

Organizing Committee:

Robyn Kaake, PhD
Assistant Adjunct Professor, Quantitative Biosciences Institute (QBI), UCSF

Nevan J. Krogan, PhD
Professor, Department of Bioengineering and Therapeutic Sciences, UCSF
Director, Quantitative Biosciences Institute (QBI), UCSF
Senior Investigator - Gladstone Institute of Data Science and Biotechnology

Klim Verba, PhD
Assistant Professor, Cellular Molecular Pharmacology, UCSF
Investigator, Quantitative Biosciences Institute (QBI), UCSF

Day 1: November 4, 2026

8:00 AM – Doors Open/Registration

OPENING SESSION
9:00 AM – Opening Remarks by **Nevan Krogan**, UC San Francisco

9:30 AM – Keynote Talk by **Lan Huang**, University of California Irvine – introduced by Robyn Kaake, UC San Francisco

10:30 AM – Sponsored Talk by **Ryan Bomgarden**, Thermo Fisher Scientific
"The Evolution of Protein Crosslinking: Simplifying Workflows for Complex Protein Samples"

10:40 AM – Break

SESSION ONE – **Cross-Linking Mass Spectrometry (Part I)** – Chaired by **Robyn Kaake**, UC San Francisco
11:00 AM – **James Bruce**, University of Washington
"Cellular stress interactome dynamics"

11:20 AM – **Leah McDermott**, University of Washington
"Mapping the Mitochondrial Permeability Transition Pore Interactome to Reveal Mechanisms of Human Aging and Disease"

11:35 AM – **Patrick Griffin**, University of Florida
"Illuminating the functional components of NR transcriptional regulatory complexes"

11:55 AM – **Franz Herzog**, IMC Krems University of Applied Sciences
"Mechanistic Insights Into Targeted Protein Degradation by Chemical Crosslinking and Quantitative Mass Spectrometry"

12:10 PM – **Changjian Feng**, University of New Mexico
"Quantitative Crosslinking Mass Spectrometry Reveals Phosphorylation-induced Changes in the Functional Protein Dynamics of Neuronal Nitric Oxide Synthase"

12:25 PM – Lunch

1:25 PM - 1:55 PM - **Platinum Sponsor Presentation by Weijing Liu**, Thermo Fisher Scientific, *"Structural Biology Mass Spectrometry: Bridging Proteoforms, Structure, and Interactions"*

SESSION TWO – **HDX/Covalent Labeling** – Chaired by **Monita Muralidharan**, UC San Francisco
1:55 PM – **Lisa Jones**, UC San Diego

2:15 PM – **Monika Priyadarshini**, Stanford University
"Integrative Structural Mapping of Chromatin Condensates by Phase-resolved HDX-MS and XLMS"

2:30 PM – **Ben Walters**, Genentech

2:50 PM – **Petr Novak**, Institute of Microbiology of the Czech Academy of Sciences
"Mapping Protein Structure with Singlet Oxygen Labeling"

3:05 PM – **David Schriemer**, University of Calgary

3:25 PM – Break
SESSION THREE – **Native/Top Down** – Chaired by **Shouling Xu**, UC San Francisco
3:45 PM – **Michael Sharon**, Weizmann Institute of Science

4:05 PM – **Neil Kelleher**, Northwestern University
"The Plasma Proteoform Assay: Tracking Progressive Liver Cirrhosis via >1500 Plasma Proteoforms across >200 Subjects"

4:25 PM – **Corinne Lutomski**, University of Oxford

4:45 PM – **Kallol Gupta**, Yale University
"Resolving Dynamic Membrane Signaling Assemblies by Single-Ion Lipid-Vesicle Native Mass Spectrome"

5:00 PM – **Ruben Luo**, Stanford University
"Clinical Testing of Monoclonal and Oligoclonal Immunoglobulins Using Top-Down Mass Spectrometry"

5:20 PM – Poster Session with Light Reception

Day Two: November 05, 2026

8:00 AM – Doors Open/Registration

SESSION FOUR - **Drug Discovery** – Chaired by **Nevan Krogan**, UC San Francisco
9:00 AM – **Malvina Papanastasiou**, The Broad Institute
"Enabling Early-stage Drug Discovery with NanoHDX-MS: Rapidly Interrogating Protein-protein and Protein-small Molecule Interactions at Scale"

9:20 AM – **Liz Hecth**, Amgen

9:40 AM – **Darragh O'Brien**, University of Oxford
"HDX-MS Reveals the Structural Dynamics Underlying Drug Activity: Insights from Deubiquitinase Inhibitors"

10:00 AM – Break

SESSION FIVE – **CryoEM/CryoET** – Chaired by **Kliment Verba**, UC San Francisco
10:20 AM – **Oleg Klykov,** UT Health San Antonio
"Approaches for Physiologically Relevant Structural Proteomics"

10:35 AM – **Michael Rout**, Rockefeller University

10:55 AM – **Mia Greeson**, Stanford University
"Structural and Proteomic Sissection of GPR143 Defines a Non-canonical GPCR-effector Interface Underlying Ocular Albinism"

11:10 AM – **Yifan Cheng**, UC San Francisco

11:30 AM – Group Picture

12:00 PM Lunch

1:00 PM – Gold Sponsor Presentation by Bruker

1:20 PM – Gold Sponsor Presentation by Trajan Scientific

SESSION SIX – **Computational Modeling** – Chaired by **Dominic Grisingher**, UC San Francisco
1:40 PM – **Andrej Sali**, UC San Francisco

2:00 PM – **Haiyuan Yu**, Cornell University
"Interactome Scale Structural Modeling of Protein Interactions and Systematic Blindspots of AlphaFold"

2:15 PM – **Roland Dunbrack**, Fox Chase Cancer Center

2:35 PM – **Ignacia Echeverria-Riesco**, UC San Francisco
"Integrative Structure Modeling of SynGAP1 in the Context of the Postsynaptic Density"

2:50 PM – **Dima Kozakov**, University of Texas

3:10 PM – **Jure Leskovec**, Stanford University

3:30 PM – Break

SESSION SEVEN – **Protein Interactions** – Chaired by **Danielle Swaney**, UC San Francisco
3:50 PM – **Francis O'Reilly**, St. Jude Children's Research Hospital

4:10 PM – **Simon Butterworth**, Massachusetts Institute of Technology
"Proteome-wide Crosslinking Mass Spectrometry Reveals Novel Components of Essential Complexes in Toxoplasma"

4:25 PM – **Jennifer Geddes-McAlister**, University of Guelph

4:45 PM – **Monita Muralidharan**, UC San Francisco
"Structural and Functional Characterization of an LtgA–LtgD Peptidoglycan-remodeling Assembly in Neisseria Meningitidis"

5:00 PM – **Michael Trnka**, UC San Francisco
5:30 PM – Gala Dinner

Day Three: November 06, 2026

8:00 AM – Doors Open/Registration (Atrium - 1st Floor)

SESSION EIGHT – **Cross-Linking Mass Spectrometry (Part II)** – Chaired by **Ignacia Echeverria-Riesco**, UC San Francisco
9:00 AM – **Arun Wiita**, UC San Francisco
“Structural surfaceomics to identify conformation-selective immunotherapy targets in cancer”

9:20 AM – **Yi He**, Thermo Fisher Scientific
"An Innovative Data-Independent Acquisition (DIA) Approach for the Quantitative Analysis of Protein-Nucleic Acid Interactions in Living Cells"

9:35 AM – **Andrew Michael**, Institute of Microbiology of the Czech Academy of Sciences
"Chromatographic Environment and Crosslinker Molecular Properties Shape the Analytical Sampling Space of Crosslinked Peptides"

9:50 AM – **Krita Kaasik**, UC San Francisco
"Crosslink Guided Modeling Reveals O-GlcNAc Dependent Conformational Dynamics of the Giant Presynaptic Scaffolds Bassoon and Piccolo"

10:05 AM – **Bjorn-Erik Wulff**, Stanford University

10:20 AM – Break

SESSION NINE – **Emerging Technologies** – Chaired by **Kliment Verba**, UC San Francisco
10:40 AM – **Ed Huttlin**, Harvard University

11:00 AM – **Darren Kahan**, UC Berkeley
Detecting Long-Range Biases in Intrinsically Disordered Proteins Using X-ray Footprinting / Mass Spectrometry

11:15 AM – **Dari Kimanius**, Chan Zuckerberg Institute

11:35 AM – **Xiaojing Sui**, Northwestern University
"Mapping the Landscape of Proteome Metastability and Structural Plasticity in Aging and Proteotoxic Stress"

11:50 AM – Presentation by **Nicolas Hartel**, Thermo Fisher Scientific
"Development of DIA workflow for Multiplex Hydroxyl Radical Protein Footprinting"

12:10 PM – Closing Remarks by **Nevan Krogan**, UC San Francisco

12:15 PM – End

14th Symposium on Structural Proteomics Abstracts

Poster Abstracts

Abstract #1
Presenter: Ryan Bomgarden
Title: An optimized enrichment strategy for improved mass spec analysis of chemically cross-linked peptides
Abstract: A major challenge of crosslinking mass spectrometry (CX-MS) is the relatively low abundance of crosslinked peptides after protein digestion. This results in very few or no crosslinked peptide identifications by MS when sample complexity exceeds a few hundred proteins. Therefore, enrichment of crosslinked peptides is necessary to identify them in complex samples. An enrichable, MS-cleavable crosslinker, Azido-A-DSBSO, has become a popular reagent for selective capture of crosslinked peptides. This crosslinker also contains two NHS amine-reactive groups for lysine crosslinking, an azido group for enrichment using Click chemistry-based methods, and an acid-cleavable group for removal of the

enrichment moiety before MS analysis. Here, we describe our efforts comparing different Click enrichment methods, reaction conditions, affinity resins, and elution conditions to optimize crosslinked peptide yields. We also developed a new dibenzocyclooctyne (DBCO) magnetic bead using a low pH-resistant polymer for the automated enrichment of DSBSO crosslinked peptides. These new beads have higher capacity than other magnetic beads and crosslinked peptide yields within 80% of traditional non-magnetic resins. SCX fractionation using spin tips and columns also increased crosslinked peptide identifications by 30% compared with enrichment alone. Overall, our optimized crosslinked-peptide enrichment workflow incorporating fractionation significantly improves the identification of crosslinked peptides in complex cell lysate samples.

Abstract #2

Presenter: Robert Chalkley

Title: Identification of Trivalent Crosslinks From Tandem Mass Spectrometry Data

Abstract: Chemical crosslinking followed by mass spectrometry is an increasingly used approach for studying protein-protein interactions. The cell also natively uses protein crosslinking to increase structural strength. Last year a novel MS-cleavable crosslinker was described, TSTO, which can react with three primary amine groups, so link three lysines in spatial proximity. In this publication MS3 data was acquired for crosslinked product analysis, which is slower than MS2 data acquisition, but provides simpler bioinformatic analysis. In the present study we describe support for analysis of MS2 crosslinked products linking three lysines using the Protein Prospector software. We contrast the results with those achieved using MS3 analysis of the same whole cell lysate crosslinked sample. We also demonstrate the software can identify native ternary crosslinks in collagen fibrils.

Abstract #3

Presenter: Alessio Di Ianni

Title: Boosting Cross-link Peptide Discovery and Validation with Boxcar-assisted Cross-linking Mass Spectrometry (BaXL-MS)

Abstract: Orbitrap (OT)-based mass spectrometers are widely regarded as the gold standard in high-resolution mass spectrometry. Yet, their relatively slow scan speed compared with time-of-flight (TOF) or linear ion trap (LIT) analysers remains a limitation. Recent developments have enhanced scan rate and sensitivity on the Orbitrap platform. However, these improvements have been largely confined to the MS2 level with either Data-dependent (DDA) or Data-Independent (DIA) acquisition methods, while ion sampling efficiency at the MS1 level remains suboptimal. To address this, Meier et al. (2018) introduced the BoxCar method, which distributes the C-trap's charge capacity across multiple narrow m/z segments within a single scan. This strategy reduces dominance of highly abundant ions, increases injection times for low-abundance species, and markedly improves their detectability and signal-to-noise ratio. The primary drawback of BoxCar is the reduced scan speed, and its scans are not used to trigger MS2 spectra. Jenkins and Orsburn later advanced this approach with BoxCar Assisted MS Fragmentation (BAMF), enabling triggered DDA-MS2 by recording the whole m/z range in two or three multiplexed targeted single ion monitoring (tSIM) scans. These MS1 scans are then used to acquire MS2 spectra. A slight variation of this approach termed Multiple Accumulation Precursor Mass Spectrometry (MAP-MS) has been introduced by Phlairaharn et al., which has been now commercialised on novel Thermo instruments as the ""enhanced Dynamic Range (eDR)"" feature. Because cross-link peptides are typically low-abundant species, we reasoned that the increased intrascan dynamic range offered by BAMF could facilitate their detection and validation, an aspect particularly critical when studying complex biological samples. In this work, we adapted BAMF for cross-link peptide identification, resulting in a novel acquisition mode termed BoxCar-assisted Cross-linking Mass Spectrometry (BaXL-MS). As a quality control sample for the evaluation of the method, we prepared bacterial E. coli ribosomes cross-linked with disuccinimidyl sulfoxide (DSSO). We designed several BaXL-MS based DDA methods in which different

number of MS1 tSIM scans, MS2 ion accumulation times and/or gas-phase fractionation (GPF) were applied. We then compared these methods to a similar DDA XL-MS method with a standard OT Full-Scan. XL-MS data were searched using XiSEARCH 1.8.7 and filtered at 5% false discovery rate (FDR) at the unique residue pair (URP) level using XiFDR 2.3.5. We found that several unique cross-links were identified only with BaXL-MS methods compared to standard settings. Additionally, we developed a pipeline for integrating the XL-MS results obtained from Xi into Skyline. The inspection of the MS1 data confirmed an enhanced feature detection of cross-link peptides, as indicated by higher isotopic dot products (idotp). Overall, combining different BaXL-MS methods enabled the identification of additional cross-links and improved validation of peptides identified exclusively by the standard method. This approach enables the construction of reliable, in-depth sample-specific XL-MS spectral libraries, which can be used for ad hoc quantitative DDA or DIA BaXL-MS measurements. Importantly, all methods can be readily implemented on any Orbitrap-based mass spectrometer without any software or hardware modifications, and XL-MS data remain compatible with established database search pipelines that rely on MS2 information.

Abstract #4

Presenter: Katelyn Erickson

Title: Unlocking Epitope Mapping of Patient-Derived HLA Antibodies Using High-Sensitivity Low-Flow HDX-MS

Abstract: HLA-specific antibodies play important roles in immune recognition and have significant clinical relevance. Hydrogen–deuterium exchange mass spectrometry (HDX-MS) enables structural epitope mapping of antigen–antibody interactions but traditionally requires substantial sample amounts, limiting analysis of scarce, patient-derived antibodies. Here, we developed a highly sensitive low-flow HDX-MS workflow that minimizes sample consumption while maintaining robust peptide coverage and data quality. HLA antigen and antibody were mixed at a 1:1 molar ratio, diluted tenfold into D_2O labeling buffer, and quenched under denaturing conditions. Automated sample preparation was performed using a Trajan CHRONET platform, followed by online Nepenthesin II proteolysis, trapping, chromatographic separation at 4 µL/min, and MS analysis on a modified Orbitrap Tribrid mass spectrometer. Data were processed using Proteome Discoverer and HDExaminer Pro. The workflow successfully analyzed as little as 2 pmol of HLA antigen per injection using a 10-minute gradient. Peptide mapping achieved 83% sequence coverage with 259 overlapping peptides, providing high spatial resolution for HDX analysis. The N-terminal region was not observed, possibly because of structural flexibility and preferential cleavage into peptides below MS detection limits. Differential deuterium uptake between apo and antibody-bound states was measured at 30, 300, and 3600 seconds, with three technical replicates per condition. Multiple patient-derived HLA antibodies were mapped using only a few micrograms of total antibody. These results demonstrate that low-flow HDX-MS can extend epitope mapping to clinically scarce antibodies, enabling detailed structural characterization of immunologically relevant samples with substantially reduced sample requirements.

Abstract #5

Presenter: Katharina Geißler

Title: Integrating cryo-ET and proximity labeling to resolve molecular interactions of rare vault subspecies in situ

Abstract: The eukaryotic vault is a large ribonucleoprotein complex whose cellular function remains enigmatic despite four decades of structural and biochemical characterization. Our in situ structural analyses using cryo ET revealed that vaults associate with endoplasmic reticulum (ER) and nuclear envelope membranes and encapsulate ribosomes. However, these functional vault subspecies are rare, comprising only 1.4% of the intracellular vault population, and the mechanistic basis for their membrane association and ribosome encapsulation remains obscure. To define the vault-associated proteome

directly in situ, we applied proximity labeling coupled to quantitative label-free mass spectrometry. Beyond the known vault component TEP1, we identified multiple poly(ADP-ribose) polymerases, numerous ER-associated proteins, and a striking enrichment of the translational machinery, including 69 of 79 ribosomal proteins. Functional enrichment analysis highlighted protein translation, ribosome-associated processes, and membrane-related pathways and proteins as dominant features of the vault interactome. Our proteomic data independently validates key observations from our in situ structural studies and provides complementary molecular evidence that vaults associate with ER and nuclear envelope membranes and encapsulate ribosomes. These results demonstrate the power of integrating cryo electron tomography with mass spectrometry-based proximity labeling to characterize the interaction landscape of large macromolecular assemblies in their native cellular context and establish a resource for future investigations into vault biology.

Abstract #6

Presenter: Michael Glocker

Title: IgG antibodies are shapeshifting proteins

Abstract: Studying protein structure dynamics is key to understanding protein function modulation. Alternative protein conformations are well discriminated from each other by nanoESI mass spectrometry. The shapeshifting IgG antibodies are present as compactly folded native conformations in neutral buffered solution, identified by molecular ions with narrow charge state distributions, relatively few charges, and small collisional cross sections. Simultaneously present extended but nevertheless native conformations produced additional ions with higher charge states, different charge state distributions, and larger collisional cross sections. Computed collisional cross sections match to experimental data, indicating equilibrium, and an “m-shape” to “Y-shape” alteration of IgGs. The two reversibly adopted antibody conformations interchange by an “m2Y” flip. Burying Fc-receptor binding sites by laying the Fab arms alongside the Fc part prevents initiating humoral and cellular immune system responses prior to antigen contact.

Abstract #7

Presenter: Clair Goul

Title: Structural mapping and interactomics of lysosomal protein complexes

Abstract: A central challenge for cell biology is to connect organelle-scale structure with the molecular identities and interaction interfaces of the proteins that build those structures. Cryo-electron tomography captures organelle context but often cannot assign all constituent proteins, while traditional proteomics approaches capture neither structural information nor weak, transient interactions. We are developing an integrated strategy using crosslinking-mass spectrometry (XL-MS) and immunoprecipitations to map the spatial and structural organization of the lysosome, a disease-relevant organelle whose ~200 resident proteins form complexes that dynamically reorganize in response to cellular state. We focus initially on mapping interactions of the lysosomal cholesterol transporter, NPC1, mutations in which cause the lysosomal storage disorder Niemann-Pick Type C. We screen native polymers for extraction of NPC1 while preserving membrane-associated interacting complexes. Initial mass spectrometry results reveal both known and novel interactors of NPC1. CryoET and single-particle cryoEM reveal NPC1 particles with additional densities that likely represent interacting proteins. In parallel, we develop end-to-end XL-MS workflows to generate cleaner, more precise interaction maps of NPC1 by stabilizing associated complexes. These can be extended to determine interaction interfaces at residue-level resolution. By integrating structural proteomics data with in-house protein interaction models, this work will support cryo-ET interpretation and particle identification and help establish a general framework for connecting molecular-scale interactions to organelle-scale architecture in situ.

Abstract #8

Presenter: Michael Karpíšek
Title: The Utilization of FluoroAlkyl Radicals for Structural Biology
Abstract: Protein footprinting (PF) is a technique used in structural biology providing low-resolution structural information on proteins and protein complexes. However, different PF reagents may exhibit distinct labeling preferences depending on their physicochemical properties. Thus, we investigated whether reagent hydrophobicity influences the preferences of reagent labeling. Specifically, we studied Togni reagents (TR) with different hydrophobicity and assessed whether there is a correlation between reagent hydrophobicity and preferences toward labeling of hydrophobic parts of proteins. The aim was to identify TR suitable for labeling of transmembrane regions of membrane proteins. We applied the Fast FluoroAlkylation of Proteins (FFAP) protocol developed in our laboratory to study TR of different hydrophobicities. Upon activation, these reagents release fluoroalkyl radical that reacts with aromatic residues and cysteines. We used myoglobin (Mb) as model protein system and Cl-/H+ transporter (ClC-ec1) membrane protein to confirm our findings. Model proteins were studied using intact mass spectrometry (Mb) and bottom-up approach (Mb, ClC-ec1). The results show that increase of TR hydrophobicity leads to an overall decrease in modification extent. Further, no shift in labeling site preferences dependent on reagent hydrophobicity were observed. In case of hydrophobic reagents, formation of specific side product of different labeling preferences was observed. Thus, our study shows that TR of comparable hydrophobicity to Togni reagent II should be preferred when performing FFAP over TR of higher hydrophobicity.

Abstract #9
Presenter: Hyunsoo Kim
Title: Conformational Changes in Plasma Proteins in Healthy Aging
Abstract: Aging involves progressive physiological deterioration, yet underlying molecular mechanisms remain poorly understood. While age-related protein expression changes are well-documented, functional aging consequences are equally influenced by alterations in protein conformation and protein-protein interaction (PPI) networks. We present a comprehensive analysis of age-dependent structural dynamics in plasma proteins, integrating experimental proteomics with computational structural biology. Using covalent protein painting (CPP) labeling coupled with multiple reaction monitoring LC-MS/MS, we assessed conformational changes across four age groups (20s-50s) in 24 healthy individuals (n=6 per decade). Through cosine similarity analysis and limma-based statistical modeling, we identified five distinct age-associated conformational patterns, from progressive structural opening to closure, encompassing 27 proteins and 57 peptides. Network analysis revealed coordinated structural remodeling among key protein interactors, suggesting synchronized conformational changes within functional complexes. We verified specific conformational change sites using AlphaFold3 Multimer, mapping precise regions undergoing age-related remodeling within protein complexes. These findings illuminate the dynamic aging plasma proteome and establish a framework for identifying conformational biomarkers of healthy aging and age-related disease susceptibility.

Abstract #10
Presenter: Kitaik Lee
Title: Structures of dynamic interactors at native proteasomes by PhIX-MS and cryo-electron microscopy
Abstract: Molecular machines rely on dynamic, low-affinity interactions to perform their functional roles. We developed PhIX-MS (photo-induced in situ crosslinking-mass spectrometry), a structural proteomics workflow to capture topological information for such transient interactions in cells by UV-activated crosslinking. Applying PhIX-MS with cryo-electron microscopy (cryo-EM) to proteasomes, we mapped the redox sensor TXNL1 at the proteasome regulatory particle (RP), including its dynamic thioredoxin-like domain near RPN2/PSMD1 and RPN13/ADRM1, where it is ideal for reducing substrates prior to proteolysis. RPs without the proteolytic core particle (CP) were structurally resolved while bound to

TXNL1 and/or the chaperone PSMD5/S5b, which inserts its C terminus into the ATPase pore, causing extensive structural rearrangements. Additionally, PhIX-MS and AlphaFold identified the ubiquitin ligase UBE3C/Hul5 at RPN2, RPN3, and a dynamic RPN10 region, tethering UBE3C above the substrate entry channel. Our integrative approach enables the localization of native, low-affinity protein interactions and is broadly applicable to dynamic macromolecular assemblies.

Abstract #11

Presenter: Jiapeng Li

Title: MS-Based Immunopeptidomics to Identify Drug-Haptenated MHC-I Epitopes on Tumor Cells

Abstract: Major histocompatibility complex class I (MHC-I) molecules present intracellularly derived peptides on the cell surface for immune surveillance by cytotoxic T cells. Covalent inhibitors can modify target proteins and potentially generate drug-haptenated peptides that are subsequently presented by MHC-I (Figure 1). Sotorasib, an approved covalent inhibitor of KRAS G12C, modifies Cys12 and can generate a sotorasib-modified KRAS peptide (VVVGAC*sotorasibGVGK; V7-Soto) that is presented as a drug-induced neoantigen. Here, we developed an LC-MS/MS-based immunopeptidomics workflow to identify haptenated MHC-I peptides presented on tumor cells. MHC-I complexes were immunoprecipitated from cancer cells using the pan-HLA class I antibody W6/32, followed by isolation of MHC-I-bound peptides. The resulting immunopeptidome was analyzed using targeted parallel reaction monitoring (PRM) and untargeted data-dependent acquisition (DDA) on a Thermo Scientific Q-Exactive Plus Orbitrap mass spectrometer. PRM analysis identified V7-Soto in KRAS G12C cells treated with 10 μM sotorasib for 24 h. The identity of the cell-derived peptide was validated against a synthetic V7-Soto standard based on precursor and fragment-ion spectra, including diagnostic $y8^{2+}$ (m/z 625.8) and $y7^{2+}$ (m/z 576.3) ions (Figure 2). Application of this workflow to elironrasib-treated KRAS G12C cells further identified a KRAS G12C-derived K5 peptide carrying a +941.5480-Da modification that was detected only following drug treatment. MS/MS fragmentation localized the modification to Cys12 of KRAS. The observed mass shift is consistent with an elironrasib-derived species and suggests that intracellular processing or metabolism of covalent inhibitors may contribute to the generation of haptenated MHC-I neoantigens. Work is underway to identify its mechanism. Together, these results demonstrate the utility of LC-MS/MS-based immunopeptidomics for direct identification and characterization of drug-haptenated MHC-I peptides and reveal previously uncharacterized drug-derived modifications that may expand the repertoire of neoantigens presented on tumor cells.

Abstract #12

Presenter: Weijing Liu

Title: Integrative Native Top-Down Mass Spectrometry for Characterization of Membrane Protein Proteoforms, Structure, and Interactions

Abstract: Comprehensive characterization of membrane proteins is essential for understanding proteoform heterogeneity, structure–function relationships, and molecular interactions, yet remains challenging because of structural complexity and limited sequence coverage. Here, we present an integrated native mass spectrometry workflow for hierarchical membrane protein characterization, spanning intact proteoform resolution to structural and interaction analysis. Charge detection mass spectrometry (CDMS) and hybrid infrared electron transfer dissociation (IR-ETD) enable proteoform-resolved analysis and structural interrogation of intact protein assemblies, while MS^n supports identification of bound ligands. CDMS resolves isotopic distributions of the AqpZ tetramer (~99 kDa) and its ligand-bound states, distinguishing apo and holo proteoforms. Whereas ETD and EThcD generate minimal fragmentation, IR-ETD directly fragments the intact tetramer predominantly from flexible regions. For β1AR, freshly prepared samples exhibit the expected intact mass, whereas one month of storage produces additional proteoform peaks consistent with degradation. IR-ETD localizes C-terminal truncation as a major degradation pathway. For a human major facilitator superfamily (MFS) transporter, cryo-EM

reveals an unresolved N-terminal region. Native top-down fragmentation predominantly originates from the C-terminal region and correlates with structural features observed by cryo-EM, providing complementary information on protein dynamics and accessibility. Native top-down analysis further releases associated detergent and lipid species, while MS^3 differentiates isobaric ligands. Together, these results demonstrate an integrated native MS workflow connecting proteoform resolution, sequence characterization, structural interrogation, and ligand identification, complementing cryo-EM and other structural biology approaches.

Abstract #13

Presenter: Riley Metcalfe

Title: A highly optimized search program for searching Astral-acquired SDA-crosslinking MS data

Abstract: Searching crosslinking mass spectrometry (XLMS) data is extremely challenging due to the enormous search space possible in a crosslinking experiment. This is conventionally referred to as the 'n2 problem' – for n possible peptides, there can be ~n2 possible crosslinked peptide pairs. This problem is amplified for heterobifunctional crosslinkers with broad reactivity such as SDA, which, unlike BS3 and DSSO, can form crosslinks involving any residue, massively increasing the search space. A final challenge is that the rate of data acquisition made possible by modern instruments, such as the Orbitrap Astral, put significantly more strain on data processing pipelines and software compared to older instruments. To address these challenges, we have developed a novel XLMS search program, LinkCatcher. LinkCatcher is written in modern Fortran and is very high performance, processing Orbitrap RAW files in ~2-3 minutes and Astral RAW files in ~10-15 minutes on consumer hardware, with modest memory requirements. We have benchmarked LinkCatcher on several published Orbitrap SDA and DSSO datasets and unpublished Orbitrap Astral SDA datasets, establishing identification performance equal to or surpassing existing search engines specializing in SDA. We have also directly compared data acquired on Orbitrap versus Orbitrap Astral, showing that, in the samples examined using our search engine, Astral data generally results in the identification of approximately twice as many crosslinks. We anticipate publicly releasing LinkCatcher in early 2027. LinkCatcher will democratize the SDA crosslinking techniques pioneered in our laboratory and aid in their adoption by other researchers.

Abstract P14

Presenter: Shivay Parihar

Title: Mapping the Aging-associated Actin Interactome: PTMs, ABP Interactions, and Tissue Specificity from Worms to Primates

Abstract: Actin is among the most abundant and dynamic proteins in eukaryotic cells, cycling between monomeric and filamentous states to support cell shape, motility, division, and transcription. This behavior is governed by two structural layers: post-translational modifications (PTMs) that control actin folding, polymerization, and ATP hydrolysis, and interactions with actin-binding proteins (ABPs) that build the filament architectures needed for its diverse functions. Actin cytoskeletal decline has recently been shown as feature of aging and age-related diseases, but most studies have focused on filament level deterioration rather than age related changes in actin's structural biology namely, its PTMs and ABP interactions. In preliminary work, I find that histidine 73 methylation, deposited by SETD3, declines with age in human skeletal muscle, and that N terminal acetylation, mediated by NAA80, is altered in mouse neutrophils during certain diseased states. I am generating C. elegans mutants for orthologs of NAA80 and SETD3 alongside actin PTM-mimetics to test the tissue specific impact of these PTMs on actin folding and function. I am profiling multiple PTMs of G vs F-actin across banked mouse, human, worm, and non-human primate muscle, brain and immune cells using IP-MS (bottom-up) to understand how PTM crosstalk and PTM-profile influence actin filament dynamics during aging. Future work will map actin's ABP interactions in muscle, neurons and intestine using LifeAct bound TurboID in worms.

Together, this work asks whether age associated PTM changes are an early, causal driver of actin cytoskeletal and proteostatic decline, rather than a downstream consequence.

Abstract #15

Presenter: Thomas Perkins, Jr.

Title: Discovery, biophysical, and structural characterization of a new family of positive allosteric modulators for MRGPRX1

Abstract: The primate specific Mas-related G protein-coupled receptors of subfamily X (MRGPRX) have emerged as critical mediators of non-histaminergic itch sensation (pruritus), inflammation and pain. Due to its intriguing dual functionality, MRGPRX1 has attracted particular attention as a novel therapeutic opportunity with the potential to treat both chronic itch and pain. MRGPRX1 is activated by endogenous enkephalin fragments, the most potent example being the BAM8-22 peptide. This activation can be further enhanced by positive allosteric modulators (PAMs). In this contribution we present how the integration of computational methods with complementary structural, biophysical and cellular techniques accelerated the discovery of MRGPRX1 allosteric modulators. Novel compounds were identified in a structure-based virtual screening campaign followed by iterative compound optimisation. A novel SPR biophysical assay, capable of distinguishing between different modes of action, demonstrated both on-target binding of selected compounds and revealed their modes of action. Positive allosteric modulation was confirmed in a cellular assay. To support the structure-based design of new chemical matter, we established robust protein production and cryo-EM workflows enabling the routine determination of high-resolution MRGPRX1 structures. Multiple complex structures, including MRGPRX1 bound to the endogenous ligand BAM8-22 and two newly identified PAMs, provided experimental insights into the respective modes of action of these PAMs and structural support for our optimisation activities. Collectively, these findings provide deeper understanding of positive allosteric modulation of MRGPRX1 and offer a platform for the rational design of targeted therapeutics for the treatment of chronic pain and pruritus.

Abstract #16

Presenter: Thomas Perkins, Jr.

Title: Nuvisan's Integrated Mass Spectrometry Platform: Advancing Drug Discovery Through Native MS, Chemical, and Quantitative Proteomics

Abstract: Nuvisan's Mass Spectrometry (MS) Platform provides a fully integrated proteomics infrastructure that supports the entire drug discovery process, from early target identification to biomarker discovery and quantification. A key strength of our platform is the combination of native MS-based protein interaction analysis, chemical proteomics and quantitative proteomics approaches. Native MS allows us to analyze proteins and protein complexes under non-denaturing conditions, providing insights into binding stoichiometry and ligand interactions. Our Affinity-Selection MS approach (MagMASS) enables high-throughput identification of binders and is particularly well-suited for complex targets, including protein complexes. For covalent drug discovery, our targeted covalent inhibitor (TCI) workflow covers the full pipeline, from broad reactivity profiling across multiple amino acids to LC-MS-based screening and kinact/KI determination. Our proteomics workflows encompass a broad spectrum of state-of-the-art quantification strategies. Using both data-independent (DIA) and data-dependent (DDA) acquisition strategies, we have experience with a broad range of quantification methods, including label-free (LFQ) and isobaric labeling approaches (TMT, iTRAQ), allowing us to select the most suitable approach for each project. This flexibility enables the robust identification and quantification of thousands of proteins across a wide range of biological samples. Downstream bioinformatic analyses, including gene set enrichment analysis (GSEA) and proteome-wide target profiling, translate large-scale proteomic data into meaningful biological insights. These analyses support a wide array of applications, including proteolysis-targeting chimera (PROTAC)-induced target degradation studies. All workflows are supported by a

modern, high-throughput instrument portfolio, delivering robust and scalable MS solutions across all stages of drug discovery, from hit identification to biomarker development.

Abstract #17

Presenter: Mark Petersen

Title: Mapping the interactions of residual water in dry protein samples by solid-state x-ray footprinting mass spectrometry (ssXFMS)

Abstract: Petersen M., Gupta S., Ralston C., and Marqusee S.

Proteins fundamentally depend on water for folding and function. As proteins are lyophilized, the water content decreases to approximately 10% (w/w) of total mass. This retained water is difficult to disassociate from protein powder, requiring temperatures over 100°C to measure by thermogravimetric analysis, which suggests that the water is tightly bound. Mapping protein-water interactions in the dry state may help elucidate why some proteins rehydrate robustly, while other proteins cannot be rehydrated and appear to misfold irreversibly. X-ray footprinting mass spectrometry (XFMS) is a well established method for studying proteins in solution. In XFMS, solvated protein is exposed to high power x-rays to generate hydroxyl radicals from surrounding water. These radicals oxidize nearby side chains, leading to +16 Da modifications. To map these modifications, samples are proteolyzed and analyzed by bottom-up LC/MS. To map protein-water interactions in dry protein, we have developed a solid-state version of XFMS (ssXFMS) in which radicals can be generated from either bound residual water or oxygen gas. X-ray dose dependence of +16 Da modifications have been observed in multiple different lyophilized proteins, allowing us to interrogate the conformations adopted by proteins in the solid milieu.

Abstract #18

Presenter: Helena Rheinbay

Title: Time-Resolved Native Mass Spectrometry for Direct, Label-Free Characterization of Protein Complex Assembly and Disassembly Kinetics In Vitro

Abstract: Protein–protein interactions and protein complex formation are fundamental to cellular function. Disease-associated mutations can disrupt these interactions, perturbing essential molecular pathways. Understanding the mechanisms governing complex assembly, stability, and kinetics is therefore crucial for elucidating disease mechanisms and identifying potential therapeutic strategies. In vitro studies of complex assembly kinetics often rely on indirect approaches such as Förster resonance energy transfer (FRET), which require fluorescent labelling and may perturb the system. Here, we investigate native mass spectrometry (native MS) as a potential label-free, direct approach for monitoring protein complex formation in vitro. We apply this strategy to the assembly of the neuronal SNARE complex, isolated and in the presence of Munc18-1 and Munc13-1, key regulators involved in SNARE-mediated membrane fusion. Native MS enables direct detection of distinct protein assemblies from small sample quantities, with measurement times ranging from approximately one minute to several hours. This provides the temporal resolution required to characterize complex formation kinetics within heterogeneous protein mixtures. Our work demonstrates the potential of native MS as a complementary approach for studying protein complex assembly and its regulation, with future applications for disease-associated mutations and potential therapeutic targets.

Abstract #19

Presenter: Jann Rusch

Title: Benchmarking crosslinking mass spectrometry to map protein interaction interfaces

Abstract: Crosslinking mass spectrometry (XLMS) is used to obtain structural information on protein complexes. However, to which extent crosslinks help identifying interacting regions between proteins and how this depends on the crosslinker used and properties such as length, disorder, and interface size of the interacting proteins is largely unclear. To answer these questions, we created a benchmark dataset of

24 protein interactions with resolved structures and different interface and protein features. We selected five different crosslinkers based on either NHS- or photo-crosslinking chemistry: photo-methionine, photo-leucine, SDA, SSDA and BS3. Crosslinking was performed either in cells or after enrichment of the transiently expressed protein pairs through co-immunoprecipitation. Our preliminary data suggest that interface size, disorder propensity, and crosslinking chemistry are important factors largely determining if and which structural information can be obtained on protein binding from the detected crosslinks. We hope that our work can guide future studies in the selection of crosslinking protocols and in the interpretation of XLMS data to accelerate structural annotation of protein interactomes.

Abstract #20

Presenter: Rona Schein

Title: Variant-Specific Remodeling of the Munc18-1 Interactome in STXBP1-Associated Autism and Epilepsy

Abstract: Autism spectrum disorder (ASD) and epilepsy frequently co-occur as neurodevelopmental conditions driven by shared genetic architecture and convergent synaptic dysfunction. We hypothesize that disease-associated variants within the same risk gene induce distinct protein-protein interaction (PPI) perturbations that may contribute to phenotypic heterogeneity. Munc18-1, encoded by STXBP1, is an essential presynaptic regulator of SNARE-mediated synaptic vesicle fusion, with pathogenic variants associated with epilepsy and ASD. To test our hypothesis, we used affinity purification–mass spectrometry (AP-MS) to map PPIs of wild-type Munc18-1, STX1A and STX1B and disease-associated missense variants in HEK293T cells. Preliminary analyses reveal variant-specific remodeling of the Munc18-1 interactome. ASD-associated variants retain or show increased association with STX2/3, whereas epilepsy-associated variants show reduced association with these proteins.. Together, our findings suggest that variant-specific perturbation of the Munc18-1 interactome may contribute to phenotypic heterogeneity across STXBP1-associated disorders.

Abstract #21

Presenter: YUQI SHI

Title: Low-scrambling Calibrations for Single-Residue HDX-MS application on Thermo Scientific Orbitrap Tribrid Platforms

Abstract: Single-residue hydrogen–deuterium exchange mass spectrometry (HDX-MS) provides detailed insight into protein structure, dynamics, and interactions by localizing exchange at the amino-acid level. This resolution requires fragmentation methods that preserve solution-phase deuterium labeling, because gas-phase hydrogen scrambling can obscure residue-specific information. Orbitrap Tribrid platforms support several fragmentation techniques, including ETD, EThcD, UVPD, IR-ETD, and IR-EThcD; however, each requires platform-specific optimization to minimize scrambling and back-exchange. Here, we systematically evaluated calibration strategies across multiple Tribrid systems and fragmentation modes. A model peptide, HHHHHHIIKIIK, was prepared in H_2O and D_2O and analyzed on Orbitrap Lumos, Eclipse, Ascend Structural Biology Tribrid, and Apex Tribrid mass spectrometer. Source, capillary, and fragmentation parameters were tuned to generate low- and high-scrambling conditions. Deuterium retention and residue-level scrambling were evaluated using HXExpress. Lumos and Eclipse showed comparable low-scrambling settings, including Source Offset 10, IRM Offset −2.5, and TL1 Offset 9,

although IRM Offset required separate optimization for peptide and protein modes. Ascend and Apex Tribrid required Source Offset 12, FIRM Offset values of −2.4 and 2.34, and an IRM Offset of −2.54. Under optimized conditions, supplemental activation in EThcD did not increase scrambling up to 40% HCD energy. UVPD produced substantial scrambling under ETD-optimized conditions, but enabling UVPD in the high-pressure ion-trap region reduced scrambling by approximately 40% for +3 precursors and permitted irradiation times up to 100 ms. On Apex, IR-ETD and IR-EThcD maintained approximately 10% scrambling at IR power below 8%, with no additional scrambling from supplemental activation. These results provide practical guidance for robust, reproducible single-residue HDX-MS across Orbitrap Tribrid platforms.

Abstract #22
Presenter: Soni Neelesh
Title: Structural Principles of the Nuclear Basket and Its Dynamic Molecular Neighborhood
Abstract: The Nuclear Pore Complex (NPC) controls transport between the nucleus and cytoplasm and supports RNA processing and nuclear organization. The nuclear basket is a flexible, cage-like structure on the nuclear side of the NPC. It provides interaction sites for proteins that control mRNA surveillance, mRNA export, chromatin organization, and transport. Using integrative modeling, we combined cross-linking mass spectrometry (XL-MS), in-cell cryogenic electron tomography (cryo-ET), and structural predictions to compute a more complete and precise structural model of the yeast NPC and its nuclear basket. Our model shows a distributed attachment network that connects Nup1, Nup2, Nup60, Nup145N, and the Mlp proteins to the NPC scaffold using a combination of structured domains, linear motifs, and intrinsically disordered regions. Analysis of individual NPC tomograms shows that NPC structural variability is larger in regions distal from the core scaffold. This result shows that the basket has a highly dynamic structure. We also determined the structure of the TREX-2 mRNA-export complex bound near the nuclear side of the central transport machinery. TREX-2 also dynamically associates with the NPC and links stable basket architecture to transient RNA-processing machinery. The modeled structure suggests that mRNP processing and transport occur together at the NPC rather than as separate steps. We also model Pml39 as a basket-associated factor and identify Cmi7 as an Mlp2-dependent basket component. These results show that the nuclear basket is a dynamic and modular platform for the NPC molecular neighborhood. It connects mRNA processing and export with nucleocytoplasmic transport and nuclear organization.

Abstract #23
Presenter: Susan Tsutakawa
Title: AlphaFold is amazingly accurate except for the important bits
Abstract: Non-favored protein side-chain rotamers, spanning allowed and outlier conformations, are frequently dismissed as only in specialized cases.. Analyzing the high-resolution TOP2018 dataset comprising 12,873 entries reveals that over 80% of PDB entries contain at least one non-favored residue. Mapping these conformations against AI/ML ligand-binding predictors and evolutionary trace scores shows that allowed and outlier rotamers are significantly enriched within functional sites and correlate with high evolutionary conservation. These findings suggest that non-favored rotamers provide critical energetic advantages for catalysis and ligand recognition. However, evaluating AlphaFold 2 and AlphaFold 3 models against experimental structures demonstrates that prediction algorithms systematically fail to capture this local strain, modeling > 75% of experimental non-favored rotamers as

favored conformations. While these strained states are biologically vital for mechanistic function, current machine learning predictors smooth over them, underscoring the need for improved structural refinement and prediction accuracy in modeling functional microenvironments.

Abstract #24

Presenter: Rosa Viner

Title: Native Top-Down MS^n for Structural Characterization of RNA–Fluorescent Dye Complexes

Abstract: Oligonucleotides are increasingly important as therapeutic agents and drug targets, and their higher-order structures play critical roles in function and biomolecular interactions. Analytical approaches capable of probing oligonucleotide conformation and ligand-induced structural changes are therefore needed. Here, we investigated RNA interactions with RNA-specific fluorescent dyes using native mass spectrometry (MS) combined with conformation-sensitive top-down MS^n fragmentation. A 59-nucleotide hairpin RNA and RNA-specific fluorescent dyes were analyzed by native nanoelectrospray ionization in positive and negative ion modes using a Thermo Scientific™ Q Exactive™ UHMR or Orbitrap™ Tribrid™ Apex Structural Biology mass spectrometer. Native top-down MS^n experiments were performed on the Orbitrap Tribrid Apex MS equipped with a 213 nm UV laser, combining low-q collision-induced dissociation (lqCID) with activated electron photodetachment dissociation (a-EPD). Data were analyzed using Thermo Scientific™ OligoFinder Software. Native MS confirmed binding of up to eleven dye molecules per RNA. Structural characterization focused on RNASelect Red (1:1 RNA) and RiboGreen (1:4 and 1:11) complexes. Top-down MS^2 (lqCID) and MS^3 (a-EPD/lqCID) revealed distinct fragmentation patterns between apo and dye-bound RNA. Apo RNA exhibited more extensive fragmentation than RiboGreen-bound RNA, indicating increased structural stability upon dye binding. Fragmentation along the main stem extended to nucleotide position 15 for apo RNA but only to position 5 with RiboGreen, consistent with stabilization through intercalation within the A-form helix. In contrast, RNASelect Red did not show reduced fragmentation upon binding, suggesting a different interaction mechanism. These results demonstrate the utility of native top-down lqCID combined with UV a-EPD for characterizing oligonucleotide higher-order structure and noncovalent interactions.

Abstract #25

Presenter: Wanpeng Wang

Title: In situ spatial proteomics reveals interactome of Ci as activator and repressor of the Hh signaling pathway

Abstract: In Drosophila, the transcription factor Ci is the sole output of the Hedgehog (Hh) signaling pathway. Depending on Hh signaling states, Ci undergoes proteolysis and post-translational modifications that alter its localization and interactome. To systematically characterize Ci as an activator or repressor of the Hh pathway, we used TurboID and APEX2 knock-in alleles to map its proximity via native in situ spatial proteomics. Combined with CUT&RUN using an endogenously GFP-tagged Ci allele, we provide a systematic functional map of Ci as the primary executor of Hh signaling.

Abstract #26

Presenter: Divya Yadav

Title: Trigger factor delays the assembly of GAPDH: a noncanonical chaperone function revealed by structural proteomics

Abstract: Divya Yadav[1 2], Jaro Schmitt[3], Alain Scaiola[4], Nenad Ban[4], Bernd Bukau[3], Stephen D. Fried[1] 1Department of Chemistry, Johns Hopkins University, 3400 N. Charles Street, Baltimore, MD 21218, USA, 2Department of Structural Biology, St. Jude Children's Research Hospital, 262 Danny Thomas Place, Memphis, TN 38105, USA, 3Center for Molecular Biology of Heidelberg University (ZMBH), Heidelberg, Germany, 4Institute of Molecular Biology and Biophysics, Department of Biology, ETH Zurich, Switzerland.

Many cytosolic proteins begin to fold, and sometimes to assemble, while they are still being made on the ribosome. Chaperones are generally assumed to help with these early steps. We used limited proteolysis–mass spectrometry (LiP-MS) to compare E. coli lacking DnaK/DnaJ or trigger factor (TF) with wild-type cells. Loss of DnaKJ structurally perturbs ~28% of the soluble proteome, whereas loss of TF affects only ~3%. Chaperone dependence in vivo is also largely unrelated to chaperone dependence during refolding in vitro (Yadav et al., Mol Syst Biol 2025). Glyceraldehyde-3-phosphate dehydrogenase (GapA) stood out among the few proteins that depend on TF. Here we show that TF acts on GapA by preventing its cotranslational assembly, not by promoting it. We tagged GapA at its native chromosomal locus and purified it from wild-type, ΔdnaKJ and Δtig cells. GapA made without TF has a distinct circular dichroism (CD) signature, a lower sedimentation coefficient and a roughly threefold higher turnover number, so it is biophysically different from wild-type GapA. Selective ribosome profiling showed that TF binds nascent GapA tightly through most of its synthesis. In wild-type cells we detect no cotranslational (co-co) assembly of GapA. In Δtig cells, nascent GapA chains engage each other near the end of translation. TF therefore enforces post-translational assembly of the tetramer. Preliminary cryo-EM of Δtig GapA (four classes, 2.3–2.9 Å) suggests a structural consequence. In one class (~18% of particles), the density around K184/T185 fits a domain swap between subunits rather than the canonical arrangement, which agrees with the profiling data. These results point to a new role for TF in controlling when oligomers assemble. They also suggest that a chaperone can shape native structure by delaying assembly rather than speeding it up.

Abstract #27

Presenter: Petrus Zwart

Title: Uniform Data Interchange and Access Across DOE Molecular-Science Beamlines and Modalities

Abstract: Users of DOE molecular-science facilities increasingly work across sites and techniques: a single project may combine rotation and serial crystallography at NSLS-II, SAXS at the ALS, and tomography or spectroscopy elsewhere. Each facility, however, exposes its data through its own layouts, logs, and conventions, so the user bears the cost of reconciling them, and no downstream tool can consume results from more than one source without bespoke adapters. Here we present a cross-facility effort to build the infrastructure to make interchange between facilities, and access across modalities, uniform.

Abstract #28

Session: SESSION NINE - Emerging Technologies

Speaker: Xiaojing Sui

Title: Mapping the Landscape of Proteome Metastability and Structural Plasticity in Aging and Proteotoxic Stress

Abstract: Aging and proteotoxic stress compromise proteome stability, leading to altered protein solubility, aggregation, and proteostasis collapse. However, the organismal magnitude of proteome-wide structural shifts and their downstream functional consequences remain uncharacterized. Here, we used quantitative structural proteomics coupling limited proteolysis with TMT-mass spectrometry (LiP-TMT-MS) to map the dynamic conformational landscape of the Caenorhabditis elegans proteome during aging and proteotoxic stress driven by metastable or polyglutamine (polyQ) proteins. Well before physiological decline, one-third of the aging proteome undergoes significant structural alterations. The primary targets of these age-associated shifts map directly to the proteostasis network, including ribosomes, chaperones, and the ubiquitin-proteasome system. Conformationally altered proteins are enriched in intrinsically disordered regions, β-sheets, and ubiquitination sites, with changes concentrated in functionally critical domains. Notably, comparing aging with non-autonomous proteotoxic stress models—animals expressing a temperature-sensitive myosin mutation or muscle-specific polyQ expansions—revealed distinct proteome-wide landscapes. For example, animals expressing metastable myosin-ts in body wall muscle

cells at permissive temperatures has organism-wide consequence affecting conformational changes in ~150 proteins both within and beyond the sarcomere, alongside a striking ~140-fold induction of small heat shock proteins (Hsp16s) that co-localize with thick filaments without broadly activating other chaperones. PolyQ35 and polyQ40 expression similarly induced widespread conformational changes across hundreds of proteins, showing minimal overlap with the myosin-ts metastable sub-proteome and promoted the aging signature. Importantly, tissue-restricted proteotoxic stress triggers systemic, cell-non-autonomous proteome instability, impacting neuronal, intestinal, and secreted proteins. Together, our findings show that early proteome-wide structural dynamics reflect both adaptive stress responses and progressive dysfunction during aging and proteotoxic stress.

Abstract #29

Session: SESSION NINE - Emerging Technologies
Speaker: Darren Kahan
Title: Detecting Long-Range Biases in Intrinsically Disordered Proteins Using X-ray Footprinting / Mass Spectrometry
Abstract: The fast dynamics and structural heterogeneity that define intrinsically disordered proteins (IDPs) and regions (IDRs) make them refractory to most traditional structural and biophysical methods. We have developed a new method for monitoring transient long-range sidechain burial in an IDP's conformational ensemble using X-ray generated hydroxyl radical footprinting monitored by mass spectrometry (XFMS). Using this approach, we identified biases in the ensemble of the IDP Cited2 which are disrupted by chemical denaturation and perturbed by distal sequence changes. Moreover, we show that these buried regions are key drivers of ligand binding and function. We find that for the human transcription factor Sox2, transient intradomain interactions in a long IDR compete with interdomain contacts; this competition is modulated by DNA-binding. In sum, we demonstrate that XFMS as an exciting new tool for characterizing interactions in IDPs and informs on the relationship between an IDP's conformational ensemble and its function.

Abstract #30

Session: SESSION ONE - Cross-Linking Mass Spectrometry (Part I)
Speaker: Changjian Feng
Title: Quantitative crosslinking mass spectrometry reveals phosphorylation-induced changes in the functional protein dynamics of neuronal nitric oxide synthase
Abstract: Nitric oxide synthase (NOS) is a family of multi-domain flavo-hemo-proteins that is responsible for biosynthesis of NO in vivo. Its function depends on extensive domain-domain docking interactions and conformational dynamics, both of which are regulated by protein-protein interactions and post-translational modifications such as phosphorylation. Its large size and intrinsic conformational dynamics have limited detailed molecular-level biophysical characterization. How NOS responds to regulatory factors at the molecular level remains largely unclear. We have led efforts to develop and apply quantitative cross-linking mass spectrometry (qXL MS), in combination with AlphaFold2 modeling and clustering analyses, to provide detailed structural dynamics information on the conformational landscape and its remodeling by external partnering proteins such as calmodulin (CaM) and mutation at domain-domain docking interfaces. We mostly focused on truncated NOS constructs that are minimal models for the NOS output state for NO production. In this work, we extend our approach to full-length neuronal NOS (nNOS) enzyme and its phosphorylated form. We compare DSBU crosslinks in four sample groups: nNOS, CaM-bound nNOS, pSer1412 nNOS, and CaM-bound pSer1412 nNOS. This is to gain insights into the effects of phosphorylation on both CaM-free and CaM-bound samples, as in vivo evidence indicates that the phosphorylation may attenuate the requirement for CaM in NOS activation by affecting conformational dynamics. In addition, we aim to use crosslinks to both validate AF2 structural models of

full-length nNOS and guide structural modeling. Our initial qXL MS data reveal notable changes in domain-domain crosslinks, particularly for the CaM-free samples. These exciting new results at the residue level point to an allosteric conformational remodeling induced by phosphorylation.

Abstract #31

Speaker: Andrés Reyes

Title: Targeted Top-Down Quantification of Histone Proteoforms by ETD-IR Proteoform Reaction Monitoring

Abstract: The Center for Research on Integrative Structural Proteomics (CRISP) at UCSF is developing advanced top-down mass spectrometry capabilities to characterize and quantify intact proteoforms, enabling direct analysis of protein modifications and their combinatorial patterns. By integrating Orbitrap Tribrid APEX instrumentation with advanced fragmentation strategies and targeted workflows, CRISP aims to provide proteoform-resolved insights into protein regulation and disease-associated alterations. To demonstrate these capabilities, we developed a histone proteoform reaction monitoring (PfRM) workflow using infrared-assisted electron transfer dissociation (ETD-IR) on the Orbitrap Tribrid APEX. We evaluated 20 data-dependent acquisition (DDA) fragmentation methods using purified histones and a 15-minute LC gradient. ETD-IR outperformed conventional ETD, CID, and HCD, generating more matched fragment ions and higher sequence coverage. Using Proteoform Studio, we identified high-confidence histone proteoforms and selected 34 proteoforms for targeted analysis. PfRM was performed using Proteoform Finder (ProSight Native). The enhanced fragmentation efficiency of ETD-IR provides more quantifiable transitions for targeted proteoform monitoring. This workflow demonstrates the potential of targeted top-down proteomics for characterizing combinatorial PTMs and provides a basis for future applications to disease-associated proteoforms in diverse biological systems.

Abstract #32

Speaker: Cao Son Trinh

Title: Mapping the Architecture of Protein Complexes Using Cross-Linking Mass Spectrometry

Abstract: The Center for Research on Integrative Structural Proteomics (CRISP) at UCSF is establishing an advanced cross-linking mass spectrometry (XL-MS) platform to map protein interaction networks and resolve the structural architecture of molecular machines at the proteome scale. By integrating complementary cross-linking strategies, including enrichable PhoX and MS-cleavable DSBSO, with advanced mass spectrometry and structural modeling, CRISP aims to characterize protein complex composition, connectivity, and architecture across diverse biological systems.

Our expertise in large-scale XL-MS is demonstrated by our recent PNAS study (Trinh et al., 2026), in which an optimized PhoX-based workflow identified 52,944 unique cross-linked peptide pairs across 5,064 proteins, revealing 3,083 protein–protein interactions in Arabidopsis. These data captured both well-characterized and previously underexplored protein assemblies, providing spatial constraints to investigate their structural organization and connectivity.

Building on this established expertise, CRISP is extending XL-MS capabilities to neuronal systems, cancer, and infectious diseases, with the goal of mapping disease-associated protein interaction networks and uncovering structural alterations underlying cellular dysfunction. By integrating XL-MS with complementary structural and computational approaches, CRISP aims to provide molecular insights into disease mechanisms and support the development of future therapeutic strategies.

Reference

Trinh, C. S*., Shrestha, R*., Mao, P.*, …Xu, S. (2026). Mapping the architecture of protein complexes in Arabidopsis using cross-linking mass spectrometry. PNAS, 123(33), e2519615123.

Speaker Abstracts (Alphabetical by Last Name)

Session: SESSION SEVEN - Protein Interactions
Speaker: Simon Butterworth
Title: Proteome-wide crosslinking mass spectrometry reveals novel components of essential complexes in Toxoplasma
Abstract: Protein–protein interactions underpin nearly all cellular processes, yet systematic definition of these networks remains limited outside a few model organisms, leaving the architectures of essential complexes across eukaryotic diversity uncharted. We developed a high-coverage crosslinking mass spectrometry (XL-MS) framework to define the proteome-wide interactome of Toxoplasma gondii, a human pathogen that chronically infects >2 billion people and diverged from conventional model eukaryotes over 1 billion years ago. Adapting sample preparation to the parasite's distinct cell biology and improving existing XL-MS methods, we identified 29,624 DSSO-crosslinked peptide pairs defining 2,859 protein–protein interactions at a 1% interaction-level FDR. This network recovered >160 protein complexes identified in Toxoplasma or inferred by homology, plus >2,000 novel interactions. We modeled each crosslinked pair with Chai-1, finding broad concordance between model confidence metrics and crosslink-derived distances, and showing how crosslink constraints can steer predictions toward accurate structures and identify alternative interfaces. Combining molecular genetics, co-immunoprecipitation, and functional assays, we validated unrecognized components of essential complexes, including a structurally distinct ATP synthase subcomplex built around a divergent, apicomplexan-specific alpha subunit essential for parasite fitness. Beyond revealing unexpected diversification of core mitochondrial machinery, this work establishes a transferable strategy to define the architecture of divergent organisms and a foundational resource for discovering lineage-specific vulnerabilities in pathogen biology.

Session: SESSION SIX - Computational Modeling
Speaker: Ignacia Echeverria
Title: Integrative structure modeling of SynGAP1 in the context of the postsynaptic density
Abstract: The postsynaptic density (PSD) is a mesoscale, phase-separated assembly of hundreds of proteins that organizes excitatory synaptic signaling, and its dysregulation underlies many neurodevelopmental disorders. The compositional complexity and structural heterogeneity of the PSD make it intractable to traditional structural characterization. Using a minimal set of PSD scaffold proteins, we investigated how perturbations such as point mutations and phosphorylations reshape the PSD scaffold, focusing on SynGAP1, a central Ras/Rap-GAP and one of the most abundant PSD proteins. SynGAP1 plays a dual role within the PSD: its GAP activity regulates receptor trafficking, while its structural and disordered regions drive PSD condensation. Consequently, to understand SynGAP1’s role in the PSD, we need to describe its structure within the PSD scaffold architecture and the material state of the condensate. We address this by building an integrative structural model of SynGAP1 within a minimal PSD, incorporating crosslinking mass spectrometry (XL-MS), cryo-electron tomography, AlphaFold3/Boltz predictions, and affinity-purification MS (AP-MS). However, none of these data sources capture the properties of the condensate through the dynamics of SynGAP1's disordered regions and the multivalent interactions that drive phase separation. We therefore performed coarse-grained slab molecular dynamics simulations to characterize SynGAP1 phase separation under different conditions, used these simulations to infer structural and dynamical properties of SynGAP1, and incorporated those

properties into our integrative models. We found that changes in the SynGAP1 trimerization region are sufficient to perturb SynGAP condensation properties and the PSD scaffold organization.

Session: SESSION ONE - Cross-Linking Mass Spectrometry (Part I)
Speaker: Changjian Feng
Title: Quantitative crosslinking mass spectrometry reveals phosphorylation-induced changes in the functional protein dynamics of neuronal nitric oxide synthase
Abstract: Nitric oxide synthase (NOS) is a family of multi-domain flavo-hemo-proteins that is responsible for biosynthesis of NO in vivo. Its function depends on extensive domain-domain docking interactions and conformational dynamics, both of which are regulated by protein-protein interactions and post-translational modifications such as phosphorylation. Its large size and intrinsic conformational dynamics have limited detailed molecular-level biophysical characterization. How NOS responds to regulatory factors at the molecular level remains largely unclear. We have led efforts to develop and apply quantitative cross-linking mass spectrometry (qXL MS), in combination with AlphaFold2 modeling and clustering analyses, to provide detailed structural dynamics information on the conformational landscape and its remodeling by external partnering proteins such as calmodulin (CaM) and mutation at domain-domain docking interfaces. We mostly focused on truncated NOS constructs that are minimal models for the NOS output state for NO production. In this work, we extend our approach to full-length neuronal NOS (nNOS) enzyme and its phosphorylated form. We compare DSBU crosslinks in four sample groups: nNOS, CaM-bound nNOS, pSer1412 nNOS, and CaM-bound pSer1412 nNOS. This is to gain insights into the effects of phosphorylation on both CaM-free and CaM-bound samples, as in vivo evidence indicates that the phosphorylation may attenuate the requirement for CaM in NOS activation by affecting conformational dynamics. In addition, we aim to use crosslinks to both validate AF2 structural models of full-length nNOS and guide structural modeling. Our initial qXL MS data reveal notable changes in domain-domain crosslinks, particularly for the CaM-free samples. These exciting new results at the residue level point to an allosteric conformational remodeling induced by phosphorylation.

Session: SESSION FIVE - CryoEM/CryoET
Speaker: Mia Greeson
Title: Structural and proteomic dissection of GPR143 defines a non-canonical GPCR-effector interface underlying ocular albinism
Abstract: Ocular albinism type 1 (OA1) is an X-linked disorder characterized by severe visual impairment with no approved therapies. OA1 is caused by loss-of-function mutations in GPR143, an atypical orphan G protein-coupled receptor (GPCR) that localizes to melanosomes, the organelles that synthesize melanin. Although GPR143 is essential for melanosome biogenesis, its signaling mechanism has remained undefined, owing to its orphan status and absence of a solved structure. To delineate GPR143's signaling mechanism, we integrated cryo-electron microscopy, cell-based signaling assays, and unbiased proximity labeling proteomics. Cryo-EM reveals a non-canonical activation mechanism in which the receptor's N-terminus occupies the orthosteric pocket as a tethered peptide agonist, stabilizing an active conformation in the absence of an exogenous ligand. BRET-based assays demonstrate that GPR143 constitutively recruits β-arrestins but not G proteins. Notably, OA1 patient mutations clustered in the third intracellular loop (ICL3) of GPR143 do not disrupt β-arrestin recruitment, suggesting an alternative disease mechanism. Unbiased proximity labeling proteomics reveals that GPR143 recruits the BLOC-3 complex—a lysosome-related organelle biogenesis factor—to endomembrane compartments in an ICL3-dependent, β-arrestin-independent manner. AlphaFold modeling predicts a GPR143/BLOC-3 interaction interface at ICL3, coinciding with the site of OA1-associated mutations. Consistent with this model, disease-associated variants selectively disrupt GPR143-BLOC-3 interactions while leaving β-arrestin recruitment intact, linking loss of this non-canonical signaling complex to disease pathogenesis. Together, these findings establish GPR143 as a structurally and functionally atypical GPCR through a

previously undescribed mode of GPCR-effector coupling. This work provides a mechanistic and proteomic framework for organelle-localized GPR143 signaling and suggests the GPR143-BLOC3 complex as a candidate therapeutic target for OA1.

Session: SESSION THREE - Native/Top Down
Speaker: Kallol Gupta
Title: Resolving Dynamic Membrane Signaling Assemblies by Single-Ion Lipid-Vesicle Native Mass Spectrometry
Abstract: Dynamic, multivalent associations between membrane proteins and their interactomes are fundamental to membrane-associated signaling. These interactions generate heterogeneous, polydisperse macromolecular assemblies on organellar membranes that drive signaling outcomes. Defining molecular progression of these events therefore requires precise, unbiased detection of the formed multimeric assemblies directly from membranes. Here, we present a single-ion lipid-vesicle nativeMS platform that reconstitutes the protein components of a signaling pathway in customizable lipid membranes and directly detects the resulting assemblies by native top-down MS. The single-ion detection capability enables detection of both abundant and rare signaling components and captures molecular progression of the membrane-associated signaling assemblies. The top-down capabilities resolve the protein constituents and stoichiometries of the range of mass detected multiprotein complexes formed during signaling, directly from membranes. Using neuronal SNAREs, we determine the dynamic, multimeric assembly of SNAREs and associated proteins that dock synaptic vesicles to plasma membrane. Next, applying this to SNAREopathic neurological disorders, we show how rare disease associated SNARE-variants abrogates formation, stoichiometry, and abundance of key molecular complexes that leads to impaired synaptic transmission. This provides a direct molecular explanation for SNAREopathic neurological disorders. Broadly, the work provides a generalizable structural-proteomics strategy for resolving heterogeneous membrane-associated signaling assemblies and how disease mutations reshape their molecular trajectories

Session: SESSION NINE - Emerging Technologies
Speaker: Nicolas Hartel
Title: Development of DIA workflow for Multiplex Hydroxyl Radical Protein Footprinting
Abstract: Nicolas Hartel1, Daniel A. Polasky2,4, Fengchao Yu2,4, Carolina Rojas Ramirez2, Rohit Jain3, Janna Kiselar3, Mark R. Chance3, Alexey I. Nesvizhskii2,4, Rosa Viner1
1Thermo Fisher Scientific, San Jose, CA , 2University of Michigan, Ann Arbor, MI, 3Case Western Reserve University, Cleveland, OH, 4Fragmatics LLC, Northville, MI
Protein footprinting coupled with mass spectrometry (PF-MS) enables characterization of protein higher-order structure through covalent modification of amino acid side chains. However, the diverse modifications generated by multiplex PF increase data-processing complexity and challenge reproducible site localization and quantification. Here, we evaluated spectral library-based data-independent acquisition (DIA) and label-free data-dependent acquisition (DDA) for multiplex PF-MS analysis of calmodulin and NISTmAb standard. Proteins were labeled with sodium triflinate and exposed to X-ray radiolysis at the NSLS-II XFP beamline. Following trypsin/Lys-C digestion and LC separation, peptides were analyzed using an Orbitrap Astral mass spectrometer with DDA and DIA acquisition. Data were processed using a pre-release build of FragPipe 25, incorporating an optimized PTMProphet-based localization workflow for multiplex PF modifications and a new Extent of Modification summarization tool supporting DDA and DIA quantification. DIA isolation windows ranging from 2–3 Th were evaluated. For NIST mAb and calmodulin 858 and 1,387 precursors were quantified with CV <20% using 3 Th windows, respectively. Increasing the isolation window from 2 to 3 Th reduced PTM peptide identification but improved quantification by scanning more points across the peak in DIA MS2. Narrow-window DIA provided comparable or improved quantitative reproducibility relative to DDA for peptide-level

conformational analysis, whereas DDA precursor-based quantification remained advantageous for single-amino-acid-resolution analysis. Together, PTMProphet localization and Extent of Modification summarization provide an integrated FragPipe workflow that streamlines multiplex PF-MS analysis while supporting complementary DIA peptide-level and DDA residue-level structural characterization.

Session: SESSION EIGHT - Cross-Linking Mass Spectrometry (Part II)
Speaker: Yi He
Title: An innovative data-independent acquisition (DIA) approach for the quantitative analysis of protein-nucleic acid interactions in living cells
Abstract: Mass spectrometry can resolve UV- and chemically induced protein–RNA crosslinks at both the peptide and amino-acid level. However, analyzing these crosslinks in complex systems remains challenging, particularly when comparing cellular conditions or states. Previous protein–RNA crosslinking studies have mostly used data-dependent acquisition (DDA) approach. Here, we developed data-independent acquisition (DIA) workflows using UV- or chemically crosslinked E. coli ribosomes. The DIA data was acquired with 2 Th or 4 Th isolation windows and searched against spectral libraries built from DDA runs of each sample. For E. coli ribosomes crosslinked with nitrogen mustard (NM), the two window schemes yielded an 86% overlap in identified nucleotide-crosslinked peptides (NuXLs) and a precursor-intensity correlation of 0.97. We then applied the workflow to ribonucleoprotein (RNP) complexes isolated from living cells, quantifying RNA-binding activity at the residue level in samples treated with or without clickable electrophilic purines (CEPs) before or after UV irradiation. Across 452 identified NuXLs, cysteine was the most frequently crosslinked residue, and CEP treatment reduced RNA-binding activity by an average of 2.3-fold, consistent with the probe occupying RNA-interaction sites. Together, these DIA workflows enable quantitative, residue-resolved investigation of protein–nucleic acid interactions in complex systems.

Session: SESSION ONE - Cross-Linking Mass Spectrometry (Part I)
Speaker: Franz Herzog
Title: Mechanistic insights into targeted protein degradation by chemical crosslinking and quantitative mass spectrometry
Abstract: Targeted protein degradation is a promising therapeutic strategy aimed at selectively eliminating disease-associated proteins through ubiquitination. A key component of this process is a class of E3 ligases, which catalyzes the transfer of ubiquitin moieties onto substrate proteins. The substrate receptor Cereblon facilitates targeted protein degradation through its interaction with the core ligase complex. Importantly, the activity of Cereblon can be modulated by Cereblon E3 ligase modulating drugs (CELMoDs), which function by altering substrate specificity and facilitating the recognition of neosubstrates. The therapeutic efficacy of CELMoD agents correlates with their ability to induce the closed conformation of Cereblon. CELMoD agents differ significantly in their capacity to promote this structural transition, highlighting the need for methods that can assess the efficacy beyond binding affinity measurements. To overcome this limitation, we applied chemical crosslinking combined with quantitative mass spectrometry (qXL-MS) to detect the structural rearrangement of protein domains within Cereblon. By systematically comparing crosslink intensities between the open and closed states of Cereblon, we identified two distinct regions showing significant conformational shifts, facilitating the quantitative assessment of local structural changes upon CELMoD binding. Chemical crosslinking and quantitative mass spectrometry provide mechanistic insights into drug-induced conformational rearrangements in E3 ligases demonstrating the potential of qXL-MS as a quantitative tool for assessing compound-induced structural transitions.

Session: SESSION EIGHT - Cross-Linking Mass Spectrometry (Part II)
Speaker: Krista Kaasik

Title: Crosslink guided modeling reveals O-GlcNAc dependent conformational dynamics of the giant presynaptic scaffolds Bassoon and Piccolo
Abstract: O-GlcNAcylation, the reversible addition of a single N-acetylglucosamine to serine and threonine residues, regulates the function of nuclear, cytoplasmic, and mitochondrial proteins. Despite its abundance at neuronal synapses, how O-GlcNAcylation influences the structure and assembly of synaptic proteins remains poorly understood. Using a conditional granule cell-specific knockout of O-GlcNAc transferase (OGT) in the mouse cerebellum, we identified presynaptic active zone scaffolds Bassoon and Piccolo as major targets of O-GlcNAc dependent regulation. Proteomic analysis of cerebellar synaptosomes revealed a reduction of both proteins following OGT loss. Bassoon and Piccolo are highly disordered proteins and remain poorly characterized structurally. To investigate their molecular organization, we integrated DSSO crosslinking with structural modeling to generate experimentally constrained models of Bassoon and Piccolo. Molecular dynamics simulations of O-GlcNAc-modified and unmodified models revealed reduced local structural fluctuations in the modified proteins. Together with the crosslink-guided models, these results are consistent with O-GlcNAcylation modulating the conformational dynamics of these intrinsically disordered scaffolds in a manner that may favor interaction-competent Bassoon–Piccolo assemblies. Consistent with disruption of active zone organization, OGT-deficient granule neurons exhibit reduced Bassoon puncta at presynaptic terminals, followed by progressive granule cell loss and cerebellar neurodegeneration. Our results identify O-GlcNAcylation as a regulator of the structural dynamics and maintenance of giant presynaptic scaffolds and suggest a mechanism by which metabolic and post-translational modification state can influence long-term active zone integrity and neuronal survival.

Session: SESSION NINE - Emerging Technologies
Speaker: Darren Kahan
Title: Detecting Long-Range Biases in Intrinsically Disordered Proteins Using X-ray Footprinting / Mass Spectrometry
Abstract: The fast dynamics and structural heterogeneity that define intrinsically disordered proteins (IDPs) and regions (IDRs) make them refractory to most traditional structural and biophysical methods. We have developed a new method for monitoring transient long-range sidechain burial in an IDP's conformational ensemble using X-ray generated hydroxyl radical footprinting monitored by mass spectrometry (XFMS). Using this approach, we identified biases in the ensemble of the IDP Cited2 which are disrupted by chemical denaturation and perturbed by distal sequence changes. Moreover, we show that these buried regions are key drivers of ligand binding and function. We find that for the human transcription factor Sox2, transient intradomain interactions in a long IDR compete with interdomain contacts; this competition is modulated by DNA-binding. In sum, we demonstrate that XFMS as an exciting new tool for characterizing interactions in IDPs and informs on the relationship between an IDP's conformational ensemble and its function.

Session: SESSION FIVE - CryoEM/CryoET
Speaker: Oleg Klykov
Title: Approaches for physiologically relevant structural proteomics.
Abstract: Crosslinking mass spectrometry (XL-MS) is a powerful tool for structural insights into proteins and their interactions. XL-MS performed on mammalian samples like cells and tissues often fails to capture full proteome complexity and ignores transient higher order protein assemblies. To access the protein machinery in a near-native state, we developed an animal-level crosslinking approach that preserves relevant information within minutes of animals' death, thus providing the most physiologically and translationally relevant information. Our approach approximates the in vivo setting and utilizes mouse transcardiac perfusion and a combination of intracranial injections, thus coined as perfusion-XL (pXL) and intracranial injection-XL (iciXL). We successfully crosslinked mouse heart and brain with membrane-

permeable DSS and increased the depth of analysis with DSBSO reagent. Targeted cryoelectron tomography imaging of the brain tissues after iciXL confirmed physiologically intact synaptic arrangement. In a mouse model of heart cystic fibrosis, pXL outperforms classic crosslinking with a higher specificity towards translationally relevant targets. We then demonstrated the utility of iciXL on example mouse models of Opioid Use Disorder (OUD) tracing region-specific structural rearrangements induced by fentanyl. Taken together, we progressed XL-MS from cell culture directly into animals and envision broad utilization of physiologically relevant structural proteomics by clinicians.

Session: SESSION ONE - Cross-Linking Mass Spectrometry (Part I)
Speaker: Leah McDermott
Title: Mapping the Mitochondrial Permeability Transition Pore Interactome to Reveal Mechanisms of Human Aging and Disease
Abstract: Mitochondrial dysfunction is a hallmark of aging and age-related diseases. A major consequence is opening of the mitochondrial permeability transition pore (mPTP), a large inner-membrane channel that induces swelling and cell death. Despite decades of study, its molecular identity and mechanism of regulation remain unresolved. Adenine nucleotide translocase (ADT), ATP synthase, and Cyclophilin D have been implicated in pore formation and activity, but structural and interaction-level changes remain incompletely characterized. To address this gap, isobaric quantitative Protein Interaction Reporter technology was used to quantify protein-protein interaction (PPI) and conformational adaptations in mitochondria during calcium stress-induced mPTP opening. These quantitative cross-linking mass spectrometry (qXL-MS) data enabled novel and direct visualization of PPI and structural remodeling that accompanies mPTP opening. Among the prominent changes across bioreplicates, cross-link level changes indicative of ADT C-state depletion and M-state enrichment revealed a distinct conformation shift during permeability transition. Moreover, treatment with both calcium and the mPTP inhibitor cyclosporin A moderated and even reversed these changes, with C-state enrichment and M-state depletion, despite identical calcium exposure. This observation links ADT interactome changes to the functional state of the mPTP, rather than calcium stress. Together, these results indicate ADT and other protein interactome remodeling as fundamental components of permeability transition and highlight qXL-MS as a powerful approach for uncovering mPTP molecular mechanisms underlying mitochondrial dysfunction in aging and disease.

Session: SESSION EIGHT - Cross-Linking Mass Spectrometry (Part II)
Speaker: Andrew Michael
Title: Chromatographic Environment and Crosslinker Molecular Properties Shape the Analytical Sampling Space of Crosslinked Peptides
Abstract: Crosslinking mass spectrometry (XL-MS) relies largely on LC-MS workflows adapted from bottom-up proteomics, despite crosslinked peptides comprising a low-abundance, physicochemically distinct fraction of digests. How strongly the chromatographic environment determines the observable crosslink population remains poorly defined. In this presentation, we explore how LC configuration, mobile-phase composition, and crosslinker chemistry shape the XL-MS analytical sampling space. BSA crosslinked with DSS or DSPU was analyzed across two different LC systems, multiple chromatographic configurations, and mobile-phases modified with either formic, acetic, or propionic acid. We followed peptides behaviour from chromatographic separation through MS1 representation, MS2 sampling and crosslink identification. Changing LC configuration altered both the yield and identity of recovered crosslinks, demonstrating that chromatography shapes the population accessible to downstream identification. Altering the chemistry of the mobile-phase through acid modifiers changed peptide retention and peak dispersion, which propagated into changes in MS1 precursor intensity and charge-state representation. These chromatographic and MS1 changes extended to the breadth, redundancy, and

temporal positioning of MS2 sampling. Increased crosslink recovery did not require increased MS2 acquisition rates; under favourable conditions, less redundant sampling broadened precursor access within the available acquisition capacity. The productivity of MS2 sampling in crosslink recovery was strongly dependent on precursor properties, increasing with precursor intensity and varying with charge-state. Finally, differences in crosslinker-specific molecular properties affected chromatographic behaviour, showing that the chemistry installed by the crosslinker itself contributes to peptide retention. Collectively, the observable XL-MS sampling space emerges from interactions across chromatographic environment, precursor properties, MS2 allocation, and crosslinker-derived molecular properties.

Session: SESSION SEVEN - Protein Interactions
Speaker: Monita Muralidharan
Title: Structural and functional characterization of an LtgA–LtgD peptidoglycan-remodeling assembly in Neisseria meningitidis
Abstract: Peptidoglycan turnover in Neisseria meningitidis depends on multiple lytic transglycosylases (LTGs), including LtgA and LtgD. Because LTG-mediated peptidoglycan turnover influences envelope integrity and the production of host-recognized cell-wall fragments, defining the structural and functional consequences of LtgA–LtgD association may inform future studies of meningococcal physiology and the potential of LTGs as antibacterial targets. However, the functional consequence of LtgA–LtgD association remains unresolved. We used a comprehensive, multiscale structural and functional approach integrating X-ray crystallography, cryo-EM modeling, native mass spectrometry, enzymatic assays, and HDX-MS to characterize composition, quaternary organization, enzymatic activity, and assembly-associated solution-state dynamics. The 2.99 Å LtgD crystal structure captured a homodimeric arrangement, whereas the ~4.9 Å complex model placed the LtgA U-domains adjacent to the α- and core domains of LtgD, suggesting a candidate interaction surface. Native mass spectrometry independently confirmed monomeric LtgA, homodimeric LtgD in solution, and formation of a 1:1 LtgA–LtgD heterodimer. Functional assays showed that LtgA and LtgD each supported robust peptidoglycan turnover when assayed separately, whereas the complex exhibited no detectable activity under the same conditions. Comparative HDX-MS, interpreted with PISA-derived interface geometry, showed that multiple overlapping LtgD peptides (aa 50–68) in the modeled contact region had reduced minor exchange populations in the complex, although LtgD changes extended beyond the predicted interface. LtgA protection was localized to a distal 38–68 region represented by multiple overlapping peptides. Together, these findings identify an assembly with no detectable activity under the assay conditions and reveal partner-specific dynamic consequences.

Session: SESSION TWO - HDX/Covalent Labeling
Speaker: Petr Novak
Title: Mapping Protein Structure with Singlet Oxygen Labeling
Abstract: Singlet oxygen (^1O_2) is a highly reactive species that can covalently modify solvent-accessible amino acid residues in proteins, making it a promising probe for mass spectrometry-based structural proteomics. Here, we developed a quench-flow platform for generating ^1O_2 under mild conditions by photodynamic activation of phthalocyanine with red laser irradiation. The labeling reaction was efficiently quenched with sodium azide. The method was evaluated using intrinsically disordered and membrane proteins to assess its ability to report solvent-accessible surface area. Protein samples were analyzed by LC-MS/MS using a Vanquish Neo system (Thermo Scientific) and timsTOF SCP mass spectrometer (Bruker Daltonics), and the data were processed using DataAnalysis, MASCOT, and R-based workflows. The labeling strategy was further applied to compare monomeric and fibrillar α-synuclein, as well as the open and closed conformations of the E. coli chloride channel. In addition, analysis of serum samples demonstrated discrimination between monomeric and oligomeric α-synuclein. Our results show that

singlet oxygen labeling effectively captures conformational differences in proteins and provides structural information for both soluble and membrane-associated systems. These findings support the potential of ^1O_2 based labeling as a complementary approach for structural proteomics and biomarker-oriented studies.

Session: SESSION FOUR - Drug Discovery
Speaker: Darragh O'Brien
Title: HDX-MS Reveals the Structural Dynamics Underlying Drug Activity: Insights from Deubiquitinase Inhibitors
Abstract: Ubiquitin-specific protease 19 (USP19) is a membrane-associated deubiquitinase implicated in protein quality control, muscle atrophy, adipogenesis and metabolic signaling, making it an attractive therapeutic target for sarcopenic obesity and related metabolic disorders. Nevertheless, the conformational flexibility of deubiquitinases and limited structural information on inhibitor-bound USP19 pose significant challenges for structure-based drug discovery and rational inhibitor optimization. Here, we demonstrate the power of hydrogen-deuterium exchange mass spectrometry (HDX-MS) for probing the solution-state structural dynamics of full-length (1,297 amino acids) USP19 and elucidating the molecular mechanism of its inhibition. We investigated USP19-INH, a 4-hydroxypiperidine small-molecule inhibitor, using an integrated biochemical, structural and computational approach. Activity-based protein profiling mass spectrometry (ABPP-MS) demonstrated the strong target engagement, potency and high selectivity of USP19-INH for endogenous USP19 compared to more than 50 deubiquitinases also identified in human MCF7 cells. HDX-MS revealed inhibitor-induced changes in deuterium uptake across specific regions of USP19, providing insight into the binding interface and associated conformational changes that are difficult to resolve using conventional static structural approaches. Integration of these solution-state measurements with computational docking enabled the development of a structural model for USP19-INH recognition and inhibition. Together, these findings establish a mechanistic framework for small-molecule targeting of USP19 and demonstrate how HDX-MS can address structural knowledge gaps for dynamic and challenging drug targets. More broadly, the study highlights the utility of an integrated ABPP-MS and HDX-MS platform to accelerate drug discovery, through mapping of ligand interactions, resolving conformational responses and providing experimentally-constrained structural information to guide the rational design and optimization of next-generation deubiquitinase inhibitors.

Session: SESSION TWO - HDX/Covalent Labeling
Speaker: Monika Priyadarshini
Title: Integrative structural mapping of chromatin condensates by phase-resolved HDX-MS and XLMS
Abstract: The organization of the genome into chromatin determines access to the underlying DNA and thereby regulates genome function. At the mesoscale, dynamic, short-range interactions among nucleosomes, the repeating units of chromatin, determine higher-order chromatin organization and compaction. In vitro and in cells, these multivalent interactions can promote chromatin phase separation in condensates. However, the molecular contacts and conformational changes that organize these condensates, and how they vary across distinct functional chromatin states, remain poorly understood. Here, we integrate hydrogen–deuterium exchange mass spectrometry (HDX-MS) and cross-linking mass spectrometry (XLMS) to map molecular accessibility and interaction networks within chromatin condensates. We developed phase-resolved HDX-MS to distinguish changes in phase-separated chromatin from those in soluble chromatin. Using this approach, we found that chromatin within condensates exhibited broad but non-uniform protection from solvent exchange, with localized increases in accessibility. XL-MS revealed corresponding changes in contacts within regions identified by HDX-MS. The restricted set of cross-links observed in the condensed state further indicates that chromatin samples a narrower conformational ensemble upon condensation. Together, these findings reveal that condensation does not simply bury chromatin surfaces but instead is a selective, regulated structural

transition that selectively remodels nucleosome interfaces and restricts the conformational ensemble sampled by chromatin. This work provides a framework for understanding how short-range nucleosome interactions regulate molecular accessibility across chromatin states and, ultimately, access to the underlying DNA. More broadly, phase-resolved HDX-MS and XL-MS offer complementary approaches for resolving structural changes within large, dynamic phase-separated assemblies.

Session: SESSION NINE - Emerging Technologies
Speaker: Xiaojing Sui
Title: Mapping the Landscape of Proteome Metastability and Structural Plasticity in Aging and Proteotoxic Stress
Abstract: Aging and proteotoxic stress compromise proteome stability, leading to altered protein solubility, aggregation, and proteostasis collapse. However, the organismal magnitude of proteome-wide structural shifts and their downstream functional consequences remain uncharacterized. Here, we used quantitative structural proteomics coupling limited proteolysis with TMT-mass spectrometry (LiP-TMT-MS) to map the dynamic conformational landscape of the Caenorhabditis elegans proteome during aging and proteotoxic stress driven by metastable or polyglutamine (polyQ) proteins. Well before physiological decline, one-third of the aging proteome undergoes significant structural alterations. The primary targets of these age-associated shifts map directly to the proteostasis network, including ribosomes, chaperones, and the ubiquitin-proteasome system. Conformationally altered proteins are enriched in intrinsically disordered regions, β-sheets, and ubiquitination sites, with changes concentrated in functionally critical domains. Notably, comparing aging with non-autonomous proteotoxic stress models—animals expressing a temperature-sensitive myosin mutation or muscle-specific polyQ expansions—revealed distinct proteome-wide landscapes. For example, animals expressing metastable myosin-ts in body wall muscle cells at permissive temperatures has organism-wide consequence affecting conformational changes in ~150 proteins both within and beyond the sarcomere, alongside a striking ~140-fold induction of small heat shock proteins (Hsp16s) that co-localize with thick filaments without broadly activating other chaperones. PolyQ35 and polyQ40 expression similarly induced widespread conformational changes across hundreds of proteins, showing minimal overlap with the myosin-ts metastable sub-proteome and promoted the aging signature. Importantly, tissue-restricted proteotoxic stress triggers systemic, cell-non-autonomous proteome instability, impacting neuronal, intestinal, and secreted proteins. Together, our findings show that early proteome-wide structural dynamics reflect both adaptive stress responses and progressive dysfunction during aging and proteotoxic stress.

Session: SESSION EIGHT - Cross-Linking Mass Spectrometry (Part II)
Speaker: Bjorn-Erik Wulff
Title: Pushing the structural resolution limits of in-vivo crosslinking through novel chemistries
Abstract: Protein crosslinking mass spectrometry is nascently able to reveal structural information with molecular detail. But progress towards this goal is often hindered by the reutilization of chemistries designed to detect protein-protein interactions rather than to resolve precise structural details. We carried out a ground-up design of novel chemistries and associated strategies aiming to break through this hindrance and resolve a protein's rich structural details at the molecular level in living cells. Using ribose-binding protein as a benchmark, we demonstrate the ability to read out dense networks of crosslinks that each reliably reflects a pair of residues in direct van der Waals contact. The resulting distance constraints therefore imply structural details with unique precision, and the data discerns the conformational changes that ribose-binding protein undergoes when the cells are fed ribose before the crosslinking. Alongside crystallography, protein NMR and cryo-EM, this is an empirical path to molecular-resolution experimental protein structure determination – one with an entirely orthogonal set of strengths and limitations.

Session: SESSION SIX - Computational Modeling

Speaker: Haiyuan Yu
Title: Interactome scale structural modeling of protein interactions and systematic blindspots of AlphaFold
Abstract: A significant portion of disease-causing mutations occur at protein-protein interfaces however, the number of structurally resolved multi-protein complexes is extremely small. Here we present a computational pipeline, PIONEER2.0, that integrates 3D structural similarity with geometric deep learning to accurately predict protein binding partner-specific interfacial residues for all experimentally observed human binary protein-protein interactions. We estimate that AlphaFold3 fails to produce high-quality structural models for about half of the human interactome; for these challenging cases, PIONEER2.0 significantly outperforms AlphaFold3 in predicting their interface residues, making PIONEER2.0 an excellent alternative and complementary tool in real-world applications. We further systematically validated PIONEER2.0 predictions experimentally by generating 1,866 mutations and testing their impact on 5,010 mutation-interaction pairs, confirming PIONEER-predicted interfaces are comparable in accuracy as experimentally determined interfaces using PDB co-complex structures. We then used PIONEER2.0 to create a comprehensive multiscale structurally informed human interactome encompassing all 352,124 experimentally determined binary human protein interactions in the literature. We find that PIONEER2.0-predicted interfaces are instrumental in prioritizing disease-associated mutations and thus provide insight into their underlying molecular mechanisms. Overall, our PIONEER2.0 framework offers researchers a valuable tool at an unprecedented scale for studying disease etiology and advancing personalized medicine.

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