significant decrease in succinylation. We speculate that succinylation of these metabolic proteins may alter their activity. Therefore, the investigation of the role of succinylation in metabolism and pathology warrants further exploration. Overall, our data provides a holistic understanding of the molecular perturbations associated with HCM.

Zhan Gao

Dr. Zhan Gao is currently a postdoctoral researcher at UW-Madison, specializing in Top-down mass spectrometry-based proteomics. His research focuses on elucidating the mechanisms of cardiovascular diseases. During his postdoctoral studies under the mentorship of Professor Ying Ge, he developed a novel Azo-enabled three-step sequential extraction method to thoroughly identify and quantify proteoforms from human heart tissue. His work has been published in several top-tier international journals and presented at academic conferences. Dr. Zhan Gao is particularly interested in the development and application of mass spectrometry-based proteomics technologies to uncover novel insights into heart diseases. His research aims to contribute to a better understanding of disease mechanisms, potentially leading to new therapeutic strategies. Dr. Zhan Gao received his BS from Beijing University of Chemical Technology. He moved to Zhejiang University where he received his PhD in analytical chemistry, focusing on the use of mass spectrometry to identify glycan isomers. In 2022, he joined Ge group and currently focuses on high sensitivity single cell proteomics. He enjoys spending time with friends, watching movies, and going to the gym.
Ceramides are a class of signaling lipids known to be involved in metabolic diseases. In cells, ceramides regulate cell death, autophagy, and insulin sensitivity, and their function depends on their acyl chain composition and their localization to specific organelle membranes. Less is known about the function of ceramides circulating in plasma, but elevated plasma ceramides are associated with conditions of metabolic stress, such as obesity and diabetes. Plasma ceramide levels also increase in cold exposure, a metabolic stress that activates nonshivering thermogenesis in brown adipose tissue (BAT). Activation of nonshivering thermogenesis improves glucose and lipid homeostasis. Nonshivering thermogenesis is an energetically expensive process where fuel is oxidized to generate heat rather than ATP. Circulating ceramides are required for body temperature maintenance in cold; reducing plasma ceramide levels by inhibiting de novo ceramide synthetic enzymes leads to hypothermia. However, the mechanism by which circulating ceramides function to support thermogenesis remains unknown. We have shown that cultured brown adipocytes take up ceramides conjugated to synthetic HDL particles from their media. Furthermore, treating these cells with ceramides leads to changes in gene expression and increases in mitochondrial membrane potential and oxygen consumption rate that support thermogenesis. Based on our preliminary observations, we hypothesize that cold exposure leads to uptake of plasma ceramides into the brown adipose tissue, where the ceramides alter signaling pathways that regulate energy expenditure. This research will improve our understanding of the function of circulating ceramides in metabolic stress, allowing us to better understand, and possibly intervene in ceramide transport and metabolism during metabolic stress.

Isabella James

Isabella James is a graduate student in the Integrated Program in Biochemistry (IPiB) at the University of Wisconsin-Madison. She is using mass spectrometry to study lipid signaling in metabolic stress. She obtained her BS at Brigham Young University, where she worked in the Price lab to develop a method to measure the folding stability of the plasma proteome in transthyretin amyloidosis. Isabella focused her passion for metabolism when she worked in the University of Utah metabolomics core facility exploring a wide range of biological questions using mass spectrometry. She is currently in the Simcox Lab studying the function of plasma ceramides using heavy isotope tracing to assess uptake in the brown adipose tissue. In 2023 Isabella was awarded the Dr. Stephen Babcock AG Chem Graduate Fellowship which currently funds her research.
High Resolution Liquid Chromatography – Mass Spectrometry (LC-MS) For Rapid, High-Throughput Clinical Testing of β2-Transferrin in Human Nasal Secretions

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Background: Leakage of Cerebrospinal Fluid (CSF) can result from trauma, lacerations, and surgery, and if untreated can result in life-threatening meningitis. CSF leaks can be diagnosed through the detection of β2-Transferrin (β2-Tf) from nasal secretions. β2-Tf is a uniquely glycosylated proteoform of Transferrin found primarily in CSF, which can be distinguished from the most abundant Transferrin proteoform (β1-Tf) by its altered electrophoretic mobility. However, gel-based detection of β2-Tf requires hours of labor and has low specificity due to the numerous alternative glycoforms of Transferrin. Higher-throughput assays are needed to improve patient care. Methods: We have developed a novel, approach utilizing high-abundance protein depletion and liquid chromatography coupled to high-resolution mass spectrometry (LCMS) to resolve Transferrin Glycoforms by retention time and m/z. This approach improves on the specificity of the gel-based method, and takes advantage of existing high-throughput liquid chromatography platforms to reduce sample-to-sample analysis time. Secretion samples were analyzed using a Vanquish HPLC system coupled to a Q-Exactive Plus Mass Spectrometer (ThermoFisher). Broadband mass spectra were collected and the deconvoluted average masses were used to identify Transferrin glycoforms. Results: Our approach was benchmarked using remnant nasal secretion samples from patients with known CSF leakage. Examining 15 samples, the resulting ESI-MS spectra revealed peaks at 79,554 Da and 78,008 Da, consistent with β1-Tf (Pred. MW: 79,554.71 Da) and β2-Tf (Pred. MW: 78,008.35 Da) within ~10 ppm mass accuracy. β1-Tf was observed in all samples, while β2-Tf was observed in 13 of 15 samples. Samples with missing β2-Tf were marked by significant hemolysis. An optimized LC-MS gradient enables rapid (~10 minute) sample-to-sample injection times. Hemolyzed samples represent an additional challenge for identification of β2-Tf, owing to ion suppression from blood-derived β1-Tf. We are developing additional enrichment approaches for β2-Tf that we anticipate will reduce or eliminate this analytical challenge.

Morgan Mann

Morgan W. Mann, PhD, is a postdoctoral researcher at Stanford University and clinical chemistry DABCC fellow at the University of California San Francisco (UCSF). His personal and professional interests involve the development of novel clinical assays to streamline medical diagnostics and address emerging challenges to our healthcare systems. Prior to his joint positions at Stanford and UCSF, Morgan earned his PhD in Cellular and Molecular Pathology at the University of Wisconsin – Madison, where he applied mass spectrometry-based proteomics to study innate inflammation signaling pathways and viral protein structure in the context of airway infection. He received dual bachelor’s degrees in Biochemistry and Mathematics from the University of Oklahoma.
Automated Container-less Sample Processing System for Single Cell Multi-omics

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Single-cell proteomics stands to reveal aspects of cell heterogeneity at a phenotypically direct level, providing new insight especially when combined with other single cell analyses (Multiomics). However, with only picograms of analytes in a cell, a major challenge is the considerable sample loss from surface binding during sample preparation. These surfaces can include pipette tips or sample containers, which not only reduce potential information, but can introduce random errors, undermining the ability to detect true heterogenous cellular events. To address these issues, we eliminate the need for these surfaces through the novel use of acoustic levitation to perform sample handling of single cell samples in the air using a platform we termed the “Levcell”. To demonstrate the improvements of a container-less processing system, we performed experiments comparing our levitation platform against tube processing. SCoPE2 was used at a 50 and single cell level, with levitated samples displaying greater intensity and identifications when using a signal to noise ratio cut-off. Further analysis of peptide intensities revealed that the Levcell displayed a significant positive correlation in recovering the longer and more hydrophobic peptides. Additionally, by incorporating DNA/RNA extraction techniques and through modification of the sample processing and acquisition methods, we simultaneously analyzed nucleic acids, proteins, metabolites, and lipids from the same single cell. This approach facilitates the ability to obtain a more comprehensive understanding of cellular heterogeneity. Most recently, through implementation of computer-vision guided feedback, the Levcell uses information of levitated samples to autonomously control various processes resulting in stable sample processing of up to 6 droplets for greater than 24 hours. With addition to a fully integrated custom built automated single cell isolation system and reagent addition arm, the Levcell can process samples from start to finish in a reliable high-throughput manner.

Cory Matsumoto

Cory Matsumoto received his Bachelor of Science in Chemistry from the University of Illinois at Urbana-Champaign in 2019, where he researched protein engineering under the mentorship of Dr. Yi Lu. During this time, he developed a passion for engineering, undertaking various small personal projects involving technologies such as 3D printing, design, and robotics. Later, he pursued a Ph.D. at the University of Illinois at Chicago under the mentorship of Dr. Yu Gao, where he was encouraged to explore projects that aligned with his engineering interests. His work involved a variety of projects focusing on addressing significant challenges in single-cell proteomics, including the development of a multi-column, multi-trap LC system for high-throughput single-cell proteomics and global peptide chemical modification strategies to enhance sensitivity. His first-author paper on the dual-column LC system was just accepted by Analytical Chemistry, and the peptide modification manuscript is currently under review. Amidst the COVID-19 pandemic, he and Dr. Gao started to develop the Levcell, an acoustic levitation device that processes single-cell samples without the need for a container, providing a solution to lossless sample preparation, which is a major challenge in single-cell analyses. Through continuous skill development and collaboration with other members of the Gao lab, he further enhanced the platform to include additional functionalities such as automation and single-cell isolation. This work resulted in a US patent and has been a major breakthrough in the Gao lab. With further development, his goal is to make the Levcell an accessible and versatile platform, enabling the ability to perform single-cell analyses by other labs and collaborators.
AutoPiMS: spatially-resolved top-down mass spectrometry enables proteoform-level spatial biology of ovarian cancer

Northwestern University

Mass spectrometry (MS) has enabled spatially-resolved omics in biological tissues. Top-down MS that investigates intact proteoforms is required to capture posttranslational modifications and sequence variations. However, direct top-down MS on tissue is challenging due to the proteome complexity and the wide dynamic range of proteoforms. We have recently developed proteoform imaging mass spectrometry (PiMS), which enables the spatial detection of 10x proteoform features at 4x molecular weight coverage from human tissues compared to conventional MS imaging techniques. PiMS utilizes nanospray desorption electrospray ionization (nano-DESI) as a sampling and ionization probe, and charge detection via individual ion mass spectrometry (I2MS) is employed in MS data acquisition. Based on the successful PiMS implementation, we developed an integrated MS-based workflow, AutoPiMS, to approach an understanding of the spatial proteoform biology of complex cancerous human ovarian tissues. The AutoPiMS workflow includes: 1) on-tissue spatial profiling to obtain proteoform-selective images; 2) region-of-interest profiling coupled to label-free quantitation to enable the discovery of proteoform signatures in cancer and stroma cell types; 3) multiplexed molecular identification of proteoform signatures using automated on-tissue MS/MS powered by in-house built algorithms newly developed for the workflow. Using high-grade serous ovarian cancer biopsies as an example, we discovered >1000 proteoform features down to 0.1% relative abundance. We achieved >60% success rate in automated on-tissue MS/MS, leading to molecular identification of >100 proteoforms up to 60 kDa mass range, including ~32 kDa tropomyosin alpha-chain isoforms with 95% identical sequence. Moreover, we used label-free quantitation to identify >200 proteoforms that are significantly upregulated in tumor or stroma cells from the same tissue biopsy, which has been validated by their corresponding PiMS images. We believe that PiMS2 workflow can be readily expanded to a variety of clinical samples to advance our understanding of disease progression and improved treatment.

Pei Su

Pei Su is an NIH/NIAID K99 postdoctoral fellow in Prof. Neil Kelleher’s research group in the Department of Chemistry at Northwestern University. He earned a Bachelor of Science in Chemistry at Fudan University in 2015. He received his Ph.D. in Analytical Chemistry in 2020 from Purdue University under the supervision of Prof. Julia Laskin. He joined the Kelleher Research Group at Northwestern University in 2021. His graduate research has been focused on instrumentation development for mass spectrometry and ion chemistry in the gas phase and at interfaces. During his postdoctoral training, he joined Neil’s instrumentation team to tackle challenges in spatial and single cell top-down proteomics and developed single molecule-based mass spectrometry technologies for proteoform detection directly from biological tissues and single cells with applications in biomarker discovery and disease diagnostics.
The health and longevity benefits of exercise are well-established, with evidence suggesting that extracellular vesicles (EVs) released during exercise contribute to these positive effects. EVs are nanometer-scale, lipid membrane-enclosed vesicles, playing crucial roles in intercellular communication by transporting functional proteins to recipient cells. This study conducted a bottom-up proteomics analysis of plasma-derived EVs from sedentary and exercised mice using a Data-Dependent Acquisition (DDA) MS approach with the timsTOF Pro, aiming to identify therapeutic proteins for skeletal muscle recovery. EVs were isolated from the blood of mice subjected to four weeks of either sedentary conditions (sedentary EVs) or voluntary unlocked wheel running (exercised EVs). Label-free quantification (LFQ) analysis revealed the differential regulation of the EV proteins between the two groups. Notably, Pm20d1 was significantly up-regulated in exercised EVs. Studies have shown that Pm20d1 is linked to brown fat oxidation and neurogenesis, with its increased expression associated with reduced Alzheimer’s disease-related pathologies. Antioxidants such as Gpx1 and Prdx3 were exclusively found in exercised EVs, highlighting their role in defending against oxidative stress. When exercised EVs were injected into a muscle-disused mouse model, both neurogenesis and neovascularization were improved. Additionally, injecting Gpx1 encapsulated in a liposome enhanced the vascular structure of the disused model. These findings provide strong evidence that exercise-induced EVs contain proteins, particularly antioxidants, that contribute to muscle recovery and overall health. This research will be extended to human subjects. In the follow-up study, human plasma EVs will be collected before and after a 6-week exercise regimen, followed by proteomics analysis. The primary goal of this extension is to explore the potential of these EVs to preserve vascular and whole-body health in humans.

Yanqi Tan

Yanqi Tan is a Research Assistant at the University of Illinois Urbana-Champaign, working in Jonathan Sweedler’s lab since August 2021. Her research mainly involves the necessity to detect and quantify low-abundant proteins and peptides within complex and dynamic biological matrices using liquid chromatography-mass spectrometry (LC-MS) approaches, with the goal of advancing our understanding of various diseases and biological processes. Yanqi’s primary work focuses on the measurement of hemorphins, which are the endogenous opioid peptides in a mouse model of sickle cell disease (SCD). This research aims to elucidate the role of hemorphins in SCD-related pain mechanisms and signaling pathways. Additionally, Yanqi’s conducts proteomics analyses of extracellular vesicles (EVs) from exercised mouse model, identifying critical proteins involved in disease models and responses to exercise-derived stimuli. Originally from China, Yanqi holds a Bachelor of Science in Chemistry from Beijing Normal University. During her undergraduate studies, she developed a keen interest in mass spectrometry and its potential in disease research, driving her current pursuits. Yanqi also worked as a project intern at Novartis, where she helped develop analytical methods for pharmaceutical process control. Beyond the lab, Yanqi enjoys a variety of non-academic interests and hobbies. She loves singing and listening to popular music, watching sports games, and traveling.
Multi-Omic Application of MALDI-MSI and MALDI-IHC for Biomolecular Profiling of Immune Cells in Tissues and Single Cells

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Immune desert tumors, characterized by their lack of immune cell infiltration and response, pose significant challenges in the treatment. These tumor types exhibit resistance to immunotherapy, underscoring the necessity for advanced diagnostic techniques to better understand their microenvironment and cellular composition. N-glycosylation and glycogen reservoirs are indispensable features of both innate and adaptive immune cells for proper development, activation, and trafficking throughout the body. To gain a better understanding of immune cell metabolic status and cellular composition, we employed Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry Imaging (MALDI-MSI) and MALDI-immunohistochemistry (IHC) for the spatially resolved analysis of biomolecular and cellular distributions at both the tumor tissue section and single-cell levels. We obtained serial clinical tissues sections of the immune cold tumors, colon, prostates and pancreatic cancers, and employed the high-throughput single cell array-based platform with antibodies for CD4, CD8, CD19, and CD14 to profile human PBMC isolates. For both tissues sections and single cells, N-glycans were released by PNGase F PRIME, glycogen accumulations were digested by isoamylase and cellular compositions were determined by established photocleavable-tagged antibodies from Ambergen Inc. We identified distinct immune N-glycan signatures, glycogen exhaustion, and a unique cellular composition of each tumor tissue section for CD20+ B cells and CD3/CD44+ T cells. In addition, PBMC analysis revealed over 40 N-glycans of distinct N-glycan structural classes present in each immune cell subtype that cleanly differentiate CD4 and CD8 T-cells, and CD19 B-cells, each also with variable glycogen levels. This multimodal interrogation enhances our knowledge of tumor biology and offers potential biomarkers for identifying patients who might benefit from specific therapeutic interventions. These insights pave the way for novel therapeutic strategies aimed at converting immune desert tumors to immune-responsive states, potentially improving the efficacy of immunotherapies and patient outcomes.

Lyndsay Young
Currently, I am a postdoctoral fellow in Dr. Richard Drake’s laboratory in the Department of Cell and Molecular Pharmacology and Experimental Therapeutics. I recently received my Ph.D. in Biochemistry in Dr. Matthew Gentry’s lab from the University of Kentucky in 2022. My current research focuses on the implementation of spatial mass spectrometry imaging for disease profiling and mechanistic investigations. The Drake Lab has pioneered MALDI-MSI to access N-glycosylation from the tissue microenvironment from FFPE and frozen sections. These N-glycan tissue maps serve as guides to target tumor-localized glycoprotein targets for proteomic analysis, as well as providing molecular determinants for histopathology applications. Previous work in the lab has revealed the importance of glycans in cancer progression. My postdoctoral work has focused on defining the N-glycome across colorectal carcinoma progression. We detected a shift in N-glycome profiles from normal to cancerous tissues, marked by a decrease in high mannose N-glycans. Further analysis of the tumor microenvironment by MALDI-MSI and MALDI-IHC identified a unique N-glycan signature indiscriminate of the cancer stage that correlates with populations of a B-cell nucleus and T-cell halo of the tertiary lymphoid structures which has been published in Frontiers in Immunology. This work will provide necessary information in spatial N-glycome, proteome, and extracellular matrix composition understanding of the tumor and tumor microenvironment in disease progression. Ultimately, I aspire to be a successful postdoctoral fellow in the field of tumor metabolism and attain a tenure-track faculty position at an R1 institution.
Deep Structural Characterization of Metabolites and Metabolic Regulation of Protein Post-Translational Modifications in Diseases

Zhijun Zhu1, Shuling Xu1, Di Zhang2, Jinjun Gao2, Luigi Puglielli1, Yingming Zhao2, Lingjun Li1
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Metabolites play vital roles in biological processes, but their complex structures, especially stereostructures, make mass spectrometry (MS) characterization challenging. Novel multifunctional chemical derivatization strategies have been developed for this purpose. Amino acids (AAs) in the D-form are essential in neurological processes, despite the predominance of their L-enantiomers. MS analysis of low-abundance D-AAs faces challenges due to enantiomeric separation difficulties, low sensitivity, and lack of internal standards. Our in-house developed N,N-dimethyl-L-leucine (L-DiLeu) tags enable chromatographic separation of 20 D/L-AA pairs, supporting a 4-plex isobaric labeling strategy for high-throughput enantiomer-resolved quantification. N,N-dimethyl-D-leucine (D-DiLeu) reagents provide internal references of L-DiLeu labeled D-AAs using their enantiomeric surrogates, D-DiLeu-labeled L-AAs. This approach, termed CHRISTMAS (chiral pair isobaric labeling strategy for multiplexed absolute quantitation), enhances the detection and quantitation of low-abundance AAs, especially D-AAs, in complex biological matrices. Ion mobility collision cross section (CCS) alignment filters out interferences from coeluting isomers/isobars, further improving accuracy. In wild-type and Alzheimer’s disease (AD) mouse brain cortexes, 20 L-AAs and 5 D-AAs were quantified, revealing significant D-AA alterations and providing molecular insights into their roles in aging, AD progression, and neurodegeneration. Simultaneously, metabolites also modify proteins through post-translational modifications (PTMs), increasing PTM diversity. Lysine L-lactylation (KL-la), a newly identified PTM driven by L-lactate, has been studied along with its structural isomers N-ɛ-(carboxyethyl)-lysine (Kce) and D-lactyl-lysine (KD-la), which can be induced by glycolysis-associated metabolites. To clarify the presence of nuclear KD-la and Kce and their relation to the Warburg effect, we developed a “digestion-derivatization” strategy for the separation and identification of these isomers. These technologies showed KL-la as the dominant lactylation isomer on cellular histones, induced by high glucose and glycolysis. These findings enhance our understanding of glycolysis-responsive PTMs and their stereochemical distinctions, with broader implications for PTMs as structural isomers.

Zhijun Zhu

Mr. Zhijun (Andrew) Zhu received his BS in Chemistry from Wuhan University in 2019. He is currently a 5th-year graduate student in the Analytical Chemistry program at the University of Wisconsin-Madison, working under the guidance of Prof. Lingjun Li. During his graduate studies, Andrew has developed advanced mass spectrometry-based analytical strategies aimed at improving the throughput and depth of identification in omics through multidimensional separation and chemical tools. His research focuses on the deeper structural characterization of metabolites, lipids, and protein post-translational modifications, with the goal of elucidating underexplored molecular mechanisms of diseases such as Alzheimer’s disease and cancer. Upon completion of his PhD studies this summer, Andrew will join Stanford University as a postdoctoral researcher. At Stanford, he plans to continue utilizing mass spectrometry to investigate the molecular bases of lysosome dysfunction in diseases.