RISING STAR ABSTRACTS

Beyond Ensemble Measurements: Resolving Antigen Binding and Conformational Heterogeneity in Multispecific Antibodies

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Multispecific antibodies (MsAbs) are a new modality in the treatment of complex diseases, i.e., cancers, neurodegenerative, and autoimmune disorders. By binding multiple epitopes on the same or different targets, they offer enhanced selectivity, improved safety, and efficacy. However, achieving desired stability and binding affinity towards a specific antigen while avoiding cross-reactivity remains a central challenge in the rational design and engineering of MsAbs. We interrogate a bispecific antibody pair (Johnson & Johnson Innovative Medicine) that recognizes a peptide epitope of the respiratory syncytial virus F (RSV F) antigen using tandem-trapped ion mobility spectrometry (Tandem-TIMS). Utilizing the ability of Tandem-TIMS to conduct non-ensemble measurements, we characterize the impact of sequence mutations and distinct protein conformations on the antigen-binding properties of the bispecific antibodies. Subsequently, structural analysis of experimental data was performed using the molecular dynamics-based structure relaxation approximation (SRA) method. We observed distinct glycoform distributions for the wild-type and AAS Fc-mutated bispecific antibodies and found that the AAS mutant adopts larger conformations (6590 Å² for the 26+ charge state) than the wild-type species (6340 Å²), despite its slightly lower mass (~220 Da). Leveraging the unique non-ensemble measurement capabilities of Tandem-TIMS, we directly interrogated the conformational dependence of RSV F peptide binding. Our findings reveal that the RSV F antigen recognizes both the wild-type and AAS-mutated bispecific antibodies in a conformation-dependent manner, with binding preferentially observed for larger conformations. Such conformation-selective interactions would likely remain masked in conventional ensemble measurements that average across multiple coexisting conformational states. Molecular dynamics-based structural relaxation approximation (SRA) calculations further support the hypothesis that distinct conformational states govern the relative binding affinities of the bispecific antibodies toward the RSV F protein.

Paired structural proteomic and phosphoproteomic analysis of CDK7 inhibition in fibrolamellar carcinoma patient-derived organoid models

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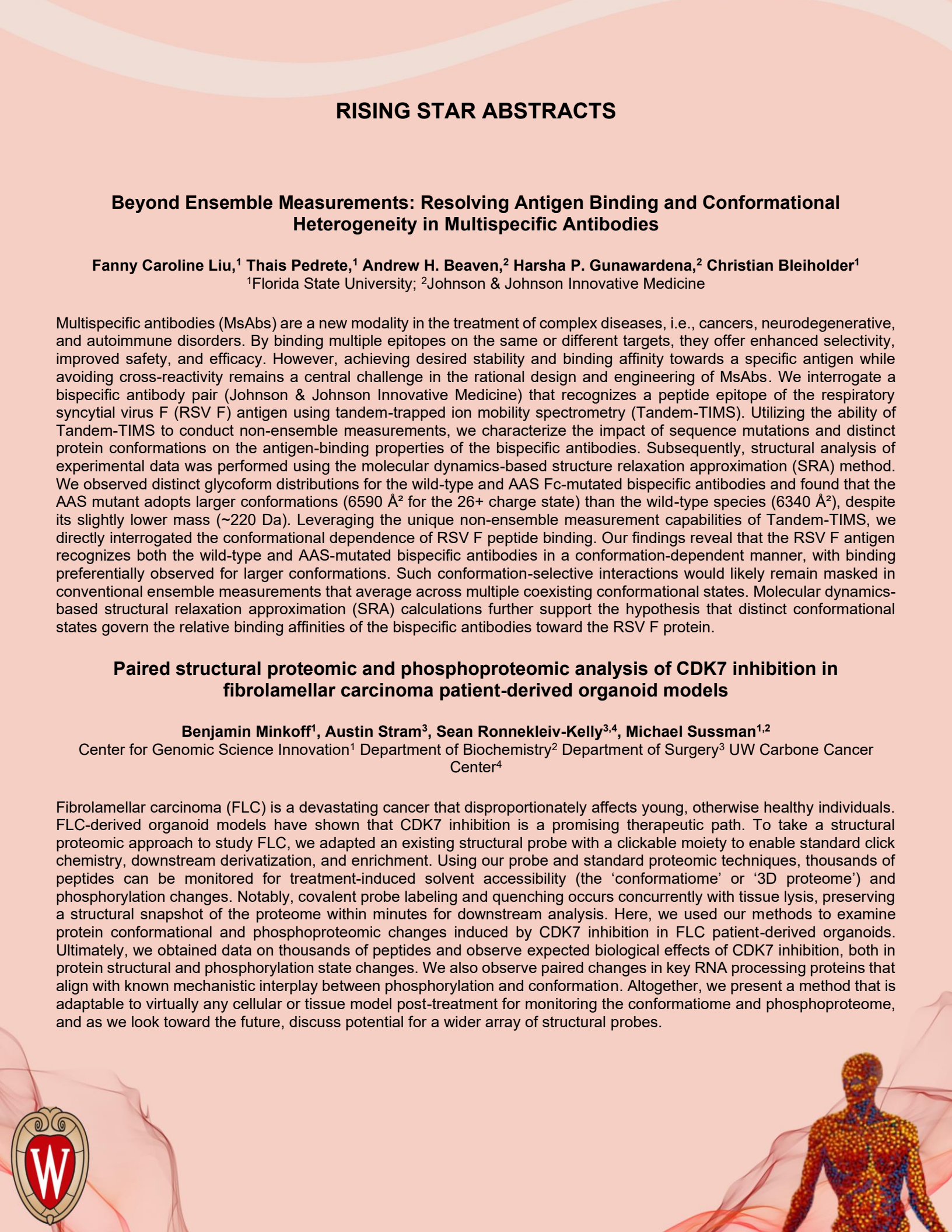
Fibrolamellar carcinoma (FLC) is a devastating cancer that disproportionately affects young, otherwise healthy individuals. FLC-derived organoid models have shown that CDK7 inhibition is a promising therapeutic path. To take a structural proteomic approach to study FLC, we adapted an existing structural probe with a clickable moiety to enable standard click chemistry, downstream derivatization, and enrichment. Using our probe and standard proteomic techniques, thousands of peptides can be monitored for treatment-induced solvent accessibility (the 'conformatiome' or '3D proteome') and phosphorylation changes. Notably, covalent probe labeling and quenching occurs concurrently with tissue lysis, preserving a structural snapshot of the proteome within minutes for downstream analysis. Here, we used our methods to examine protein conformational and phosphoproteomic changes induced by CDK7 inhibition in FLC patient-derived organoids. Ultimately, we obtained data on thousands of peptides and observe expected biological effects of CDK7 inhibition, both in protein structural and phosphorylation state changes. We also observe paired changes in key RNA processing proteins that align with known mechanistic interplay between phosphorylation and conformation. Altogether, we present a method that is adaptable to virtually any cellular or tissue model post-treatment for monitoring the conformatiome and phosphoproteome, and as we look toward the future, discuss potential for a wider array of structural probes.



Optimization of Oxylipin Quantification using Bio-Inert Chromatography Systems and Mass Spectrometry

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Oxylipins are a class of low abundance signaling lipids that regulate numerous facets of disease, including inflammation, platelet aggregation, and vascular stiffness. Oxylipins are produced from polyunsaturated fatty acids by a variety of enzymes including cyclooxygenase, lipoxygenase, and cytochrome p450[JM1.1] enzymes. Oxylipins are typically quantified with liquid chromatography mass spectrometry (LC-MS), and difficulties arise due to their low abundance and rapid degradation. In this study we optimize LC-MS methods to improve the quantification of oxylipins. Plasma samples were used as the matrix, and all samples were obtained from a healthy control. Oxylipins were extracted from plasma using solid-phase extraction. A reversed phase targeted LC/MS-MS method utilizing an Agilent 6495D QqQ was used for analysis. Ion funnel voltages were set at 50 or 100 volts. We compared both standard and bio-inert columns, as well as standard and bio-inert LC setups. Ion funnel parameters significantly alter detectable levels of oxylipins within LC-MS/MS. By decreasing voltages applied to ions inside the ion funnel, most oxylipin species had increased signal while peak quality was maintained. We also demonstrated that fully bio-inert setups quantify more compounds and show increased levels of some compounds, but less EpODE species, a kind of epoxide oxylipin. Next, we injected analytical grade alpha-linoleic acid, the direct precursor of EpODE and observed more formation of EpODEs within the instrumentation when using stainless steel columns. We demonstrate that oxylipins are significantly affected by pre-mass analyzer parameters such as ion funnel voltage. Our data also shows that oxylipin analysis benefits from fully bio-inert systems. The stainless-steel components of the column may also be contributing to epoxidation reactions of PUFAs, creating oxylipin species from PUFAs during analysis. Altogether, we demonstrate that oxylipin levels must continue to be analyzed and interpreted with care, precision, and caution.

Building a Quantitative Neuropeptidomic Toolkit

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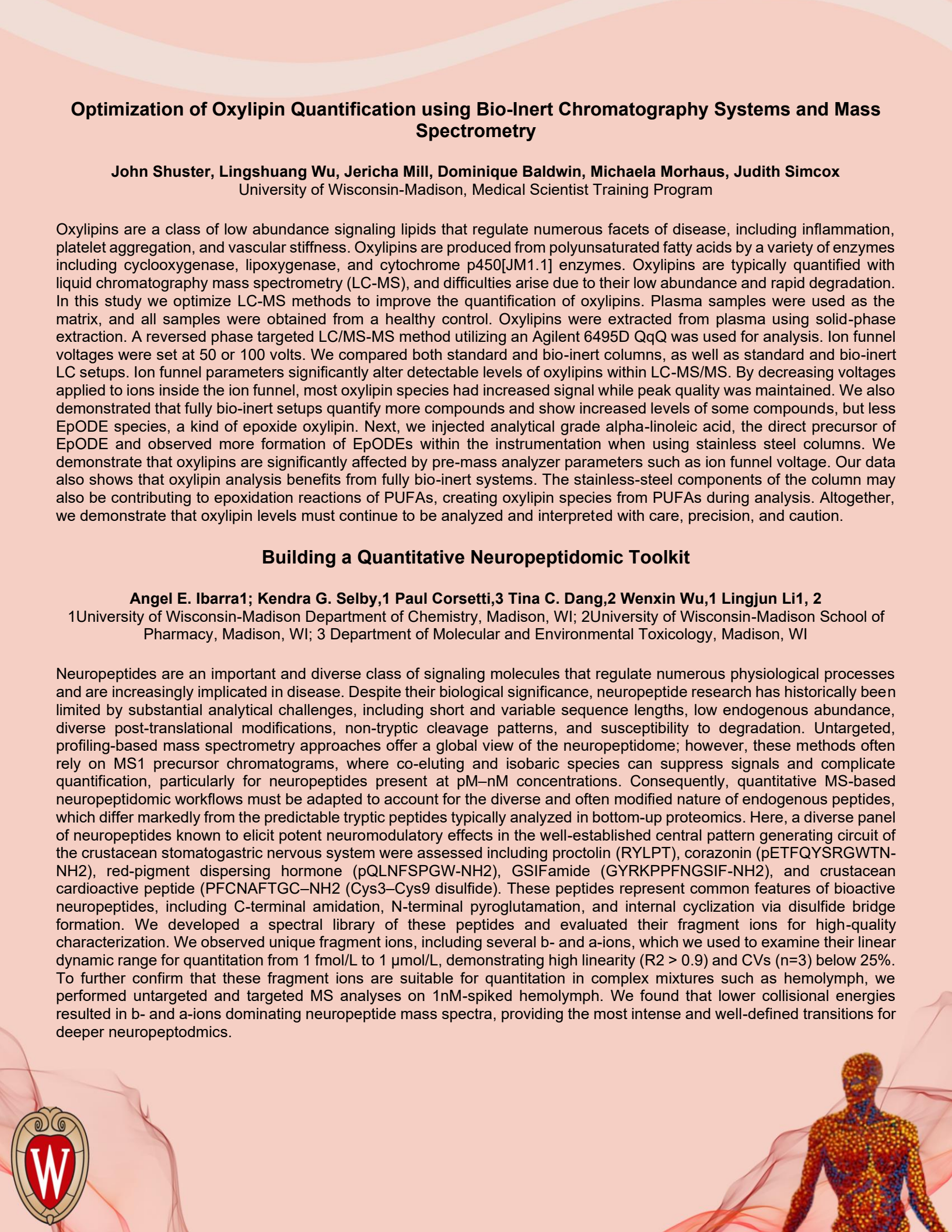
Neuropeptides are an important and diverse class of signaling molecules that regulate numerous physiological processes and are increasingly implicated in disease. Despite their biological significance, neuropeptide research has historically been limited by substantial analytical challenges, including short and variable sequence lengths, low endogenous abundance, diverse post-translational modifications, non-tryptic cleavage patterns, and susceptibility to degradation. Untargeted, profiling-based mass spectrometry approaches offer a global view of the neuropeptidome; however, these methods often rely on MS1 precursor chromatograms, where co-eluting and isobaric species can suppress signals and complicate quantification, particularly for neuropeptides present at pM–nM concentrations. Consequently, quantitative MS-based neuropeptidomic workflows must be adapted to account for the diverse and often modified nature of endogenous peptides, which differ markedly from the predictable tryptic peptides typically analyzed in bottom-up proteomics. Here, a diverse panel of neuropeptides known to elicit potent neuromodulatory effects in the well-established central pattern generating circuit of the crustacean stomatogastric nervous system were assessed including proctolin (RYLPT), corazonin (pETFQYSRGWTN-NH2), red-pigment dispersing hormone (pQLNFSPGW-NH2), GSIFamide (GYRKPPFNGSIF-NH2), and crustacean cardioactive peptide (PFCNAFTGC–NH2 (Cys3–Cys9 disulfide). These peptides represent common features of bioactive neuropeptides, including C-terminal amidation, N-terminal pyroglutamation, and internal cyclization via disulfide bridge formation. We developed a spectral library of these peptides and evaluated their fragment ions for high-quality characterization. We observed unique fragment ions, including several b- and a-ions, which we used to examine their linear dynamic range for quantitation from 1 fmol/L to 1 µmol/L, demonstrating high linearity (R2 > 0.9) and CVs (n=3) below 25%. To further confirm that these fragment ions are suitable for quantitation in complex mixtures such as hemolymph, we performed untargeted and targeted MS analyses on 1nM-spiked hemolymph. We found that lower collisional energies resulted in b- and a-ions dominating neuropeptide mass spectra, providing the most intense and well-defined transitions for deeper neuropeptodmics.



The Proteoform Landscape of HIV-1 Virions

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Human immunodeficiency virus type 1 (HIV-1) maturation is a tightly regulated process that depends on proteolytic processing of the Gag polyprotein and post-translational modifications (PTMs) that influence viral assembly, budding, and infectivity. While bottom-up proteomics has identified numerous viral and host PTMs, the intact proteoforms that define mature virions remain incompletely characterized. We have leveraged state-of-the-art proteoform analysis to fully characterizing HIV-1 virions at the proteoform-level including incorporation of PTMs identified by deep multiprotease bottom-up proteomics and the use of multiple fragmentation methods with top-down proteomics to generate Class 1 proteoform identifications. Across three complementary top-down search engines, MetaMorpheus, TopPIC, and Proteome Discoverer, we identified 3,645 proteoforms spanning both HIV-1 proteins and packaged host factors, including Cyclophilin A. These proteoforms revealed diverse combinations of PTMs and proteolytic truncations. PTMs were detected throughout all Gag domains in the bottom-up dataset, with many occurring at sufficient occupancy to be observed as distinct intact proteoforms in the top-down analysis, especially methylated and phosphorylated proteoforms. Proteolytic truncations confirmed previously proposed processing events while uncovering novel sites of regulation, including an internal truncation and sequential trimming of HIV-1 p6 separating hydrophilic and hydrophobic regions involved in host-factor recruitment, as well as cleavage of the host translation regulator PABPC1. To investigate proteoforms associated with viral maturation, virions were generated in the presence of the maturation inhibitor Bevirimat or the capsid inhibitor Lenacapavir. Relative quantification revealed that Lenacapavir, the more effective therapeutic, induced broader proteoform perturbations than Bevirimat. Complementary peptidoform analysis further identified altered phosphorylation at capsid residue S281, implicated in uncoating, and Gag residue S499, associated with viral budding and Vpr recruitment. These findings establish the first comprehensive proteoform-level atlas of mature HIV-1 virions and demonstrate how integrated top-down and bottom-up proteomics can reveal coordinated PTM and proteolytic regulation underlying HIV-1 maturation and antiviral drug response.

Proteome-wide QTL mapping enables gene-protein-phenotype network construction in a genetically diverse MASLD mouse model

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Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD) is a heritable condition, affecting nearly 40% of adults worldwide, whose molecular mechanisms remain poorly defined. Here, we present the largest tissue-level proteomic quantitative trait loci (pQTL) mapping study to date, leveraging a genetically diverse mouse cohort to decode the genomic architecture underlying liver protein abundance and MASLD susceptibility. By integrating proteomic and genomic data to identify candidate genetic markers correlating to the abundance of individual proteins, pQTL mapping informs on the mechanistic underpinnings of heritable diseases. We profiled the liver proteomes of 444 genetically and metabolically diverse mice with high quantitative precision, identifying over 12,000 unique protein groups across the cohort. Integrating these data with genomes and robust metabolic phenotyping, we mapped over 4000 genomic loci to the abundance of at least one protein. Over 85% of these associations are trans-acting, implicating widespread distal regulatory networks, and revealing genomic hotspots of coordinated protein regulation. By overlaying pQTL networks with metabolic trait correlations, we identified putative causal genes and protein networks underlying MASLD susceptibility. Among these, we highlight the E3 ubiquitin ligase UBR1 as a candidate determinant of hepatic steatosis susceptibility, correlated with multiple established drivers of metabolic dysfunction. To assess translational relevance, we cross-validated these findings in a cohort of human liver samples spanning the spectrum of MASLD severity, confirming UBR1's role and identifying candidate substrates of UBR1-mediated ubiquitination. This multi-omics framework establishes a powerful platform for mechanistic hypothesis generation and lays the groundwork for genomic marker-based disease subtype stratification in MASLD.

Quantitative Neuropeptidomics Reveals Spinal Cord Neurochemical Signatures of Disease-Modifying Osteoarthritis Drugs

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Osteoarthritis (OA) is the most prevalent arthritic condition in the United States with chronic pain mechanisms under investigation. Disease-modifying osteoarthritis drugs (DMOADs) aim to intervene in biological processes driving OA progression rather than merely mitigating symptoms. Despite these efforts, no FDA-approved DMOADs or effective pharmacological strategies offer long-term relief from OA pain. This study applied quantitative neuropeptidomics to characterize DMOAD-induced alterations in the rat spinal cord peptidome. Fifty-one rats were assigned to 5 groups, including Naïve, OA-Placebo, OA-HA (hyaluronic acid), OA-BB (candidate bottlebrush polymer), or OA-BBHA (BB and HA combination), and sacrificed at day (D) 42 (n = 4-5/group) and D84 (n = 5-6/group). Spinal cord neuropeptides were extracted, spiked with isotopically labeled bradykinin, and analyzed via Thermo Orbitrap Fusion Lumos. Data was searched against a rat prohormone database using PEAKS Studio v.13.1. and filtered at 1% FDR. Nearly 2,000 endogenous peptides were identified and 700 were quantifiable. GO analysis confirmed bioactive secretome enrichment, while KEGG pathways suggested a neuro-immune crosstalk in central sensitization. Global PCA separated Naive and OA-Placebo animals at D42 and D84, indicating the spinal neuropeptidome remodeling during OA progression. Treatment-specific effects on the spinal neuropeptidome were evident at the functional peptide category level. OA-BB showed broad neuropeptide downregulation versus OA-Placebo at D42 and converged toward the Naive profile at D84, consistent with reduced pain sensitivity observed behaviorally. HA induced marked early neuropeptide changes that were not sustained to D84, paralleling its shorter-lived structural benefits. Notably, BBHA changes were largely restricted to several neuropeptides at later stages, suggesting attenuation of BB-related neuropeptidomic effects when combined with HA. These findings highlight neuropeptidomics as a valuable tool to provide molecular insights into how emerging DMOADs modulate central pain pathways in a drug- and time-dependent manner, extending beyond local joint preservation to long-term stabilization of the spinal nociceptive environment.

Resolving the Complex Human Cardiac Proteome by Gel-Based Prefractionation and Top-Down Proteomics

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Cardiac contraction and signaling rely on sophisticated regulation of proteins within the sarcomere, sarcoplasmic reticulum, and mitochondria. The functions of these proteins are governed by post-translational modifications (PTMs), sequence variation, and structural organization. Mass spectrometry (MS)-based top-down proteomics provides a bird's-eye view of the proteoform landscape. However, comprehensive characterization of the cardiac proteoforms remains challenging due to sample complexity, proteoform heterogeneity, large molecular sizes, and the hydrophobic nature of many cardiac proteins. Here, we report a strategy that integrates gel-based prefractionation with top-down proteomics to resolve these challenges and facilitate proteoform characterization directly from human heart tissues. The gel-based prefractionation, adapted from Passively Eluting Proteins from Polyacrylamide gels as Intact species for MS (PEPPI-MS) workflow, separates intact proteins by polyacrylamide gel electrophoresis followed by protein recovery from gels, providing an orthogonal separation dimension prior to LC-MS. Our platform enabled the detection of multiple proteoforms larger than 50 kDa, including two large multidomain proteins, cardiac myosin binding protein C (cMyBP-C, 141 kDa), a key regulator of sarcomere organization and contractility, and myosin heavy chain (MyHC, 223 kDa), the primary motor protein in thick filaments. We also detected phospholamban (PLN), a critical transmembrane regulator of calcium handling. By omitting heating during gel separation, we preserved the pentameric assembly of PLN (detected at the 25–35 kDa fraction), yielding the first characterization of PLN proteoforms within its oligomeric state, including mono-phosphorylated, bis-phosphorylated, and palmitoylated forms. Furthermore, this strategy enabled the first-time detection of a highly heterogeneous protein species comprising at least 15 proteoforms. Using sequence tag-based de novo sequencing, this species was identified as calsequestrin-2 (CASQ2), revealing a complex landscape of glyco- and phosphoproteoforms. Overall, this gel-based prefractionation and top-down MS workflow provides a robust platform for comprehensive proteoform profiling in cardiac biology.

Integrated Lipidomics and Subcellular Proteomics Reveal Trans Fat-Induced Hepatic Organelle Remodeling

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Dietary trans fatty acids are strongly associated with cardiovascular disease, yet the underlying hepatic mechanisms remain unclear. To address this, human (HepG2) and mouse (Hepa1-6) hepatoma cells were exposed to BSA, 100 μM BSA-oleic acid (oleate, cis), or BSA-elaidic acid (elaidate, trans) for 48 hours and analyzed using untargeted lipidomics, subcellular proteomics, imaging, and biochemical assays. Untargeted lipidomics quantified 1,369 lipid species and revealed extensive trans-fat-induced mitochondrial remodeling. Compared with the oleate, elaidate exposure resulted in a 24.7% depletion of total C18:1-containing cardiolipins (n=3, $p<0.0001$), a major phospholipid class in the mitochondrial inner-membrane. These changes were accompanied by a 31.4% increase in mitochondrial superoxide levels (n=5, p=0.0135) and a 57% reduction in mitochondrial membrane potential (n=5, $p<0.0001$), indicating impaired mitochondrial integrity and bioenergetic dysfunction. Subcellular proteomics quantified 5,025 proteins across the cytosolic, ER/PM, and mitochondrial fractions. Compared with the oleate, elaidate exposure broadly suppressed OXPHOS/TCA proteins across fractions, with ER/PM showing the strongest depletion (KEGG NES=−2.8, padj=5e-17). MitoCarta3.0 marker quantification revealed ~28% less mitochondrial mass deposited into ER/PM fraction, indicating lost mitochondria-ER contacts. Furthermore, elaidate induced a pro-fission mitochondrial signature. Together, these findings suggest that trans-fat exposure disrupts organelle interactions and mitochondrial fusion-fission balance, consistent with the membrane-rigidifying effects of trans-fat incorporation. Organelle-specific alterations in hepatic lipid metabolism were another remarkable feature. Elaidate strongly induced SREBP-driven lipogenic and cholesterogenic programs, consistent with the accumulation of cholesteryl-esters observed in lipidomics. Gene set enrichment analysis further indicated a shift from mitochondrial lipid utilization toward peroxisomal β-oxidation and apolipoprotein export. Neutral lipid staining revealed a 36.8% decrease in lipid droplet abundance (n=3, p=0.0030). Together, these findings indicated that trans fats reprogram hepatic lipid storage and utilization, potentially redirecting neutral lipid flux toward secretion pathways. Overall, our findings support a model in which trans-fat treatment shifts hepatic metabolism toward a pro-atherogenic state.

Integrated Multiomics Enabled by Sequential Extraction for Comprehensive Molecular Profiling of Small Extracellular Vesicles

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Small extracellular vesicles (sEVs), including exosomes, are a subset of extracellular vesicles with a diameter less than 200 nm. In cancer, sEVs promote metastasis and immunosuppression, and in cardiovascular disease, sEVs are integral to apoptosis and angiogenesis. Isolation and characterization of sEVs from bodily fluids and/or cell media permits direct analysis of disease-derived material while overcoming the classic dynamic range problem posed by these media. Consequently, sEVs show great promise for biomarker discovery. However, comprehensive characterization of sEVs is complicated by the extremely limited material available in sEVs. To address this challenge, we present an integrated mass spectrometry-based multiomics platform for simultaneous characterization of lipids, metabolites, and proteins from a single sEV sample enabled by sequential extraction, maximizing sample utilization. To enhance molecular coverage and analytical depth, the platform combines iterative tandem mass spectrometry for improved small-molecule fragmentation and nanoflow proteomics with data-independent acquisition. We achieve deep and reproducible multiomic characterization of proteins, lipids, and metabolites using 10 million sEVs. We further demonstrate the compatibility of our multiomics platform with sEVs isolated from plasma by ultracentrifugation, size exclusion chromatography with ultrafiltration, and polymer precipitation, revealing purification-dependent differences in molecular profiles associated with trade-offs in yield and purity of sEVs. By enabling integrated multiomics from the same sample, this strategy addresses a key challenge in low-input sEV analysis and establishes a robust analytical foundation for synergistic biomarker discovery and therapeutic applications.

POSTER SESSION ABSTRACTS

Poster #1

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

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The dermal extracellular matrix (ECM) proteome offers structural support and regulates cellular function. Skin protects and defends against disease, and dysregulation of the skin's ECM is linked to various conditions from wrinkling to melanoma. The skin ECM is a promising biomarker source due to its abundance of structural and secreted proteins involved in aging and disease. Our lab developed a single-step Azo-enabled extraction to recover ECM proteins from lung tissue for high-throughput analysis. Adapting this method for skin tissue, we observed differing ECM protein distribution across the chest and abdominal regions of young (>30 years) and aged (50+ years) individuals, suggesting alternative aging mechanisms in response to regional environmental exposure. Human skin samples from adult donors without skin conditions were obtained from chest and abdominal incisions. The adipose-rich hypodermis was removed, and the remaining epidermis/dermis was cryo-pulverized. Proteins were extracted using a modified, one-pot Azo extraction method, digested with trypsin, and analyzed on a Bruker timsTOF Pro operating in parallel accumulation-serial fragmentation combined with data-independent acquisition (diaPASEF) mode. We identified 3133 proteins in young chest samples, 2750 proteins in young abdominal samples, 3495 proteins in aged chest samples, and 3240 proteins in aged abdominal samples (n=4 per group). 223 ECM proteins were identified across the cohort, several of which were differentially expressed across both age and tissue region. Considering all differentially expressed proteins, comparisons across regions within age groups showed changes in proteins associated with lipid and small molecule metabolism, while comparisons across age ranges within regions showed changes in metabolic and transport mechanisms. In conclusion, our modified Azo extraction effectively recovers ECM proteins and reveals regional differences in ECM composition, which may be implicated in changes to aging pathways in response to solar ultraviolet (UV) radiation exposure.

Poster #2

Comparative Analysis of Human and Mouse Eosinophil Proteomes

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Eosinophils play roles in multiple human diseases, including asthma. Mouse models are used to study these roles. Similarities and differences in the two species' eosinophil proteomes obtained by the same methods have not been directly compared. We have generated human and mouse proteomic datasets. Analyses include using the MGI ortholog database and visualization of abundances in selected protein families. Label-free baseline proteomes showed a strong correlation of human to mouse one-to-one orthologs (r=0.67, p=2e-16). However, a number of abundant human proteins, including CLC (galectin-10), did not have an ortholog detected in mouse. Within protein families, comparisons indicate that ITGA5, -AV, and -B5 integrins were detected in mouse but not human eosinophils, and LGALS12 (galectin-12) was detected in human but not in mouse eosinophils. Ongoing/future analyses will also include our tandem mass tag datasets, comparisons of eosinophil activation signatures, and bioinformatic analyses of proteins differentially expressed and/or activated between the species. We aim to offer a framework for an improved understanding of the applicability of results on mouse eosinophils and models to human disease.

Poster #3

Single-shot Multi-omic Analysis with an Intelligent Ion Source and Micropillar Array Columns

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Previously we described a nanoflow-based multi-omic single-shot technology (nMOST) that enables simultaneous proteomic and lipidomic profiling across diverse biological samples, as well as cross-ome correlation analysis within a single LC–MS run. However, incorporation of isopropanol into the mobile phase for lipid separation generates column backpressures exceeding 1,200 bar when using conventional C18 columns, approaching the pressure limits of standard LC systems and potentially compromising long-term instrument performance. Here, we leverage Thermo Scientific OptiSpray Technology in combination with OptiSpray µPAC Neo cartridges to substantially reduce chromatographic backpressure while maintaining robustness and reproducibility. Using HEK293 cells, we evaluated flow rates of 200, 250, and 300 nL/min at column temperatures of 50 and 60°C. Comparable peptide identifications were obtained across all conditions, while the highest lipid coverage was achieved at 300 nL/min and 60°C with 750 annotated lipids. Peptide and lipid chromatographic performance, including full width at half maximum (FWHM) and peak capacity, remained comparable across all conditions. We benchmarked the optimized workflow using mouse brain, C. elegans, yeast, and plasma, identifying approximately 300–5,000 proteins and 400–1,000 lipids in a single run. Median coefficients of variation were ~7% for proteins and 15–29% for lipids, demonstrating robust quantitative reproducibility. To demonstrate biological utility, we applied nMOST to quantify protein and lipid alterations in drug-sensitive and drug-resistant Candida auris following fluconazole treatment. Samples were collected at 0, 30 min, 1 h, and 2 h after treatment. Across all time points, 171 proteins and 41 lipids were significantly altered between the sensitive and resistant isolates. Proteins involved in ergosterol biosynthesis were consistently more abundant in the resistant isolate, consistent with established mechanisms of fluconazole resistance. This optimized nMOST workflow enables robust, reproducible, single-run multi-omic profiling and provides an effective platform for integrated proteomic and lipidomic analyses across diverse biological samples.

Poster #4

High-Sensitivity Top-Down Proteomics of Phospholamban Proteoforms from Biopsy-Equivalent Cardiac Tissue

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Phospholamban (PLN) is a transmembrane protein whose genetic variants are directly associated with dilated cardiomyopathy. PLN is dynamically regulated by post-translational modifications and serves as a critical regulator of cardiac relaxation by controlling calcium uptake into the sarcoplasmic reticulum. Although we previously established a mass spectrometry (MS)-based top-down proteomics platform for comprehensive characterization of PLN proteoforms, the amount of tissue required limited its application to clinically relevant biopsy samples. Here, we report a ten-fold improvement in analytical sensitivity that enables robust extraction and characterization of PLN proteoforms from only 1 mg of human cardiac tissue. Using a simplified Azo-based extraction, 1 mg cardiac tissue samples were processed and compared with our previously established 10 mg extraction method, which required additional sample preparation steps. Following reversed-phase LC-MS/MS analysis on a Bruker Impact II instrument, PLN proteoforms were identified, characterized, and quantified. PLN was consistently extracted with excellent reproducibility with both methods. MS1 analysis enabled identification and quantification of all phosphorylated and palmitoylated PLN proteoforms. The total phosphorylation level was 1.89 ± 0.03 Pi/mol protein for the 1 mg samples and 1.91 ± 0.02 Pi/mol protein for the 10 mg samples. Palmitoylation levels were also consistent between both extraction workflows. Targeted tandem MS using collision-induced dissociation (CID) produced comparable fragmentation patterns for both sample preparation methods, confirming phosphorylation at Ser16 and Thr17 and palmitoylation at Cys36. The bis-phosphorylated proteoform achieved 71.2% sequence coverage with both workflows, demonstrating that reducing the tissue input did not compromise PTM localization or proteoform characterization. Together, these results establish that biopsy-scale tissue samples are sufficient for quantitative proteoform analysis and PTM mapping, advancing the clinical applicability of top-down proteomics for cardiovascular disease. Future

work will extend this platform to native top-down MS analysis of the PLN pentamer to elucidate its structural and functional roles in disease.

Poster #5

Accessible IMAC Strategies for Discovery of Endogenous Phosphorylated Neuropeptides in Crustacean Neural Tissues and Hemolymph

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Neuropeptides regulate diverse physiological processes, and PTMs such as phosphorylation can alter their function. LC-MS/MS analysis in the American lobster (Homarus americanus) leverages simple neurocircuitry and rich peptidome to study phosphorylation-dependent signaling. Because commercial IMAC columns vary in cost and accessibility, we optimized a low-cost enrichment strategy for endogenous, size-excluded neuropeptides rather than tryptic digests. Lobster neural tissues and hemolymph were processed by sonication, cellulose filtration, 10 kDa fractionation, desalting, and phosphopeptide enrichment using commercial Fe-IMAC columns. Laboratory Ti-IMAC tips were prepared by phosphorylating cotton fibers with urea/phosphoric acid, thermal activation, titanium sulfate loading, tip packing, and TFA equilibration. Standards were validated by MALDI on a Bruker rapifleX. Lobster samples were analyzed on a ThermoIn-house Ti-IMAC cotton tips were validated using phosphorylated and non-phosphorylated neuropeptide standards. A seven-peptide mixture containing 25% phosphopeptides showed reduced total peptide concentration after enrichment, consistent with selective retention. MALDI analysis showed depletion of non-phosphorylated signals, while phosphorylated sequences remained detectable with increased relative intensities. Additional 17-peptide mixtures containing 10% or 5% phosphopeptides produced similar results, supporting selective Ti-IMAC enrichment. Commercial Fe-IMAC enrichment of thoracic ganglion and hemolymph neuropeptides identified 20 unique phosphopeptides from pooled thoracic ganglion samples at 1% FDR, including seven high-confidence assignments with clear sequence identity, phosphorylation number/localization, and serine/threonine modification. Hemolymph analysis yielded 11 phosphopeptides, including two high-confidence candidates. Identified phosphopeptides spanned CPRPs, RYamides, RFamides, and allatostatin A-type peptides. CPRPs were the most common phosphorylated family, suggesting phosphorylation-dependent regulation. Many sequences appeared as truncated or modified forms of known crustacean neuropeptides and were often detected only as phosphorylated variants. Future zirconium/hafnium IMAC platforms may expand coverage and enable comparison with commercial systems across lobster/crustacean tissues.

Poster #6

Rapid complexomics with native gel-mass spectrometry

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The key to accessible precision medicine lies in omics-level understanding of the molecular mechanisms of disease at the levels of tissues, patients, and populations. Protein complexes are key to these mechanisms; proteins form complexes to drive a range of critical biological functions, including gene regulation and metabolism. Blue native polyacrylamide gel electrophoresis (BN-PAGE) is valuable for its ability to separate protein complexes while preserving native protein structure and binding partners. Previously used to isolate specific complexes, BN-PAGE followed by mass spectrometry (BN-PAGE-MS) is growing to be a standard technique in complexomics, the study of all complexes in a sample. After BN-PAGE, lanes are separated and sliced widthwise into fractions that are processed for LC-MS/MS analysis. Proteins found in the same fraction potentially co-migrate in the gel as part of the same complex(es), with confidence increasing with fractionation resolution. Analysing the differences in complexation between samples perturbed by putative complex-remodelling factors, such as environmental stressors or PTM-modifying enzymes, yields mechanistic insights into the assembly of a sample's complexes. BN-PAGE-MS is, by nature, low-throughput. From PAGE to fractionation by hand-slicing to sequential analysis of each fraction by LC-MS/MS, the analysis of one sample can require days of effort. However, this is inefficient and impractical for diagnostics or large-scale assays. To adapt BN-PAGE-MS for higher throughput, we have used bovine heart

mitochondria, the BN-PAGE standard, to optimise 1) BN-PAGE resolution; 2) precision and reproducibility of fractionation with a custom-made device; 3) rate of LC-MS/MS analysis, streamlining LC separation; and 4) computational analysis, developing pipelines to identify protein bovine-human homology and biological functions. With these methods, we capture known mitochondrial complexes and potentially novel interactions. Currently, we are working to automate gel processing and optimise BN-PAGE for human and non-mitochondrial samples, aiming to develop BN-PAGE-MS for reliable and rapid complexomics.

Poster #7

Deep Proteoform Mapping in Human Heart Enabled by Top-Down Proteomics with Online Multidimensional Liquid Chromatography and Machine Learning

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Top-down proteomics (TDP) has become an indispensable tool for the comprehensive characterization of proteoforms, including isoforms and post-translational modifications (PTMs). However, the dynamic range and complexity of the human proteome pose major challenges, limiting coverage achievable using conventional 1D liquid chromatography (LC). To address these challenges, we have developed online multidimensional LC (MDLC) methods for native and denaturing TDP. These methods substantially increase the separation efficacy, enabling deeper coverage and characterization of proteoforms. Denatured TDP is the gold-standard technique for proteoform characterization including PTM localization. In denatured mode, we have developed an MDLC method coupling size exclusion chromatography (SEC) with reversed-phase liquid chromatography (RPLC), yielding significant gains in peak capacity and enabled detection of large proteoforms (up to 223 kDa). Valve-based dimensional coupling automates this approach to facilitate analysis of larger sample cohorts. To analyze this dataset, we developed a novel machine learning-based rescoring strategy which identified 33% more unique proteins and 85% more unique proteoforms compared to a conventional top-down search. This approach was able to identify large proteoforms from sparse fragmentation evidence. Native top-down mass spectrometry (nTDMS) can capture the highest levels of protein structural complexity, including non-covalent interactions. However, the requirements of moderate temperatures, aqueous mobile phases, and near-neutral pH restrict the use of online LC, limiting nTDMS analyses to simple samples. We have developed online separation methods that enable detection of endogenous protein complexes from complex extracts. Our SEC-IEC method detected 133 native proteoforms and endogenous protein complexes (up to 350 kDa) from human heart tissue in less than 2 h. A multistage nTDMS approach enabled comprehensive structural characterization within the chromatographic time scale, capturing intact noncovalent complexes, released subunits/cofactors, and backbone fragments. To further increase separation power in native proteomics, we are currently developing a 3D-LC method by incorporating hydrophobic interaction chromatography (HIC) to SEC-IEC.

Poster #8

A Reference Compendium of Human Cardiac Histone Proteoforms

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In this study, we developed a streamlined top-down proteomics method to decipher the “histone code”. In a single LC-MS run, we achieved baseline separation of all four core histones (H2A, H2B, H3, and H4) together with linker H1 directly from human myocardium. By selectively enriching histones while depleting interfering proteins, our workflow boosts histone signal intensity and enables broad coverage. From the same LC–MS run, we also achieved proteoform-level quantification of histone-variant composition and PTM stoichiometry. Importantly, top-down proteomics preserves PTM connectivity on intact

histones, revealing combinatorial histone codes at the chromatographic scale. Our results show that combinatorial PTMs—not individual marks—define histone proteoform states in the human heart. We next applied top-down tandem MS (MS/MS) to characterize intact histone proteoforms. Electron-capture dissociation (ECD)-MS/MS enabled PTM site localization and direct resolution of combinatorial PTMs on intact H3 and H4 proteoforms. For H4, methylation was mapped to R3 (me1/me2) and K20 (me1/me2/me3), with acetylation assigned to the N-terminus (S1). Importantly, we resolved two isobaric proteoforms that share the same intact mass but differ in acetylation site, carrying acetyl groups at either K12 or K16. For H3, we resolved multiple N-terminal methylation states spanning ~4–7 total methylations. In parallel, top-down fragmentation provided diagnostic ions to distinguish and quantify closely related histone sequence variants that differ by only a few residues, including highly homologous H2B isoforms (H2B1C/1O/1J) that can collapse into shared MS1 features. Notably, the most unexpected was the detection of multiple truncated macroH2A proteoforms (~22kDa) in human myocardium by top-down proteomics, retaining the H2A-like domain and H2A-typical acetylation/phosphorylation. Overall, in a single LC–MS analysis, we integrate variant separation and quantification, PTM stoichiometry, and isoform discrimination, overcoming limitations of peptide-centric methods. This streamlined workflow is broadly applicable for histone proteoform analysis across diverse human tissues and biological systems.

Poster #9

Leptin in Patients with Acute Leukemia: Associations with Body Composition and Systemic Inflammation

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Background: Leptin is an adipokine involved in energy homeostasis, immune regulation, and inflammatory responses. In patients with acute leukemia, the interplay between leptin, body composition, and systemic inflammation remains poorly understood. This study investigated the association of circulating leptin concentrations with body composition, resting energy expenditure, and inflammatory status. Methods: This cross-sectional study included patients with acute leukemia. Body composition was assessed by bioimpedance spectroscopy, and resting energy expenditure was measured by indirect calorimetry. Associations between leptin and continuous variables were evaluated using Spearman's rank correlation, while group comparisons were performed using the Mann–Whitney U test. Statistical significance was set at $p < 0.05$. Results: Ten participants were observed. Leptin concentrations were not significantly associated with Body Mass Index, Fat Mass , Fat free mass, phase angle, Resting energy expenditure, albumin, age, or CRP levels (all $p > 0.05$). However, patients with elevated CRP showed higher leptin concentrations than those with normal CRP (13.88 [9.33–17.66] vs. 4.13 [3.31–7.21] ng/mL), although the difference did not reach statistical significance ($p = 0.095$). A similar nonsignificant trend was observed according to sex ($p = 0.114$). Conclusions: Although leptin was not significantly associated with body composition or resting energy expenditure, patients with higher inflammatory status tended to exhibit increased circulating leptin concentrations. The absence of clear relationships with body composition, together with the higher leptin levels observed in individuals with elevated CRP, suggests that, in this patients with acute leukemia, leptin may be more closely linked to systemic inflammation than to adipose tissue mass alone. Larger studies are warranted to clarify the role of leptin as a potential inflammatory biomarker in acute leukemia.

Poster #10

A Bottom-Up Proteomics Workflow for Characterizing Exercise-Associated Changes in the Extracellular Vesicle Proteome During Aging

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Aging is associated with progressive cellular dysfunction characterized by loss of proteostasis, protein dysregulation, and cellular senescence. Extracellular vesicles (EVs), small membrane-bound particles released by cells, carry proteins that are reflective of their parent's cellular state. They have emerged as a promising source of biomarkers for aging and age-related physiological adaptations. While exercise is known to attenuate several indicators of aging, its influence on the EV proteome

remains incompletely understood. To characterize exercise-associated changes in EV protein cargo, we implemented a bottom-up proteomics workflow employing nanoflow liquid chromatography coupled to trapped ion mobility spectrometry tandem mass spectrometry (nanoLC-timsTOF MS/MS) using a data-dependent acquisition (DDA) strategy. EVs were isolated by size exclusion chromatography (SEC) from young adult (4-month-old) mice and aged (24-month-old) mice that underwent a four-week intervention consisting of either voluntary exercise (wheel running) or sedentary conditions (locked wheel). Biological replicate numbers ranged from 4 to 7 animals per group. Following EV isolation, proteins were extracted, enzymatically digested, and purified using solid phase extraction prior to LC-MS/MS analysis. Protein identification and label-free quantification (LFQ) were performed using PEAKS Online with a 1% false discovery rate (FDR) threshold at the peptide and protein levels. This analytical workflow provided reproducible characterization of EV-associated proteins, with approximately 350 proteins identified on average per experimental group. Differential abundance analysis revealed proteins associated with senescence-related biological processes, including regulation of proteolysis and ion transport. Several proteins involved in these pathways exhibited reduced abundance in EVs from exercised aged mice compared with sedentary aged controls, suggesting that exercise may modulate EV protein cargo linked to cellular aging. These findings demonstrate the utility of high-sensitivity mass spectrometry-based proteomics for profiling EVs and provide insight into how exercise influences age-associated extracellular signaling.

Poster #11

Comparative Proteomic Analysis of Keratinocytes Expressing Individual Human Versus Mouse Papillomavirus Oncogenes

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Liquid chromatography-mass spectrometry (LC-MS)-based proteomics provide a powerful approach to understand global signaling and protein abundance changes following the introduction of viral oncogenes into epithelial cells. Here, we are using LC-MS based approaches as means to dissect papillomavirus oncogene functions in vitro. Prototypic high risk mucosal human papillomavirus type 16 (HPV-16) and prototypic cutaneous HPV8 E6 or E7 viral proteins, as well as mouse papillomavirus type 1 (MmuPV1) E6 or E7 viral proteins, were expressed in human or mouse keratinocytes, respectively (matching virus to natural host species), and subjected to quantitative proteomic profiling. Downstream gene set enrichment analysis (GSEA) of differentially expressed proteins revealed pathways altered upon expression of viral oncoproteins, including cell cycle regulation, DNA damage response, metabolic regulation, and immune response. Furthermore, a functional comparison between human and mouse proteomic profiles at gene set level revealed both shared and divergent oncogenic properties across the different viral oncogenes. Notably, proteome-level pathway enrichment demonstrated greater similarity between MmuPV1 and HPV8 oncogene-driven profiles than with HPV-16, suggesting that cutaneous human papillomavirus and murine papillomavirus engage more overlapping behaviors regulating host response compared to high-risk mucosal HPV. This study will be further expanded through comparative phosphoproteomic analysis between the same sets of viral oncogene expressing keratinocytes to strengthen and refine those conclusions. Overall, this study helps clarify how different papillomavirus oncogenes rewire host cell pathways and supports understandings of the use of MmuPV1 as a relevant in vivo model for performing studies related to HPV-driven diseases.

Poster #12

Mapping Autophagy-Associated Lipid Remodeling at Single-Cell Resolution in C4 Maize Using Mass Spectrometry Imaging

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Nitrogen (N) is essential for plant growth, with up to 75% of cellular nitrogen allocated to chloroplast photosynthetic machinery. Under nitrogen limitation, plants recycle cellular components through intracellular degradation pathways, including chloroplast turnover. Although canonical autophagy contributes to this process, the viability of autophagy-deficient plants suggests that additional recycling mechanisms may also be involved. In C4 plants such as maize, mesophyll (M) and bundle sheath (BS) cells contain structurally and functionally distinct chloroplasts, providing an opportunity to examine cell-type-specific responses to nitrogen stress. Here, we use single-cell mass spectrometry imaging (MSI) to investigate lipid

remodeling associated with nitrogen deprivation in wild-type and autophagy-deficient maize. The second leaf from W22 wild-type and atg12-1 maize grown under nitrogen-depleted (0.15 mM NO_3^-) and control (15 mM NO_3^-) conditions was flash-frozen, embedded in 10% gelatin, and sectioned at 20 µm using CryoJane tape-assisted cryosectioning to preserve tissue morphology. On-tissue chemical derivatization with EDC followed by 4-APEBA was performed to enhance the detection of membrane-associated lipids. MSI data were acquired on a Bruker timsTOF fleX at 10 µm spatial resolution using DHB for positive-ion mode and NEDC for negative-ion mode. High-resolution optical images were co-registered with MSI data in SCiLS Lab, and regions of interest corresponding to upper and lower mesophyll and bundle sheath cells were manually defined based on C4 leaf anatomy. Preliminary results show a consistent increase in the signal intensity of several lipid species, including PG 38:5, PC 36:7, and PG 42:4, in bundle sheath cells under nitrogen-depleted conditions compared with controls. This enrichment is most pronounced for phosphatidylglycerol species, major components of chloroplast and thylakoid membranes, and is consistent with lipid remodeling associated with nitrogen stress. Ongoing analyses will compare wild-type and atg12-1 tissues to evaluate the contribution of autophagy to these cell-type-specific lipid changes.

Poster #13

Global Profiling of Deamidation Susceptibility and Isoaspartate Formation via PIMT-Mediated Labeling

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Deamidation is a ubiquitous non-enzymatic post-translational modification that influences protein stability, structure, and lifespan, yet the rules governing susceptibility across whole proteomes remain poorly defined. By converting isoaspartate (IsoAsp)—the primary product of deamidation—into a detectable mass shift, we can effectively map the deamidome and identify aging processes. We developed an enzymatic labeling method using protein L-isoaspartyl methyltransferase (PIMT) and data-independent acquisition (DIA) mass spectrometry, enabling global mapping of asparagine deamidation propensity. PIMT selectively methylates isoaspartate (IsoAsp)—the primary hydrolysis product of the succinimide intermediate by converting it into methylester and succinimide species with distinct and quantifiable mass shifts. HEK293 lysates were subjected to forced degradation conditions (pH 9.8, 55°C) to accelerate asparagine turnover, then tryptically digested and labeled our PIMT method prior to high-resolution DIA-MS analysis on an Orbitrap Astral, allowing us to quantify conversion at hundreds of sites. Method performance was benchmarked using synthetic peptide standards probing steric accessibility, achieving >98% labeling efficiency at accessible sites versus ~85% at a sterically hindered sequence, confirming the assay's sensitivity to local structural environment. Applying this workflow to native, forced-degradation, and non-labeled control HEK293 lysates enabled confident discrimination of endogenous from stress-induced IsoAsp. We identified over 1,000 unique endogenous IsoAsp sites and calculated site-specific occupancy rates spanning a broad dynamic range, distinguishing highly labile hotspots from resistant residues. By correlating occupancy with sequence motifs and local structural topology, we are constructing a global deamidation propensity atlas that extends classical single-protein decay kinetics to full proteome scale.

Poster #14

Modeling ER+ Breast Cancer Liver Metastasis and FGFR-Driven Resistance in Rats

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Estrogen receptor–positive (ER+) breast cancer accounts for over 70% of all breast cancers and is treated with endocrine therapy. However, approximately 50% of patients eventually relapse, and the five-year survival rate is 27% for metastatic ER+ disease. Endocrine therapy is often combined with CDK4/6 inhibitors, yet acquired resistance remains a significant clinical challenge. Current genetically engineered mouse models are predominantly ER-negative, limiting their utility for studying ER+ metastatic progression. To address this gap, we developed a novel ER+ metastatic breast cancer model in immunocompetent August Copenhagen Irish (ACI) rats. Estrogen implantation in female ACI rats induces ER+ mammary tumors at 100% incidence with luminal B profiles resembling human ER+ cancers. From these tumors, we derived MG-1, an estrogen-dependent cell line sensitive to fulvestrant and CDK4/6 inhibitors. Orthotopic MG-1 injections rarely produce

distant lesions, but intravenous injection efficiently yields lung, bone, lymph node, and liver metastases. Metastatic sublines show epithelial-to-mesenchymal transition signatures and increased resistance to endocrine and CDK4/6 therapies. Liver metastasis is an underexplored complication of ER+ breast cancer with no current preclinical models. MG-1 liver-derived sublines overexpress FGFR1 and amplify FGFR2, exhibit enhanced migratory and clonogenic capacity, increased liver metastasis in vivo, and resistance to fulvestrant and CDK4/6 inhibitors. Knockdown of FGFR1 or FGFR2 partially restores drug sensitivity and suppresses metastatic traits. Given that FDA-approved FGFR inhibitors exist, this MG-1/ACI model provides a robust platform to evaluate FGFR1/2 as liver-tropic drivers and test FGFR- and CDK4/6-targeted therapies to improve outcomes for metastatic ER+ breast cancer patients.

Poster #15

Label-Free Thermal Proteome Profiling to Identify Metabolite-Protein Interactions

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Thermal proteome profiling (TPP) is an LC-MS method used to screen protein-ligand interactions with small molecules of interest. By quantifying non-denatured proteins from a sample over different temperature points, thousands of melting curves can be fit across the proteome. When a cell lysate or live cells are treated with a molecule of interest, interacting proteins are identified by alterations in their melting temperatures. In the simplest case, non-covalent interactions with the molecule of interest will stabilize a protein, increasing its melting temperature. However, signaling pathways stimulated by the molecule of interest, can also cause melting temperature alterations in downstream proteins due to changes in protein complexation or post-translational modification. For example, TPP was recently applied to map the signaling pathways downstream of the GPCR melanocortin 3 receptor in response to different agonists in HEK293 cells. Typically, TPP experiments are quantified with isobaric tagging. Reporter ions from the tags are used to determine the abundance of a protein at all temperature points from the same MS/MS scan. However, with the development of faster scanning high-resolution, accurate mass instruments, label-free quantification has become more reliable using data-independent acquisition (DIA) methods. Applying this to TPP allows individual injections from each temperature point to yield consistent protein identifications and reliable quantitation for melting curve generation, without the additional effort required by isobaric tagging. We applied label-free TPP to identify proteins affected by citrate in cultured mouse adipocyte lysates. Over 108 injections at 12 temperature points and two citrate concentrations, we quantified 8400 proteins and fit reliable melting curves ($R2 > 0.8$, lower plateau < 0.3) to 6400. Across treatment concentrations, we identified 135 proteins with significantly altered stability upon citrate treatment, including known interactors. Gene set enrichment analysis revealed pathways with multiple proteins affected by treatment, such as ROS pathways and adipogenesis.

Poster #16

Triple-guard chemoproteomic platform for reliable citrullination mapping with staggered DIA and DDA detection in Alzheimer's disease

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Citrullination, the enzymatic conversion of arginine to citrulline, remains difficult to profile because of the mass similarity of these amino acids. While some chemical probes have been devised, they are still prone to a high false-discovery rate (FDR). We developed a triple-guard chemoproteomic platform coupling a citrulline-selective probe, Bz_2-PYA, with data-dependent acquisition (DDA) and staggered data-independent acquisition (DIA) detection. Bz_2-PYA features enhanced ionization, excellent water solubility, and an alkyne moiety enabling biotinylation by click reaction. Three organically integrated guards address the scientific challenge of high FDR in citrullination identification. Bz_2-PYA enforces isotopic 1:1 MS1 doublets by premixing H_6/D_6 probes, discarding peptides lacking the dual signal (Guard I). An irreversible citrulline-selective reaction with stringent ammonia elution ensures the removal of residual reversible adducts (Guard II). Finally, by using cobalt(II) to mask arginine, we prevent off-target labeling (Guard III). The method was meticulously validated at the amino acid, peptide, and protein levels. Staggered DIA, featuring good reproducibility and higher citrullination identification

numbers, ensures that the isotopic precursor ion can be covered in the same fragmentation window, and the DIA data are deconvoluted via a spectral library established from massive DDA data. On average, serum from Alzheimer's disease patients exhibited significantly higher levels of citrullination than that from healthy donors, with 274 versus 170 sites identified per sample, respectively. These citrullinated proteins are enriched in immune system and lipid regulation pathways, underscoring their strong association with AD.

Poster #17

High-Throughput Relative Quantitation of Phosphatidylcholines via 4-plex Isobaric DiLeuEN Labeling

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Phosphatidylcholines (PCs) are essential membrane components and important disease biomarkers. However, their precise quantitation at the individual species level remains challenging due to the diversity of fatty acyl chain compositions and linkage types. Moreover, high-plex isobaric-based relative quantification is hindered by the lack of reactive functional groups and interference from alkali metal adducts. To address these challenges, we developed a high-throughput and high-sensitivity platform using 4-plex N,N-dimethyl leucine-derivatized ethylenediamine (DiLeuEN) isobaric tags. By targeting the phosphate headgroup, this approach enables simultaneous relative quantification of PC species from four biological samples in a single MS run, thereby facilitating efficient lipidomic profiling and biomarker discovery.

Poster #18

Improving the Quantitative Performance of Isobaric Tags with IRMPD

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Isobaric tagging enables multiplexed quantitative proteomics, but accurate quantification depends on efficient and sensitive reporter ion generation. We previously demonstrated that infrared multiphoton dissociation (IRMPD) can enhance reporter ion production from TMT tags on a Lumos Orbitrap mass spectrometer. Here, we extend this strategy to DeAla, a cost-effective diethylalanine-based isobaric tag designed for high-performance multiplexed proteomics, and evaluate whether IRMPD improves DeAla reporter ion generation compared with conventional higher-energy collisional dissociation (HCD). To enable IRMPD, we modified a Thermo Scientific Orbitrap Ascend Tribrid mass spectrometer by installing a 10.6 μm CO_2 infrared laser and updating instrument control methods to improve scan speed and parallelizable injection time. In-house synthesized multiplex DeAla tags were validated by intact tag masses and corresponding reporter ions. We first optimized peptide fragmentation using standard peptides with known sequences and found that CID at normalized collision energy 30 provided efficient backbone fragmentation while preserving DeAla tag integrity. We then compared HCD- and IRMPD-based reporter ion generation using DeAla-labeled tryptic BSA samples in SPS-MS3 workflows, followed by evaluation across sample loading amounts from 10 ng to 500 ng. Lean and diabetic mouse pancreatic islet samples were prepared similarly, with additional high-pH fractionation, to assess applicability in complex biological samples. IRMPD produced substantially higher DeAla reporter ion intensities than HCD while reducing secondary fragmentation of reporter ions at higher activation energies. In BSA analyses, IRMPD generated approximately two-fold higher reporter ion intensity and signal-to-noise ratio compared with HCD, and this improvement was consistently observed across the tested input range. These results demonstrate that IRMPD enhances DeAla-based reporter ion generation and improves quantitative sensitivity. Overall, this work establishes an IRMPD-enabled workflow for robust, cost-effective isobaric tag-based quantitative proteomics, particularly for low-input and complex biological samples.

Poster #19

Phosphoproteomic Profiling Reveals a TRPV4–AMPK Signaling Axis Governing Choroid Plexus Barrier Integrity in Hydrocephalus

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Hydrocephalus is characterized by the abnormal accumulation of cerebrospinal fluid (CSF) in the brain ventricles, resulting from disrupted CSF secretion, circulation, or reabsorption. These processes are governed by ion and water channels in the choroid plexus epithelium, which produces most of the CSF. Among these channels, the mechanosensitive cation channel TRPV4 (transient receptor potential vanilloid 4) is a candidate regulator of CSF dynamics. Inhibiting TRPV4 halts congenital hydrocephalus in rats, yet how its activity is controlled remains unclear. Because TRPV4 is both regulated by phosphorylation and capable of triggering downstream phosphosignaling, we applied mass spectrometry-based phosphoproteomics to map its regulatory network in HIBCPP cells, a well-characterized human choroid plexus epithelial model. Using titanium dioxide phosphopeptide enrichment, we profiled the phosphorylation changes induced by pharmacological activation (GSK1016790A) and inhibition (RN1734) of TRPV4. This revealed altered phosphorylation of proteins controlling epithelial barrier integrity and cellular metabolism, including Zonula Occludens-1 (ZO-1), Claudin-7, and AMP-activated protein kinase (AMPK). Notably, TRPV4 activation increased inhibitory phosphorylation of AMPK at Ser496. We validated this axis functionally by electrophysiology. AICAR-mediated AMPK activation increased inhibitory phosphorylation at Ser496 while reducing phosphorylation at the canonical activating site Thr172, resulting in increased epithelial permeability. Conversely, AMPK inhibition with Compound C reduced Ser496 phosphorylation and preserved barrier integrity. Sequential inhibition of PKA and AMPK before TRPV4 activation further reduced permeability, and phosphorylated ZO-1 was consistent with TRPV4-driven tight-junction disassembly. Together, these findings define a functional coupling between AMPK signaling and TRPV4-mediated ion transport that governs choroid plexus barrier permeability and, potentially, CSF regulation. By resolving this mechanism at site-specific resolution, our work demonstrates the power of phosphoproteomics to expose signaling nodes and positions the TRPV4-AMPK axis as a promising, non-invasive therapeutic target for hydrocephalus.

Poster #20

Thiol-Directed Isobaric Tagging for Chemoselective Labeling and Quantification of Phosphorylation-Derived Dehydroamino Acids in Tau Proteoforms

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Serine and threonine phosphorylation are central to human cell signaling, but in Alzheimer's disease they become part of a pathological proteomic landscape characterized by disrupted kinase/phosphatase activity, altered protein structure, aggregation, and neurodegeneration. Tau is the clearest example: disease-associated hyperphosphorylation occurs largely on serine and threonine residues, producing heterogeneous phosphorylation patterns that are difficult to resolve in complex human proteomic samples using conventional LC–MS/MS. Standard phosphoproteomics is a powerful approach, but it is primarily designed to detect intact phosphopeptides. Once phosphoserine (pSer) or phosphothreonine (pThr) undergoes β-elimination, the phosphate group is lost, and the residue is converted to dehydroalanine (Dha) or dehydrobutyrine (Dhb). These products retain information about the original phosphorylation site but fall outside the conventional phosphopeptide-centered workflow. We developed a thiol-directed isobaric tagging strategy to enable quantitative analysis of these phosphorylation-derived products in human proteomes. Controlled β-elimination converts pSer and pThr into the Michael acceptors Dha and Dhb. A custom four-plex thiol-isobaric reagent then reacts with these electrophilic residues through Michael addition, installing a stable reporter-encoded adduct at the former phosphorylation site. The workflow was evaluated using synthetic phosphopeptide standards, phosphoprotein digests, and human HeLa lysate as a model of a complex human proteome. These experiments confirmed the expected precursor mass shifts following β-elimination and tagging, efficient reporter-ion formation under standard HCD conditions, and accurate multiplexed quantification across both balanced and non-equimolar mixtures. In HeLa lysate, the method recovered thiol-tagged candidate peptides from a complex proteomics background while maintaining peptide-identification quality across the label-free, β-elimination, and tagging workflow stages. By targeting products of pSer and pThr chemistry rather than relying solely on global peptide amines labeling or intact phosphopeptides analysis, thiol-directed isobaric tagging provides a complementary approach to human phosphoproteomics with direct potential for studying Alzheimer's disease-relevant proteome remodeling.

Poster #21

Mass spectrometry-based characterization of phosphorylation sites and interacting proteins of the Dachsous intracellular domain

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Dachsous (Ds), a large atypical protocadherin, is a key regulator of the Hippo cell proliferation pathway. While phosphorylation of the Ds intracellular domain (ICD) is thought to influence its signaling activity, the specific phosphorylation sites and associated protein interaction network remain poorly characterized. To investigate post-translational regulation of Ds, we employed two complementary mass spectrometry-based proteomic strategies. First, FLAG-tagged Ds ICD was expressed in Drosophila S2R+ cells, IP purified, digested, and analyzed by bottom-up liquid chromatography-tandem mass spectrometry (LC-MS/MS) to identify phosphorylation sites. Second, IP purification coupled with mass spectrometry (IP-MS) was performed to define proteins associated with the Ds ICD and generate a candidate interactome. Bottom-up LC-MS/MS identified multiple phosphorylation sites within the Ds intracellular domain, providing direct evidence that Ds undergoes extensive post-translational modification. AP-MS identified hundreds of candidates interacting proteins spanning diverse functional categories, including cytoskeletal, signaling, trafficking, and nuclear-associated proteins. Together, these complementary datasets establish a resource for investigating how phosphorylation influences Ds protein interactions and downstream Hippo pathway regulation. This study demonstrates the utility of integrating phosphoproteomics with IP-MS to characterize both post-translational modifications and protein interaction networks of Dachsous. These findings provide a foundation for mechanistic studies aimed at determining how phosphorylation regulates Ds function during tissue growth and development.

Poster #22

A Pipeline for the Epitranscriptomic Analysis of MicroRNAs by Liquid Chromatography/Mass Spectrometry

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MicroRNAs (miRNAs) are small noncoding RNAs composed of 18-25 nucleotides that play a crucial role in regulating gene expression and several critical biological processes, such as cellular differentiation, metabolism, apoptosis, and cancer metastasis. The regulatory role of miRNAs is exerted not only through their nucleotide base sequences, but also through a variety of post-transcriptional modifications (PTrMs). Current understanding of these epitranscriptomic modifications and their biological roles is limited due to lack of technology to identify and quantify unknown modifications, particularly in miRNA. To analyze the epitranscriptomic landscape of miRNA we developed a pipeline consisting of four stages: miRNA extraction, separation using ion-pairing reverse-phase liquid chromatography (IP-RP-LC), sequencing by high-resolution mass spectrometry, and data analysis via the open-source search software MetaMorpheus. For initial development of the pipeline, we spiked a mixture of unmodified and modified synthetic miRNAs (22 mers), containing the following PTrMs: methyladeosine, N6-methyladenosine, and 2'-O-dimethyladenosine, into total RNA from Jurkat cells following TRIzol extraction. RNA was separated into two fractions: long RNA (~>200 nucleotides) and short RNA (~< 200 nucleotides) by solid phase extraction incorporating the use of glass fiber filters and different percentages of ethanol. The short RNA fraction was subjected to offline fractionation (IP-RP-LC) to isolate miRNA. The resulting miRNA fraction was then further separated and sequenced using IP-RP-LC coupled to data-dependent tandem mass spectrometry. Spectral files were analyzed utilizing MetaMorpheus, which recognizes and identifies RNA fragmentation and PTrMs. Employing this pipeline, we were able to isolate, separate, and sequence standard miRNAs spiked into a biological sample, as well as identify and localize their different methylation modifications. Current work involves analyzing the miRNAs isolated from wild type DU 145 prostate cancer cells compared to knockout DU 145 cells, lacking the gene CHD1, which has been found to play a role in promoting invasive prostate cancer.

Poster #23

MOF-Enabled Selective Depletion of Abundant Plasma Proteins for Integrated Proteomics

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Introduction (48): Metal–organic frameworks (MOFs) are crystalline porous materials used in separations, catalysis, and biomedicine. Plasma proteomics is hindered by extreme protein concentration ranges. We developed a native-compatible MOF depletion strategy that preserves protein structure and modifications, enabling native top down MS for intact proteoform and protein complex characterization in plasma. Methods (112): Proteins were incubated in a solution of 2-methylimidazole, 50 mM HEPES, and 1X HALT protease inhibitor. Protein@ZIF-8 biocomposites were grown by combining the methylimidazole solution with zinc acetate dihydrate and incubating for 20 minutes. Growth was quenched using 0.5 M EDTA, and biocomposites were removed via centrifugation, leaving uncaptured (UC) proteins in the supernatant. Captured (C) proteins were recovered by dissolving in 0.5 M EDTA. UC and C fractions were buffer-exchanged into 200 mM ammonium acetate and concentrated with 10 kDa MWCO filters for native top-down MS analysis. Intact proteins were analyzed by online size-exclusion chromatography (SEC) on an Agilent 1290 Infinity II 2D-LC System coupled to an Agilent 6545XT AdvanceBio LC/Q-TOF. Preliminary data (97): We developed an in situ protein@MOF strategy that selectively encapsulates highly abundant plasma proteins to improve biomarker detection in proteomics. Encapsulation efficiency depends on protein isoelectric points, with more acidic proteins showing greater uptake. In this one pot process, abundant proteins drive ZIF 8 formation and are removed by centrifugation, leaving low abundance proteins for MS analysis. Our method depleted >95% of 19 of the 22 most abundant serum proteins and enabled identification of hundreds of proteins. We optimized buffer conditions and tested protease inhibitors, then demonstrated compatibility with native top down MS, preserving structures and non covalent interactions for future plasma applications. Novel aspect (15): First native top-down proteomics study of proteins after selective depletion via in situ MOF growth.

Poster #24

Hybrid De Novo Sequencing and Database Searching Reveal Endogenous Peptides in Crustacean Models

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Endogenous peptides are critical regulators of signaling and immunity but remain difficult to characterize in organisms with incomplete genomic annotation. We developed a hybrid discovery platform that integrates transformer-based de novo sequencing (Casanovo), neuropeptide-focused database searching (EndoGenius), and empirical false discovery rate estimation via NovoBoard. This pipeline enables confident identification of endogenous peptides while expanding coverage beyond conventional database-only or de novo-only approaches. Applied to neuroendocrine tissues from Callinectes sapidus and Cancer borealis, the workflow revealed numerous high-abundance novel peptides and provided structural and genomic support for their biological relevance. Notably, we report the first histone-2A–derived antimicrobial peptide in the C. sapidus and characterize naturally occurring sequence variants. We also identified unexpected peptide homologies between crustaceans and Rattus norvegicus, enabling annotation of conserved housekeeping proteins in sparsely annotated genomes. To facilitate broad adoption, the pipeline is implemented as both a lightweight local workflow and a scalable distributed version using the Center for High Throughput Computing (CHTC), enabling efficient analysis of datasets ranging from individual experiments to large-scale studies. This hybrid platform establishes a scalable, open-source strategy for advancing neuropeptidomics and endogenous peptide discovery in emerging model organisms.

Poster #25

A comprehensive atlas of ubiquitination across mouse tissues

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Introduction Ubiquitination is a highly conserved post-translational modification involved in virtually all cellular functions. Ubiquitin is a 76-amino acid, 8.6 kDa protein that attaches covalently to lysine residues and leaves a characteristic GlyGly remnant (+114.042 Da) after tryptic digestion. Ubiquitination regulates proteasomal degradation, DNA damage response, signaling, and protein trafficking. Dysregulated ubiquitination is implicated in neurodegenerative disease, cancer, cardiovascular disease, and metabolic dysfunction. Despite its biological relevance, most large-scale studies of ubiquitination rely on cultured cells, leaving the tissue-specific landscape of ubiquitination in vivo largely uncharacterized. Methods Fifteen tissues from 3 male and 3 female wild-type mice were cryopulverized, lysed (8 M urea, 100 mM Tris pH 8, 2x phosphatase inhibitor), and precipitated in 90% methanol. Proteins were reduced/alkylated (TCEP/CAA), diluted to 2 M urea, and digested with trypsin (1:25). Desalted peptides were enriched via the Cell Signaling PTMScan HS K-ε-GG kit (5 mg input) and analyzed by nLC-MS/MS on an Orbitrap Astral Zoom in DIA mode (12–58% B, 30 min active gradient; NCE 25%; MS1 380–980 m/z, 240k resolving power; MS2 150–2000 m/z, 4 Th isolation). Data were processed in Spectronaut 21. Results We generated the first tissue-resolved atlas of the mouse ubiquitinome, observing 115,480 ubiquitination sites on 13,201 proteins, more than tripling the known mouse ubiquitinome and exceeding the harmonized human reference ubiquitinome built from aggregated public data (~108,000 sites). Ubiquitination was tissue-selective, with single-tissue sites comprising a quarter of all sites, and PCA/hierarchical clustering separating samples by tissue identity. We profiled trends in solvent accessibility, disorder, and secondary structure across the ubiquitinated proteome. Motif analysis revealed patterns tied to enzymatic function, including large-scale evidence of a conserved regulatory motif at kinase active sites. Together, this atlas offers an unprecedented, tissue-resolved foundation for dissecting ubiquitin-driven regulatory biology across organ systems and disease states.

Poster #26

Trapped Ion Mobility Spectrometry Enhances Top-Down Proteomics of Intact Proteins

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Top-down proteomics (TDP) has emerged as a powerful mass spectrometry (MS)-based approach for characterizing and quantifying intact proteoforms directly from biological samples. However, online separation of complex mixtures results in coeluting species and convoluted spectra, while achieving sufficient sequence coverage for PTM localization remains challenging. Ion mobility spectrometry (IMS) holds potential to improve TDP by increasing signal-to-noise (S/N) and separating overlapping species. Trapped ion mobility spectrometry (TIMS) further enables parallel accumulation–serial fragmentation (PASEF). PASEF has greatly increased MS2 scan rates without compromising sensitivity in bottom-up proteomics, yet IMS-based approaches remain underutilized in TDP. Here, we develop TIMS-enabled TDP analyses of heart tissue extracts using a timsTOF. Initial LC-MS experiments showed optimal protein loading requires balancing signal intensity with efficient ion transmission. Loading too much (>200ng) leads to space charge effects while too little (<50ng) results in poor S/N. We further found that shorter TIMS accumulation times (3–20ms) improved transmission of protein ions. Although this necessitated shorter ramp times, the resolution was sufficient to separate coeluting species and improve S/N. Subsequent experiments showed that PASEF can be applied to proteins, generating six additional MS2 scans per TIMS cycle. Further analysis revealed 38.2% sequence coverage for myosin light chain 2, comparable to results obtained on instruments without TIMS. Future work is focused on optimizing transmission for larger proteins and refining PASEF parameters for precursor selection. Because PASEF increases MS2 acquisition, we will investigate scan-summing strategies to improve sequence coverage and database searching of TIMS-enabled TDP data. Finally, we will compare TIMS-enabled acquisition with transmission-mode auto-MS2 to directly assess the impact of TIMS separation on TDP performance.

Poster #27

Proteome-Wide Consequences of Aneuploidy During Entry into Quiescence in Budding Yeast

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Aneuploidy, where cells harbor an incorrect number of chromosomes, is associated with significant health outcomes such as Down Syndrome. Individuals with DS often exhibit premature aging phenotypes. However, the molecular mechanisms linking chromosome amplification to premature aging remain poorly understood. Using yeast as a model system, Escalante et al. showed that aneuploid yeast exhibit defects in entering quiescence, an important growth state associated with growth control and life span. We are analyzing complete proteomes from isogenic euploid and aneuploid yeast strains during both exponential growth and quiescence to characterize the proteome-wide consequences of aneuploidy. TMT labeling was used for quantitative proteomic comparisons. Four strains were included: a wild-type euploid, an aneuploid carrying a full duplication of chromosome XII, and their corresponding strains lacking the RNA-binding protein Ssd1. Each strain was harvested during logarithmic growth (LOG) and quiescence (Q) with six biological replicates. Cells were lysed by bead milling in 8 M urea, digested in solution, and labeled with TMTpro reagents. Pooled samples were separated by high-pH fractionation prior to LC-MS/MS analysis. Proteins were identified and quantified in FragPipe using a virtual reference channel to normalize across sets. A total of 3632 proteins were quantified across all three TMT sets. Consistent with known biology, cells underwent extensive proteome remodeling through aging. 2,901 of 3,632 quantified proteins significantly changed between LOG and Q. Within the same age, samples with the same ploidy more closely resemble one another. In addition to direct contrasts between samples, we performed statistical tests on their differential aging responses, defined as differences in proteome changes between paired LOG and Q samples. Comparing wild-type euploid and aneuploid strains, 913 proteins changed differently during the transition between LOG and Q. These proteins implicate possible pathways that contribute to the observed defects of aneuploid cells in entering quiescence.

Poster #28

Comprehensive proteomic profiling of CDK7 inhibition in fibrolamellar carcinoma

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Fibrolamellar carcinoma (FLC) is a rare liver malignancy typically effecting young adults with a 5-year survival rate of ~30%. There are currently no proven therapeutic options for patients that don't qualify for surgical resection. FLC is characterized by a fusion event between DNAJB1 and PRKACA, driving aberrant PKA activity, chromatin remodeling, increased transcriptional output, and oncogenic survival signaling. Our group has used in vitro and in vivo models to shown CDK7 inhibition is a lethal vulnerability in DNAJB1-PRKACA expressing cells and patient derived FLC models. Patient derived xenografts (PDX) have been shown to preserve the histology, genomic heterogeneity, and therapeutic sensitivity of the original tumor. Here, we characterize the in vivo proteomic and phosphoproteomic response of FLC PDX tumors to CDK7 inhibition. We identified >500 differentially expressed proteins and >300 phosphosites. Pathway enrichment revealed strong downregulation of translational and RNA binding proteins. Significant decreases in phosphorylation were also seen in these proteins. Interestingly, histones were strongly upregulated, pointing to chromatin reorganization with CDK7 inhibition. Kinase activity enrichment of phosphosites demonstrated dysregulation of cell cycle kinases including CDK2 and CDK1, both known targets of CDK7. This work provides mechanistic insight into CDK7 inhibition in FLC as it progresses as a therapeutic target. Future work includes validating this response across multiple FLC PDX models and characterizing the chromatin shifts induced by CDK7 inhibition compared to the remodeling induced by oncogenic DNAJB1-PRKACA signaling.

Poster #29

A Proteomics Exploration in Mice Lacking the Collagen Receptor MRC2

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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. MRC2 is a transmembrane receptor protein that binds and internalizes collagen and collagen fragments into stromal cells for lysosomal degradation. Lungs from Mrc2 knockout (Mrc2 KO) mice appear histologically normal but have increased lung stiffness, suggesting a role for MRC2 in the regulation of matrix homeostasis. Here, we sought to characterize differences in the matrisome of WT and Mrc2 KO mouse lungs to understand the role of MRC2 in matrix composition using a bottom-up proteomics workflow. Along with lung tissues, bronchoalveolar lavage fluid (BALF) was analyzed using a combined bottom-up and top-down proteomics approach to identify changes to the secreted lung proteome in mice lacking MRC2. We used bottom-up proteomics to identify differences in the lung matrisome between WT and Mrc2 KO lungs. Notably, polymerase and spindlin-2c were significantly upregulated and fibronectin type III domain containing 3B and the core ECM protein matrilin-1 were significantly downregulated in Mrc2 KO lungs. Although matrilin-1 was originally identified as a cartilage matrix protein implicated in formation of filamentous networks in the ECM, proteomics analysis by other investigators has shown its expression in the lung. Top-down proteomics analysis of BALF necessitates the depletion of albumin to facilitate the detection of lower abundance proteins, including collagen and collagen fragments. We adapted a metal-organic framework-depletion of albumin to detect and identify proteins in BALF. Preliminary analyses show the detection of truncated transthyretin proteoforms and unidentified proteins with extensive glycosylation, corresponding to approximately 162 kDa mass shifts. Together, these proteomics results provide a methodology for the comprehensive analysis of mice lacking MRC2.

Poster #30

Proteoform-Level Analysis of Mitochondrial Respiratory Complexes from Human Cardiac Tissue

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Mitochondria are essential metabolic organelles responsible for ATP synthesis, the TCA cycle, amino acid biosynthesis, and fatty acid oxidation. Mammals contain two major functionally distinct subpopulations of mitochondria in striated muscle: subsarcolemmal (SSL) and interfibrillar (IF) mitochondria. A proteoform-level characterization of the oxidative phosphorylation (OXPHOS) respiratory complexes by top-down proteomics remains unexplored. This is an analytical challenge as there are roughly 100 subunits making up the 5 OXPHOS complexes with a variety of properties. OXPHOS respiratory complexes drive mitochondrial energy production, so defining the proteoform landscape of their subunits is critical for understanding mitochondrial function and regulation. The cohort consisted of 6 SSL and IF mitochondrial isolates (n=4 SSL, IF pairs, n=2 independent patient samples) obtained from pediatric patients with hypoplastic left heart syndrome-related heart disease. In addition, mitochondria were isolated in triplicate from frozen tissue of a control, adult heart. Mitochondria lysis and protein extraction was achieved with either Azo, a photocleavable surfactant, or n-Dodecyl-β-D-maltoside (DDM). Methanol-chloroform-water precipitation was used for cleanup before RPLC-MS using a Waters M-Class UPLC coupled to a Bruker maXis II QTOF-MS with CID for tandem MS (MS/MS). Data were analyzed using TopPIC v1.7.9, MASH Native v1.1, and Bruker Compass DataAnalysis v4.3. Mitochondrial proteins were identified using two complementary approaches: TopPIC database searching of MS/MS spectra and intact mass matching of MS1 data against an in-house established mitochondrial proteoform library. Using our protocol, we detected 59 of the OXPHOS subunits (64%), or 48%, 50%, 70%, 88%, and 88% coverage of Complexes 1 through 5 respectively. We also observed proteoforms including truncated, oxidized, acetylated, succinylated, methylated, and S-nitrosylated species. Finally, we illustrated how we aim to quantify the relative proteoform levels between SSL and IF mitochondria. In the future, we aim to expand the coverage of the OXPHOS complexes and mitochondrial proteome with multidimensional chromatography.