KEYNOTE SPEAKER ABSTRACTS

Big Data, Health and COVID-19

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Recent technological advances as well as longitudinal monitoring not only have the potential to improve the treatment of disease (Precision Medicine) but also empower people to stay healthy (Precision Health). We have been using advanced multiomics technologies (genomics, immunomics, transcriptomics, proteomics, metabolomics, microbiomics) as well as wearables for monitoring health in 109 individuals for up to 12 years and made numerous major health discoveries covering cardiovascular disease, oncology, metabolic health and infectious disease. We have found that individuals have distinct aging patterns that can be measured in an actionable period of time as well as seasonal patterns of health markers. We have also explored the effects of fiber using multiomics profiling and profile dynamics during pregnancy. Finally, we have used wearable devices for early detection of infectious disease, including COVID-19 and built an alerting system for detecting health stressors that is scalable to the entire planet. We believe that advanced technologies have the potential to transform healthcare.

Two Sides of Precision Medicine: Individualizing Assessment and Therapies

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Underlying precision medicine is the concept that an individual's Omic (proteomic) signature will provide a physician with clinically actionable diagnosis and a subsequent mechanistic therapeutic route. The challenge remains in the identification of treatable diagnosis for each specific individual. To accomplish this will require i) having an array of mechanistic therapies for each disease and ii) a means to diagnosis (identify) which therapy (or combination) will be appropriate for a particular person. We have developed a multiple modal (plasma and dried blood) high throughput LC-MSMS proteomic pipeline which we are introducing into CLIA-CAP laboratory. Simultaneously, we are developing scalable methods for high-through methods for mechanistic drug screening on iPSC-derived cultured tissue or single cell isolated from tissue biopsies from target individuals who could be beneficial in the identification of new and or the assignment of the appropriate drug treatment. Only by addressing both individual's assessment and development of individual mechanistic treatment will precision medicine be successful.

SPEAKER ABSTRACTS

Targeting Bacterial Virulence through Two-component System Signaling

Erin E. Carlson
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Bacterial two-component systems (TCSs) are crucial for the translation of complex molecular environments into microbial action. Prokaryotes have 20-120 distinct TCSs per organism and although ~50,000 TCS proteins have been identified from genomic sequences, most have not been characterized. In particular, much remains to be learned about the TCSs that control pathogenesis and virulence in numerous organisms. This knowledge is critical as the TCSs may serve as new antibacterial targets, which are desperately needed. We have focused on *P. aeruginosa* infections, which have reached a "critical" threat status making novel therapeutic approaches required. We have demonstrated the potential of TCS inhibition with benzothiazole-based molecules that perturb multiple virulence pathways in the burn wound *P. aeruginosa* isolate, PA14. While phenotypic investigations show promising decreases in multiple virulence mechanisms, we sought to obtain a deeper mechanistic understanding of their function through the combined use of RNA-Seq, to assess global transcript regulation, and activity-based protein profiling, to identify the direct protein targets of our devised inhibitors. These data indicate significant decreases in the expression of genes associated with motility pathways such as Type IV pilius production, flagellar proteins, and chemotaxis. They also showed decreases in the expression of genes associated with nitrate respiration, which is important for anaerobic growth of *P. aeruginosa* as occurs in chronic Cystic Fibrosis lung infections. Finally, we have identified key protein targets of our inhibitors.

Discovery and Utilization of Protein Markers in Niemann Pick Type C

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Niemann-Pick Type C (NPC) is a fatal, neurodegenerative lysosomal storage disorder with no FDA-approved therapy. NPC arises from mutations in either the *NPC1* or *NPC2* gene which results in lipid mistrafficking through the endo/lysosomal system. In an effort to better understand the downstream consequences of this genetic disorder and to identify potential markers that represent varying aspects of the disease pathophysiology, our laboratory has performed differential proteomic analysis in multiple NPC model systems and in patient species. In this presentation, multiple examples of protein biomarker studies will be shared along with our translational efforts to validate these findings.

Identifying the Mechanism of Metabolic Regulation During Macrophage Immune Response

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Emerging studies demonstrate that dynamic reprogramming of cellular metabolism plays a crucial part in immune response. Using multi-omic approach and isotopic tracing, we revealed that the mitochondrial metabolism in macrophages undergoes a two-stage remolding upon stimulation with classical stimuli. Specifically, we identified the metabolic transition from the early, pro-inflammatory stage to the late, more immunosuppressive stage is driven by the profound inhibition of pyruvate dehydrogenase complex (PDHC) and oxoglutarate dehydrogenase complex (OGDC). Both PDHC and OGDC belong to the mitochondrial α-ketoacid dehydrogenase family, which uses a lipoic arm covalently attached to the E2 subunit to transfer acyl-group to coenzyme A (coA) to produce acyl-coA. We discovered that reactive nitrogen species (RNS), which macrophages produce in large quantity upon activation for pathogen killing, can cause a series of novel thiol-modifications of the lipoic arm and therefore inactivate its catalytic activity. Mechanistically, we found that coA, the thiol-containing natural substrate of the E2 subunit, plays a key role in efficiently delivering RNS mediated modifications onto the lipoic arm in a targeted manner. In macrophages, the profound and partially reversible inhibition of mitochondrial α-ketoacid dehydrogenases can cause dynamic modulation of TCA cycle flux, rise-and-fall in several immunoregulatory metabolites, and influence the fluctuation of their downstream effectors such as HIF-

1α. This reveals a novel biochemical mechanism capable of substantially regulating mitochondrial lipoic arm-dependent enzymes, which function at important crossroads of the metabolic network, and have broad potential relevance for a range of physiological and pathological conditions involving high RNS production.

Leveraging Proteomics and Metabolomics for Precision Medicine during the COVID Pandemic

Akhilesh Pandey

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The global nature, severity and extended duration of the COVID pandemic was largely unanticipated and resulted in significant mortality and morbidity worldwide. Unlike other pandemics, however, it also provided an opportunity to develop and deploy a variety of novel tools and technologies designed to prevent, diagnose, predict and monitor disease caused by the SARS-CoV-2 virus. I will describe our efforts using mass spectrometry-based approaches to develop a highly sensitive assay for accurate diagnosis of SARS-CoV-2 infection from nasopharyngeal swab samples. This effort is significant because although MALDI-TOF mass spectrometry is already used in clinical microbiology laboratories throughout the world for identifying bacteria and fungi, direct detection from clinical specimens using MALDI-TOF without culture is not possible. Our approach could also be extended to detect sequence variants of the SARS-CoV-2 virus. We compared the analytical sensitivity and specificity of RT-PCR, digital droplet PCR, mass spectrometry and several point of care lateral flow assays and fluorescence assays to detect the virus, which revealed a significant difference in overall sensitivity across the platforms. Finally, by combining proteomic profiling of cytokines and other important soluble proteins with glycoproteomics, metabolomics and lipidomics through machine learning, we developed a 102-analyte signature that was predictive of severe COVID-19 based on blood samples collected at baseline. Importantly, this signature was significantly superior to the classic 'cytokine storm' panel of cytokines that are normally believed to be associated with viral infections, highlighting the importance of multi-Omics analyses that could provide a more individualized approach to treating patients.

Metabolomics for Precision Medicine: The Road to 25k

Gary Patti, Ph.D.

Chemistry, Washington University in St. Louis, St. Louis, MO

The field of metabolite profiling has evolved considerably over the past two decades. Among the most important advances has been the capability to perform untargeted metabolomics on tens of thousands of specimens. Such large-scale profiling enables the sampling of a sufficient number of subjects to stratify cohorts into subgroups based on individual variability, which is necessary to fulfill the vision of precision medicine. This presentation will provide our perspective on innovations that are enabling the application of untargeted metabolomics to precision medicine. We will focus on data processing, which is often recognized as rate limiting. We will argue that the key to success for large-scale applications is complete annotation of data derived from reference samples. As a representative example, two case studies will be discussed. The first is a longitudinal analysis of patients with COVID-19 that identified specific metabolites to predict an individual's disease course early after infection. The second is an aging study that examines families enriched with exceptional longevity.

Chemistry and Biology of Protein ADP-Ribosylation

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[TBA]

RISING STAR SESSION ABSTRACTS

Sample Agnostic Spectral Libraries: An Open Framework Enhanced Data Independent Analysis Profiling Depth

Graham Delafield*, Xiaofang Zhong*, Qinying Yu, Chris Sauer, Henrich Zetterberg, Lingjun Li

Data-independent acquisition mass spectrometry has gained significant attention in biomolecular investigations for its capacity to provide deep, unbiased proteomic profiling. Spectral library-based approaches that correlate precursor and fragment ions to previously identified peptides in adjacent datasets have cemented themselves as an accurate and robust strategy for data deconvolution. However, spectral libraries are traditionally constructed as part of the experimental design, tying the profiling depth that may be achieved to the time, resource availability, and instrumentation needed to generate comprehensive spectral libraries. Here we report the feasibility and efficacy of sample agnostic spectral libraries. Utilizing empirical retention time information to train a capsule network machine learning model, predicted retention time of previously unseen peptide sequences are cast into the comprehensive MassIVE-KB spectral library, effectively calibrating this library to a given experiment. Utilizing this approach in the analysis of cerebrospinal fluid, we quantified >9,300 proteins with 1,642 being dysregulated across control, mild cognitive impairment, and Alzheimer's Disease patient cohorts, with protein dysregulation mirroring previous literature. We present the framework of sample agnostic spectral library construction as a modular, open strategy that is provides no constraint in software or machine learning architecture. Through validating the efficacy of our approach, we demonstrate the feasibility and advantages of utilizing community-driven experimental data to construct relevant spectral libraries in order to augment biomolecular discovery and rapidly advance research in human health.

A Low Isoleucine Diet Promotes Lifespan Extension and Improves Healthspan in Genetically Heterogeneous Mice in a sSex-dependent Manner

Cara Green, University of Wisconsin – Madison

Low protein (LP) diets improve metabolic health in rodents and humans, and increase the lifespan of mice. It is becoming clear that sex, genetic background, age of onset and amino acid composition have a role to play, particularly the branched chain amino acids (BCAAs). We wanted to determine if the BCAA isoleucine recapitulates the metabolic and lifespan extending benefits of a LP diet in a genetically diverse strain of male and female mice. We used HET3 mice, which due to their allelic variation, more accurately reflect the genetic diversity of humans. We placed mice on either a Control, Low Amino Acid (LowAA, essential amino acids reduced by 2/3rds), or Low Isoleucine (LowILE, isoleucine reduced by 2/3rds) diet starting at 9 weeks (short-term) or 6 months (long-term) of age. We found in young HET3 mice 12 weeks of LowILE, but not LowAA reduced body weight gain in males and females despite increased food consumption, which may be due to increased energy expenditure. Only LowILE was able to improve glucose tolerance in males, but LowAA and LowILE improved glucose tolerance in females. Many of these responses were recapitulated when started at middle age and were maintained throughout life. At 5 and 24 months old the hepatic transcriptome, metabolome and lipidome showed distinct age, sex and diet related signatures with distinct differences in inflammatory pathways in old LowILE fed males. LowAA and LowILE males had less age-associated frailty than Controls, but females were unchanged. Urinary dysfunction was significantly improved in LowAA and LowILE males and their long-term memory trended towards improvement. While LowAA males were less frail, they did not have increased lifespan, and neither did any female groups. LowILE males, however, had a 33% increase in median lifespan. In HET3 mice a LowILE diet outperformed the LowAA diet, which may suggest that in a genetically diverse population such as humans, there are drawbacks to the reduction of all AAs, and specific AA reduction may optimize metabolic health and lifespan in a sex-specific manner.

Multi-Omic Single-Shot Technology for Integrated Proteome and Lipidome Analysis

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Liquid chromatography – mass spectrometry (LC-MS) provides a comprehensive approach for multi-omics analysis. To have a clearer insight of the complex network of diverse biomolecules, an integrated multi-omics workflow is needed. Efforts have been made to streamline sample preparation and data analyses. There is, however, very little integration on the instrument level. Here, we propose multi-omic single-shot technology (MOST) to achieve integrated proteomics and lipidomics simultaneously using a single column and one LC-MS system. The robust and reproducible technology eliminates the inconvenience of maintaining multiple MS instruments and allows for more efficient multi-omics analyses. Here we document the utility of MOST for both high and nanoflow separations – showing integrated omic single shot analysis.

To test the idea that proteome and lipidome can be analyzed in a single-shot format, we started with micro flow LC (60 µL/min) and micro-bore C18 RP BEH column (1 mm I.D.), using extracts from yeast and HAP1 cell line. Upon the 90 min gradient elution, tryptically digested peptides were observed from 0-70% mobile phase B and a Q Exactive HF mass spectrometer collected tandem mass spectral data using conventional data-dependent acquisition (DDA). Then the gradient strength was increased and lipid elution begins. At this point the mass spectrometer was placed into polarity switching mode and lipid tandem mass spectral data were collected using optimal DDA settings. With this micro flow chromatography setup we detected more than 2200 protein groups and 150 lipids from yeast, more than 2600 protein groups and 500 lipids from HAP1 cell line with relative quantification – comparable to results with individual analysis from this single-shot multi-omics method. We achieved excellent reproducibility in terms of retention and quantification. Chromatographic separation of peptides and lipids together did not interfere with quantification of each molecular class as compared to using the same chromatography and loading lipids and proteins separately. To gain the benefits of sensitivity and to reduce the consumption of samples and solvents, we moved to nanoflow (0.275 µL/min) and capillary column (0.075 mm I.D. column), coupling with an Orbitrap Tribrid Eclipse mass spectrometer. Using a 120 min gradient, we detected about 6000 protein groups and 1000 lipids from HEK293 cell line with relative quantification in a single shot. By expanding to nano flow, nano electrospray, and a Tribrid MS system, the identifications were more than doubled as compared to our old method.

Deciphering the Mechanisms of BRD4-dependent Innate Inflammatory Activation using PASEF Mass Spectrometry and Multi-Omic Analysis

Morgan Mann, Xiaofang Xu, David S. Roberts, Ying Ge, Allan R. Brasier

Introduction: Bromodomain-containing Protein 4 (BRD4) is a transcriptional regulator associated with malignant transformation, inflammation, and fibrosis. In airway viral infection, BRD4 interacts with the NF-kB subunit RelA/p65 via its acetyl-lysine binding bromodomains to activate secretion of chemokines and interferons (e.g. IL6). This signals nearby immune cells and forms an early antiviral defense. However, in severe infections, hyperstimulation and chronic inflammation can result in tissue damage and airway obstruction, often requiring hospitalization. While the RelA·BRD4 complex is known to activate immediate-early genes through transcriptional elongation, this program of gene expression is characterized by waves of intermediate and late gene expression. Consequently, it is unclear how the protein complex facilitates coordination of gene expression programs exhibited during innate induction.

To address this knowledge gap, we utilized high-resolution, Parallel Acquisition – Serial Fragmentation (PASEF) Mass Spectrometry to probe BRD4's dynamic interactome and its effects on the global proteome and transcriptome in human small airway epithelial cells (hSAECs) infected with RSV. To determine the component of these changes that is dependent on bromodomain interactions, experiments also included treatment with the competitive BRD4 inhibitor ZL-0454. Results were validated using independent IP-Western Blot, and the impact of novel interaction partners on chemokine expression was explored using

Quantitative-Reverse Transcriptase-PCR (q-RT-PCR) and Crosslinking Chromatin Immunoprecipitation (xCHIP) to measure transcript abundance in overexpression and inhibitor models.

Methods: hSAECs were grown to ~80% confluency and treated with DMSO or the BRD4-specific inhibitor ZL0454 (10uM) overnight (n=3 per group). The next day, cells were treated with PBS (Mock) or infected with the Respiratory Syncytial Virus (RSV) long strain for 24 hours (MOI of 1). Cells destined for interactome analysis were processed according to Mann, et. al. ; cells used for global proteome analysis were lysed in 0.2% Hexylphenylazosulfonate (Azo), standardized to 0.5 mg/ml, and digested with trypsin for 1 hour. UV light was used to degrade Azo after digestion, and samples were desalted using C18 tips. Each sample was analyzed using a nanoElute LC system coupled to a timsTOF Pro Mass Spectrometer (Bruker Daltonics) operating in diaPASEF mode. RNA-sequencing data was acquired from Xu, et. al.

Results: Interactome analysis revealed 101 proteins which were significantly increased in abundance within the RSV-stimulated complex, and also decreased in abundance when treated with the BRD4 inhibitor. These proteins were heavily enriched in transcriptional regulators, including members of the AP1 transcription factor complex (e.g. cJUN, JUNB). AP1 is a ubiquitous transcription factor that participates in the activation of pro-inflammatory cytokines. This suggested that BRD4 and AP1 may interact to facilitate activation of a subset of innate inflammatory genes; This hypothesis was confirmed by xCHIP analysis of hSAECs, wherein the AP1-inhibitor SR11302 blocked the Poly(I:C)-induced translocation of BRD4 to the IL6 promoter.

Global protein analysis revealed similarly interesting findings when contrasted against parallel RNA-sequencing data. 2-Dimensional Enrichment analysis indicated that BRD4 inhibition (BRD4i) was associated with notable disagreement between the protein- and transcript-level measurements in several biological pathways – most notably mRNA splicing. At the protein-level, BRD4i was associated with 14 significant changes to spliceosome in the Mock condition, and 37 in the RSV condition. Further investigation of the RNA-seq dataset demonstrated that BRD4i was associated with significant changes to the alternative splicing of ~800 genes, including XBP1 and IFRD1 – genes associated with the unfolded protein response and innate inflammation, respectively.

Advancing Mass Spectrometry-Based Multiomic Analyses Through New Approaches Towards Precision Medicine

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The ‘omics revolution has been paramount in advancing the basic, mechanistic understanding of biological systems towards precision medicine. The implementation of mass spectrometry-based multiomics approaches have shown useful in the improved elucidation of altered biomolecules implemented in multiple signaling pathways, cellular processes, and phenotypes. Here, I will present multiple studies that demonstrate the veracity of combining individual ‘omic analyses to better understand biomolecular alterations in human diseases. Mapping the altered proteome and lipidome in a mouse model of Niemann-Pick, Type C (NPC) was the primary focus of my graduate career. In our bottom-up analysis of NPC brain tissue, we observed alterations in multiple enzymes of the phosphatidylinositol signaling pathway. Serendipitously, we observed an impressive overlap when combining these results with our mass spectrometry imaging data of the brain of NPC mice. Building on this, we integrated our proteomic and lipidomic data from the brain and liver of NPC mice and observed altered fatty acid metabolism, including the polyunsaturated fatty acids, docosahexaenoic and arachidonic acid. The main goal of my postdoctoral training is to advance mass spectrometry-based multiomic analyses towards precision medicine. Recently, we have implemented a multiomics platform integrating denatured top-down proteomics, bottom-up proteomics and untargeted metabolomics to profile global protein and metabolite alterations in human pluripotent stem cell (hPSC)-cardiomyocytes (CM) aged cultures in which we identified key metabolic pathways and biomarkers for monitoring hPSC-CM maturation. Moreover, native top-down mass spectrometry provides new measurements regarding different proteoforms and

their bound ligands, such as lipids and other small molecules which may be pertinent for understanding disease mechanisms. To incorporate native top-down data into our established multiomics workflow, we will integrate data output from our current software tool, MASH Native.

Revealing the Molecular Structures of Low-Abundance Protein Biomarkers by Top-Down Proteomics and Nanotechnology

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Top-down mass spectrometry (MS)-based proteomics is the premier technology for characterizing proteoforms to decipher post-translational modifications (PTMs) together with genetic variations and alternative splicing isoforms toward a proteome-wide understanding of biological functions. Many strides have been made in the past decade to enable the application of top-down proteomics for understanding basic biological functions, unraveling disease mechanisms, and discovering new biomarkers. However, major challenges related to the proteome dynamic range, proteome complexity, and establishing proteoform–function relationships persist and limit the broader application of the top-down strategy for challenging biological systems. This talk will describe how, by bridging the diverse silos of nanotechnology and MS-basedproteomics, novel, unconventional, and effective solutions to addressing these challenges, and other chemical and biological problems, emerge. In one tale, this talk will detail how rationally designed surface functionalized multivalent superparamagnetic nanoparticles (NPs) can be used as a general affinity platform to capture and enrich low-abundance proteoforms with high specificity for top-down MS applications. I will highlight the first generation use of this nanomaterials platform for the enrichment of low-abundance phosphoproteins, the recent development of a “nanoproteomics” platform to enrich cardiac troponin I (cTnI), the gold-standard biomarker for acute myocardialinfarction (also known as a heart attack), from human serum with high specificity and sensitivity, and the ongoing work for targeting important endogenous receptor membrane glycoproteins. In the second tale, I will introduce new methodologies leveraging ultrahigh-resolution Fourier transform ion cyclotron resonance (FTICR)-MS, using a 12T solariX FTICR, and trapped ion mobility spectrometry (TIMS), using a timsTOF, to advance the comprehensive molecular characterization of complex native glycoproteins by top-down MS. This hybrid top-down MS approach is capable of providing detailed molecular insights to characterizing the structures and heterogeneity of complex glycoproteins, and specific examples involving variants of the SARSCoV-2 Spike receptor-binding domain (S-RBD) protein will be illustrated.