HDX

HYDROGEN DEUTERIUM EXCHANGE
MASS SPECTROMETRY

4th International Conference on Hydrogen Deuterium Exchange Mass Spectrometry

April 21st - 24th

2024

Monterey Conference Centre
Monterey, California, USA

WELCOME

Dear Friends and Colleagues:

We welcome you to IC-HDXMS 2024, the 4 th International Conference on HDX-MS.

In 2017, the HDX-MS community gathered in Gothenburg, Sweden, for the 1st conference of the newly-fledged International HDX-MS Society thanks to the efforts of Tineke Papavoine, Rebecca Rae, Glenn Masson, Kasper D. Rand, and Per Malmberg. The success of the 1st conference led to the organization of subsequent biennial meetings, including our 2nd conference in 2019 in Banff, Canada, led by David Schriemer and John Burke, followed by the 3rd conference in 2022 in London, UK, led by David Weis and Argyris Politis. We are excited to continue the legacy of success with this 4th conference in Monterey, USA.

The conference provides an opportunity for the entire community to share new developments and ongoing work related to the use of HDX-MS in solving essential problems in biochemistry and biomedical sciences. Our diverse program covers computation, structural biology, drug discovery, disease-related proteins, and new workflows. We will hear from many established investigators, postdoctoral fellows, and graduate students, and look forward to vibrant poster sessions. The society board will also implement community workshops covering data analysis and structural modeling, developments and best practices in automation, and tackling challenging protein analytes. We encourage you all to participate in these discussions.

The conference program also includes social events. We have an opening reception, sponsored lunch workshops, and a sponsored conference dinner. We are excited to be able to have these events and are grateful to all of the conference sponsors. We have a new student and invited speaker lunch that provides students with an extra networking opportunity. We are excited to have you all here in Monterey, and we trust that you will enjoy the conference.

Regards,
Sheena D'Arcy and Mike Guttman
Co-Chairs, IC-HDXMS 2024

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International Society for HDX-MS

The overarching aim of the Society is to support the International Conference for HDX-MS (IC-HDXMS) by providing a responsible legal entity for organizing the conference. As a framework for the IC-HDXMS, the organization seeks to unite users of HDX-MS from academia, industry, and instrument vendor companies and support the HDX-MS scientific community activities. We believe this to be critical to the continued development of the HDX-MS field. As a central principle, the Society functions fully transparently for all members through democratic principles based on standard rules for non-profit institutions. See www.hdxms.org for more information.

Conferences of the Society

The board of the Society has organized or is currently organizing the following IC-HDXMS conferences:

2017, 1st IC-HDXMS meeting in Gothenburg, Sweden
2019, 2nd IC-HDXMS meeting in Banff, Canada
2022, 3rd IC-HDXMS meeting in London, United Kingdom
2024, 4th IC-HDXMS meeting in Monterey, United States

Other Activities of the Society

The Society has supported the writing of a community-based scientific paper entitled “Recommendations for performing, interpreting, and reporting hydrogen-deuterium exchange mass spectrometry (HDX-MS) experiments.” This paper provides a set of recommendations arising from community discussions emerging out of the IC-HDXMS, 2017. With contributions from expert HDX-MS users across the community, the paper seeks to represent a consensus viewpoint to help novices entering the HDX-MS field. These recommendations were published in Nature Methods in 2019. In 2021, the society organized the Online Seminar Series in HDX-MS, somewhat making up for the lack of a physical conference due to COVID-19 constraints. In 2024, the society launched the IS-HDXMS Slack Workspace with several channels to give members a platform to ask questions and discuss ongoing challenges associated with HDX-MS.

Current Board Members

Chair – Benjamin Walters
Vice-Chair – Malvina Papanastasiou
Member – Zainab Ahdash
Member – Miklos Guttman
Supporting Member – David Weis
Supporting Member – Kasper Rand

Four positions will be turned over in 2024. At the 2024 meeting of the general assembly, an election will be held. Membership is open for all interested in HDX-MS and costs 50 euros for two years. Membership is automatically registered when you attend the upcoming IC-HDXMS conference. Are you interested in organizing a future IC-HDXMS? Contact any board member.

We acknowledge that IC-HDX in Monterey is on the traditional, ancestral territories and homelands of the Esselen, Rumsen, Costanoan and Ohlone people. To recognize the ancestral lands is an important step in honoring the people who have called the Monterey Bay home from time immemorial and acknowledging their continued presence today. Consistent with our values of community and inclusion, we have a responsibility to acknowledge, honor and make visible those whose land we work upon. We are grateful for the opportunity to host community gatherings here. As we acknowledge, the Monterey Bay as the homeland of these tribes, we ask that you, as visitors, treat this place with the same respect as those who walked before you.

To learn more and support the indigenous communities of Monterey County please visit:
https://www.esselentribe.org/
https://www.rumsenohlone.com/
https://www.costanoanrumsen.org/

4th INTERNATIONAL CONFERENCE ON HYDROGEN DEUTERIUM EXCHANGE MASS SPECTROMETRY (IC-HDX 2024)
APRIL 21-24, 2024
THE MONTEREY CONFERENCE CENTER
MONTEREY, CALIFORNIA, USA

PROGRAM

Sunday, 21 April 2024	
16:00-17:00	Registration *Steinbeck Lobby*
Opening, Closing, & all Technical Sessions take place in Steinbeck 2/3	
17:00-17:10	Opening Remarks Miklos Guttman, University of Washington Sheena D'Arcy, The University of Texas at Dallas
17:10-18:00	**Open Plenary** **Jane Dyson**, The Scripps Research Institute *Protein Disorder in Signaling*
18:00-18:40	**Technical Session 1: Protein Unfolding** Chair: Eric Underbakke, Iowa State University **Tobin Sosnik**, University of Chicago *HDX-MS finds that partial unfolding with sequential domain activation controls condensation of a cellular stress marker* **Ellie James,** University of Washington *Interrogating tau conformation and aggregation pathways by HDX-MS* **Ellie Holden**, University of Oxford *Capturing Mechanosensitivity in Cardiac Proteins* **Highlighted by Silver Sponsor Waters™**
19:00-20:30	Opening Catered Reception *Steinbeck Lobby & Stevenson Terrace*

Monday, 22 April 2024	
08:00-09:00	Late Registration *Steinbeck Lobby*
08:30-09:00	Morning Coffee *Steinbeck Lobby* Sponsored by **Bruker**
09:00-10:30	**Technical Session 2: Computation** Chair: Jonathan Phillips, University of Exeter **Daniel Deredge,** University of Maryland Baltimore *Integrative HDX: from Post Hoc Ensemble Reweighting to HDX-MS guided enhanced sampling MD simulations.* **Benjamin Walters**, Genentech *Exploring dynamic pockets: HDX-Driven MD Simulations for Structure-Based Drug Design* **David Schriemer**, University of Calgary *AutoHX: Reinventing the HDX experiment using Data-Independent Data Acquisition (DIA)* **Lisa Tuttle**, University of Washington *pyHXExpress: A Python implementation and expansion of HX-Express*
10:30-10:55	Coffee Break *Steinbeck Lobby*

Monday, 22 April 2024	
10:55-12:30	**Technical Session 3: Non-standard Workflows** Chair: Elyssia Gallagher, Baylor University **Judith Klinman**, University of California *Functional Dynamics from Temperature-Dependent HDX-MS* **Masaru Miyagi**, Case Western Reserve University *Exploring the Potential of Histidine Hydrogen–Deuterium Exchange Mass Spectrometry in Analyzing Complex Protein Mixtures* **Prabavi Dias**, The University of Texas at Dallas *Extended Applications and Improved Efficiency of HDX Using Stable Incorporation of Isotopes* **Damon Griffiths**, University of Manchester *Cyclic ion mobility for hydrogen/deuterium exchange-mass spectrometry applications* **Highlighted by Silver Sponsor Eli Lilly** **Allan Ferrari**, Northwestern University *Leveraging HDX-MS to Decipher Protein Stability and Dynamics in Varied Energy Landscapes across Natural and Designed Sequences* **Poster Flash Talks** **Maria Janowska**, University of Washington *HDXBoxeR: R package for statistical analysis and visualization of multiple protein states in differential HDX-MS* **Gillian Dornan**, Leibniz Forschungsinstitut für Molekulare Pharmakologie *Endophilin B1 recruits and activates PI3KC2B to mediate mitochondrial fission during mitophagy* **Didier Devaurs**, University of Edinburgh *A Rosetta-based pipeline for molecular modelling guided by experimental HDX-MS data*
12:30-13:30	**Lunch Seminar** - Sponsored by **Waters™** *Steinbeck 2/3* **Ganesh S. Anand**, The Pennsylvania State University *Bimodal deuterium exchange loci by Cyclic-IMS-HDXMS reveal intentional defects and ensemble behavior in virus assemblies* **Ellie Holden,** University of Oxford*Probing conformational changes in cardiac mechanosensitive proteins*
14:00-15:30	Monday Poster Session – **All EVEN numbered poster presenters are to be present.** Afternoon Coffee/Tea Service *Steinbeck 1*

Monday, 22 April 2024	
15:30-17:00	**Technical Session 4: Protein Complexes** Chair: John Burke, University of Victoria **Nikolina Sekulic**, University of Oslo *HDX-MS studies on the interaction between Shugoshin and chromosome passenger complex (CPC)* **Malvina Papanastasiou**, The Broad Institute of MIT and Harvard *Lessons from automation: optimized workflows for high-throughput HX-MS experiments* **20-minute Trajan Gold Sponsor Talk** **Siavash Vahidi**, University of Guelph *HDX-MS of supra-molecular systems, why bother?* **Alexzandrea Woudenberg**, Iowa State University *Mapping the conformational dynamics and scaffolding regulating the Pyk2/Src kinase activation complex with H/D exchange mass spectrometry* **Sushant Suresh,** University of Victoria *Molecular Basis of Regulation of Phosphatidylinositol 4-kinase III alpha (PI4KA)*
17:00-17:15	Group Photo
17:15-18:00	Free Time
18:00-22:00	**Conference Dinner** Sponsored by **Trajan** *Monterey Beach Station* *285 Figueroa St, Monterey, CA 93940* Detailed directions detailed in program.
	

Tuesday, 23 April 2024	
08:30-09:00	Morning Coffee *Steinbeck Lobby* Sponsored by **Bruker**
09:00-10:30	**Technical Session 5: Drug Discovery** Chair: Frank Sobott, University of Leeds **Deepa Balasubramaniam**, Eli Lilly and Company *Applications of HDXMS in large molecule drug discovery: defining epitope landscape across a target* **Derek Wilson**, York University *Really Paying Attention to Your HDX Data Yields Big Dividends in Drug and Vaccine Development* **Alessio Bortoluzzi**, iBET *Pushing the Boundaries of HDX-MS for Low Affinity Fragments Interactions Studies* **Lindsay Cole**, Applied Photophysics *FastHDX (ms2min HDX-MS) system applications and future commercialisation* **10-Minute Applied Photophysics Silver Sponsor Talk**
10:30-11:00	Coffee Break *Steinbeck Lobby*

Tuesday, 23 April 2024	
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Tuesday, 23 April 2024	
12:30-13:30	**Lunch Seminar** - Sponsored by **Thermo Fisher Scientific** *Steinbeck 2/3* **Yuqi Shi,** Marketing Manager, Structural Biology, Thermo Fisher Scientific *Exploring New Frontiers in Hydrogen Deuterium Exchange Mass Spectrometry: Latest Developments and Applications*
14:00-15:30	Tuesday Poster Session **All ODD numbered poster presenters are to be present.** Afternoon Coffee/Tea Service *Steinbeck 1*
15:30-17:30	**Workshop Rotations** 15:30-16:00 16:10-16:50 17:00-17:30 **Workshop A -** *Steinbeck 2* *HDX-MS of Challenging Proteins* Moderated by Zainab Ahdash, UCB Pharma & Yoshitomo Hamuro, Johnson and Johnson **Workshop B –** *Steinbeck 3* *Extracting the most from your HDX-MS Experiment: Data Analysis and Computational Modelling* Moderated by Kasper Rand, University of Copenhagen & David Schriemer, University of Calgary **Workshop C –** *Colton* *Optimizing Automation for HDX: Tips/Tricks for Reproducibility & Best Practices* Moderated by David Weis, Bristol Myers Squibb & Jamie Moroco, Photys Therapeutics
17:30	**End of Day – Free Time**

Wednesday, 24 April 2024	
08:30-09:00	Morning Coffee *Steinbeck Lobby* Sponsored by **Bruker**
09:00-10:30	**Technical Session 7: Viral Proteins** Chair: Ganesh Anand, The Pennsylvania State University **Susan Marqusee**, University of California Berkeley and National Science Foundation *Exploiting the awesome power of hydrogen exchange to uncover hidden and transient conformations in protein folding, degradation and SARS-CoV-2 Spike.* **Kelly Lee**, University of Washington *An atlas of structural and dynamic variation throughout the HIV-1 Env glycoprotein* **Valeria Calvaresi**, University of Oxford *Structural dynamics and macromolecular assembly of the Ebola virus fusion glycoprotein GP* **Sean Braet**, Pennsylvania State University *Asymmetric Packaging of Genomic RNA in RNA Viruses Promotes Egress by Integrative Cryo-EM and HDXMS* **Sophie Shoemaker**, University of California Berkeley *Mapping the Conformational Landscape of the Rabies Virus Glycoprotein*
10:30-11:00	Coffee Break *Steinbeck Lobby*

Wednesday, 24 April 2024	
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15:00-15:15	Coffee Break *Steinbeck Lobby*
15:15-16:05	**Closing Plenary** **Patrick Griffin**, The Wertheim UF Scripps Institute *A sequential priming mechanism of the progesterone receptor revealed by structural mass spectrometry*
16:05-16:15	Closing Remarks Co-Chair: Miklos Guttman, University of Washington Co-Chair: Sheena D'Arcy, The University of Texas at Dallas

Safe Travels… see you in 2026!

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ABSTRACTS – INVITED & CONTRIBUTED TALKS

1 *Protein Disorder in Signaling*

Jane Dyson, Peter E. Wright
Scripps Research

Protein sequences that do not independently form well-folded structures in solution are abundant in eukaryotes. Termed intrinsically disordered proteins (IDPs), or intrinsically disordered regions (IDRs) if they form only part of a larger protein, these polypeptide sequences play an essential role in numerous biological processes, including the regulation of signaling and transcription pathways. Early work on large multi-domain transcription factors relied on characterization of fragments of the proteins, but we realized that true insight into the functional significance of disordered regions requires evaluation of the inter-domain interactions within and between the full-length proteins. Detection and evaluation of these interactions has been an important focus for recent work in our laboratory and has given rise to new hypotheses on the functions of several sequence motifs that occur in disordered regions of proteins involved in signaling and transcription processes in the cell. Examples of the various roles of IDRs and IDPs in signaling will be described.

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2 *HDX-MS finds that partial unfolding with sequential domain activation controls condensation of a cellular stress marker*

Tobin Sosnick, Ruofan Chen, Hendrik Glauninger, Darren N. Kahan, Julia Shangguan, Joseph R. Sachleben, Joshua A. Riback, D. Allan Drummond
University of Chicago

Eukaryotic cells form biomolecular condensates to sense and adapt to their environment (1, 2). Poly(A)-binding protein (Pab1), a canonical stress granule marker, condenses upon heat shock or starvation, promoting adaptation (3). The molecular basis of condensation has remained elusive due to a dearth of techniques to probe structure directly in condensates. Here we apply Hydrogen-Deuterium Exchange/Mass Spectrometry (HDX-MS) to investigate the molecular mechanism of Pab1's condensation. We find that Pab1's four RNA recognition motifs (RRMs) undergo different levels of partial unfolding upon condensation, and the changes are similar for thermal and pH stresses. Although structural heterogeneity is observed, the ability of MS to describe individual populations allows us to identify which regions contribute to the condensate's interaction network. Our data yield a picture of Pab1's stress-triggered condensation, which we term sequential activation (Fig. 1A), wherein each RRM becomes activated at a temperature where it partially unfolds and associates with other likewise activated RRMs to form the condensate. Subsequent association is dictated more by the underlying free energy surface than specific interactions, an effect we refer to as thermodynamic specificity. Our study represents a methodological advance for elucidating the interactions that drive biomolecular condensation. Furthermore, our findings demonstrate how condensation can use thermodynamic specificity to perform an acute response to multiple stresses, a potentially general mechanism for stress-responsive proteins.

3 *Interrogating tau conformation and aggregation pathways by HDX-MS*

Ellie James, Abhinav Nath, Miklos Guttman
University of Washington

Many neurodegenerative diseases exhibit pathological aggregation of one or more intrinsically disordered proteins (IDPs). Tau is an IDP that is implicated in Alzheimer's Disease (AD) and approximately 20 other diseases. IDPs do not fold into a single well-defined 3D conformation, but instead populate dynamic and interchanging conformational ensembles. Interestingly, tau forms disease-specific fibril morphologies that can propagate like prions, meaning that fibril structure is preserved when tau aggregation is seeded. We do not know how these differences in tau fibril conformations arise, which limits our understanding of these diseases and hinders development of therapeutics to treat AD. To address this gap, we harness an in vitro tau aggregation model consisting of tau4RD, a tau truncation, or 0N4R, one of tau's six full-length isoforms. These constructs form fibrillar aggregates in the presence of polyanionic inducers in vitro, with different inducers reproducibly inducing distinct fibril morphologies. While in vitro fibril conformations are distinct from pathological fibrils seen in vivo, they will enable us to determine whether and how changes in pre-fibrillar conformations of tau correlate with differences in fibril morphology. Here, we employ pulsed HDX-MS with proteolysis or ETD to follow tau's structural changes as it aggregates from its monomeric state into various fibrillar conformations. Further, we use millisecond-HDX of monomeric tau to explore how selected inducers alter the structure and dynamics of tau's solution-state ensemble. Finally, we extend this analysis to tau aggregated in the presence of tryptanthrin, a novel tau aggregation inhibitor that we hypothesize interacts with tau's aggregation nucleus.

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4 *Capturing Mechanosensitivity in Cardiac Proteins*

Ellie Holden, Navoneel Sen, Miranda Collier, Lucia Parolin, Dirk Aarts, Katja Gehmlich, Justin Benesch
University of Oxford

The perpetual contraction and relaxation of cardiac muscle tissue creates a high-force environment to which proteins must adapt. Mechanosensitive proteins are able to convert this constant cycle of force into intramolecular conformational changes that induce partial unfolding under physiologically accessible forces. Once such protein is the actin-crosslinker filamin C, which harnesses its mechanosensitive properties to regulate the forces exerted on the network of actin filaments during muscle contraction. However, exactly how filamin C, and mechanosensitive proteins as a whole, change their conformations under force remains uncharacterised. By using HDXMS we are able to identify key structures that contribute to its mechanosensitive function, such as the isoform-unique d20 insert and sites of beta-strand donation. Although structural prediction indicates little secondary structure, the insert presents complex HDXMS data with EX1 characteristics, suggesting two distinct structural populations. By investigating exchange timepoints down to 250 ms we probe deeper into structures that appear saturated at conventional timepoints. Furthermore, when we compare the WT with a single amino acid variant (W2164C) associated with hypertrophic cardiomyopathy, we see clear localised differences in the uptake profile. This has allowed us to map this mutation's effect from amino acid interactions to phenotypic presentations providing pivotal insights into the underlying disease mechanism. We compliment these data with Native MS and Ion Mobility MS to provide a holistic picture of mechanosensitivity and the important structures associated with the physiological unfolding of filamin C in both states of health and disease.

5 *Integrative HDX: from Post Hoc Ensemble Reweighting to HDX-MS guided enhanced sampling MD simulations.*

Daniel Deredge
University of Maryland Baltimore

An accurate understanding of the structure and dynamics of proteins is of paramount importance for understanding their proper function, how disruptions can lead to dysfunction or to develop therapeutic countermeasures. Hydrogen-deuterium exchange mass spectrometry (HDX-MS) is unique in its ability to simultaneously provide localized and time-resolved information on the structural environment and dynamics of the backbone amide hydrogen of every residue of a given protein except Prolines. As such, HDX-MS has been successfully applied to provide insights into the native state ensembles of proteins, macromolecular interactions, protein-small molecule interactions or protein folding and unfolding studies. However, the structural information reported by HDX-MS remains largely limited to peptide-level resolution and, in some cases, residue-level resolution. Our efforts have aimed at demonstrating the applicability of quantitative and integrative approaches that leverage HDX-MS data to model atomic resolution structural ensembles generated by computational modeling and/or molecular dynamics (MD) simulations. HDX ensemble reweighting (HDXer) is one such approach which uses a post hoc maximum entropy approach to adjust the weights of individual frames of a structural ensemble to conform to HDX-MS data. Here, we demonstrate the applicability of HDXer within the perspective of small molecule drug discovery in two ways. First, we used HDXer to model the native ensemble of the putative therapeutic target PhuS, the cytoplasmic heme binding protein from P.aeruginosa, prior to structure-based drug design approaches to successfully target novel, cryptic drug binding sites. Second, we demonstrate the specific applicability of HDXer in structure-based drug design in leveraging lower resolution structural information from HDX-MS to model protein-small molecule drug interactions in the absence of high-resolution drug bound structures. Finally, in an effort to extend beyond post hoc ensemble reweighting, we are currently developing an HDX-MS-steered adaptive sampling workflow to guide enhanced sampling MD simulations using calmodulin as a model protein.

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6 *Exploring dynamic pockets: HDX-Driven MD Simulations for Structure-Based Drug Design*

Benjamin Walters, Alexander W. Patapoff, James R. Kiefer, Weiru Wang
Genentech

This study presents a novel method that uses data from HDX experiments to guide small molecules into dynamic and hidden pockets to within crystallographic resolution, achieving a success rate exceeding 90% over multiple targets. The method is capable of yielding these levels of accuracy, even when starting from unbound structural information where substantial alterations are necessary for binding. It will be described using a dynamic kinase, HPK-1, through comparison to solved X-ray structures, and a collection of ligands, representing a range of different binding modes. These findings will then be compared with a popular experimentally directed flexible binding program. Further utility for pharmaceutical drug discovery will also be explored through series of results from a fragment-based drug discovery program where multiple pockets were discovered and the technique enabled structure-based drug design.

7 *AutoHX: Reinventing the HDX experiment using Data-Independent Data Acquisition (DIA)*

David Schriemer, Vladimir Sarpe, D. Alex Crowder, Frantisek Filandr, Arthur Semague, Morgan Khan, Mariam Hassannia
University of Calgary

Our current practice of manually curating HDX-MS data seriously restricts the application of HDX-MS to simple protein systems and low throughput applications. It also introduces human error. These two restrictions have rendered HDX-MS a niche technique in protein analysis. We present AutoHX, a software package that completely removes the need for manual data assessment, and we illustrate its power in the analysis of complex differential analysis projects. AutoHX operates on DIA data, and it uses fragment deuteration measurements to endorse or override the peptide deuteration in a fully automated fashion. We will describe the algorithmic basis for this claim of fully automated data curation, and illustrate how AutoHX enables two DIA-based methods for data collection: one that is based on overlapping DIA transmission windows and one that is based on contiguous DIA transmission windows. The shift from MS to MS2 based methods of data collection is straightforward, promoting easy adoption. We will illustrate the power of DIA and AutoHX using data from several projects, notably a multi-ligand analysis of allostery in DNA-PKcs, a massive conformational switch involved in DNA repair pathway choice.

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8 *pyHXExpress: A python implementation and expansion of HX-Express*

Lisa M Tuttle, Miklos Guttman
University of Washington

HX-Express is an Excel visual basic based application that allows for analysis of overlapped and noisy HDX-MS data and can perform polymodal binomial fits of the data with statistical tests. A general use case for HX-Express is to analyze specific peptide/charge-states that are not well-described by a single binomial fit in software such as HDExaminer. Here we introduce pyHXExpress: a python implementation of the features of HX-Express in a highly customizable Jupyter Notebook. This allows for a polymodal analysis of complete HDX-MS projects (all mutants, peptides, charges) with minimal user input. To reduce over-fitting and to provide meaningful error estimates on the fit parameters (e.g. #D and populations) we implement a data regularization method of performing multiple fits of the raw data to which Gaussian noise has been added. The goal of pyHXExpress is to provide a user-friendly notebook for complete HDX-MS polymodal data analysis that additionally has outputs that users can easily interact with to create custom plots or to interface with other software as desired.

9 *Functional Dynamics from Temperature Dependent HDX-MS*

Judith Klinman
University of California

Temperature variation of HDX-MS in enzymes is being pursued, as a tool for defining thermal networks that originate at protein/solvent interfaces and act to initiate active site chemistry. The introduction of function-altering, site specific mutants simplifies the analysis of observed rate constants for HDX-MS (under the EX-2 regime), leading to a cancellation of the enthalpic barrier for the intrinsic chemical exchange reaction and an ability to focus on trends in ΔHo that represent the pre-equilibrium term, Kop=kop/kcl. Emerging results indicate a site specificity in regional ΔHo values that correlates with the ΔH± for the chemical reaction being catalyzed. Results from two very different enzyme reactions, catalyzed by lipoxygenase and adenosine deaminase, will be highlighted. (Supported by finding from the NIGMS)

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10 *Exploring the Potential of Histidine Hydrogen–Deuterium Exchange Mass Spectrometry in Analyzing Complex Protein Mixtures*

Masaru Miyagi
Case Western Reserve University

There are numerous hydrogen atoms in proteins that undergo exchange with deuterium upon exposure to deuterium oxide (D2O) solvent. Among them, the C-2 hydrogen of the histidine (His) imidazole group is the only hydrogen bonded to carbon but still exchanges with deuterium. The exchange rate of the C-2 hydrogen with deuterium is significantly slower than that of the backbone amide hydrogen, with a half-life on the order of days at 37 °C and physiological pH. The approach that monitors the His imidazole group's hydrogen-deuterium exchange through mass spectrometry (His-HDX-MS) was initially developed to determine the pKa values and solvent accessibility of histidine imidazole groups in proteins. Since then, His-HDX-MS has been primarily applied to relatively pure, isolated proteins. Nonetheless, His-HDX-MS is well-suited for analyzing complex protein mixtures due to the slow back exchange of imidazole C-2 D to C-2 H. This slow back exchange allows for protein digestion at 37 °C by proteases with strict substrate specificities (e.g., trypsin) and enables long chromatographic separation at ambient temperature in LC-MS/MS analysis to enhance peptide separation. We will share our efforts in applying His-HDX-MS to complex protein samples, along with a discussion on its potential applications in the field of structural proteomics.

11 *Extended Applications and Improved Efficiency of HDX Using Stable Incorporation of Isotopes*

Prabavi Dias, Javier Flores, Darby Ball,
Oladimeji Olaluwoye, Sheena D'Arcy
Chemistry and Biochemistry, University of Texas at Dallas

Hydrogen-deuterium exchange coupled with mass spectrometry (HDX) provides information on protein dynamics in solution. HDX experiments are often comparative analyses and are technically challenging as pH, temperature, and time must be precisely controlled between samples. We have used a modified HDX workflow with mixtures of proteins distinguished by stable isotopic labeling with 13C or 15N. This workflow extends the applications of HDX and improves the efficiency and consistency of HDX experiments. Our workflow can provide insight into multimeric protein systems where different copies of the same protein are in a unique environment. We have established the workflow using histones H2A-H2B, which package genomic DNA in eukaryotes. We compare the dynamics of H2A-H2B to histone variant H2A.Z-H2B. We can use this workflow to compare histones, mono-nucleosomes, and individual nucleosomes within a multi-nucleosome array. We expressed 15N- and 13C-labeled Xenopus laevis histones (H2A and H2B) using minimal media with 15NH4Cl and 13C6H12O6, respectively. To quantify any isotopic effect on the chemical exchange rate, we compared the exchange of wild type (WT), 15N, and 13C H2A and H2B in an unfolded state. To determine any isotopic effect on H-bonding, we compared the exchange of WT, 15N, and 13C H2A-H2B in a folded state. Our results validate using stable isotopes in an HDX workflow. Using the established workflow, we compared the dynamics of canonical Xl H2A-H2B to variant Human (Hs) H2A.Z-H2B. We observed significant differences in their dynamics, which is surprising given their high structural homology.

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12 *Cyclic ion mobility for hydrogen/deuterium exchange-mass spectrometry applications*

Damon Griffiths, Malcolm Anderson, Keith Richardson, Satomi Inaba-Inoue, William J. Allen, Ian Collinson, Konstantinos Beis, Michael Morris, Kevin Giles, Argyris Politis
University of Manchester

Hydrogen/deuterium exchange-mass spectrometry (HDX-MS) has emerged as a powerful tool to probe protein dynamics. As a bottom-up technique, HDX-MS provides information at peptide-level resolution, allowing structural localisation of dynamic changes. Consequently, HDX-MS data quality is largely determined by the number of peptides that are identified and monitored after deuteration. Integration of ion mobility (IM) into HDX-MS workflows has been shown to increase data quality by providing an orthogonal mode of peptide ion separation in the gas-phase. This is of critical importance for challenging targets such as integral membrane proteins (IMPs), which often suffer from low sequence coverage and/or redundancy in HDX-MS analyses. The increasing complexity of samples being investigated by HDX-MS, such as membrane mimetic reconstituted and in vivo IMPs, has generated need for instrumentation with greater resolving power. Recently, Giles et al. developed cyclic ion mobility (cIM), an IM device with racetrack geometry that enables scalable, multi-pass IM separations. Using 1-pass and multi-pass cIM routines, we use the recently commercialised SELECT SERIES™ Cyclic™ IM spectrometer for HDX-MS analyses of 4 detergent solubilised IMP samples and report its enhanced performance. Furthermore, we develop a novel processing strategy capable of better handling multi-pass cIM data. Interestingly, use of 1-pass and multi-pass cIM routines produced unique peptide populations, with their combined peptide output being 31 to 222% higher than previous generation SYNAPT G2-Si instrumentation. Thus, we propose a novel HDX-MS workflow with integrated cIM which has the potential to enable the analysis of more complex systems with greater accuracy and speed.

13 *Leveraging HDX-MS to Decipher Protein Stability and Dynamics in Varied Energy Landscapes across Natural and Designed Sequences*

Allan Ferrari, Sugyan Dixit, Jane Thibeault, Scott Houliston, Robert Ludwig, Mario Garcia, Pascal Notin, Claire Phoumyvong, Lauren Carter, Cheryl H. Arrowsmith, Miklos Guttman, Gabriel Rocklin
Northwestern University

Proteins dynamically fluctuate among a collection of conformational states with individual energy landscapes encoded by their sequence. Understanding how protein sequence and structure shape the protein energy landscape is crucial not only to understand protein function but also in the design process to build customized proteins. However, traditional biophysical methods either only measures global stability or they lack the ability to evaluate stability and dynamics in large scale. As a result, we are unable to learn the determinants of protein energy landscapes in a data driven way. Here, we leverage the throughput applicability of HDX mass spectrometry by developing a methodology to measure protein stability and dynamics for thousands of protein sequences from intact protein HDX experiments. We introduce a new computational pipeline, HDX-LIMIT, created to process the data, deconvoluting and assembling time-dependent mass distributions and modeling exchange rates and ΔG-open distributions for each protein in the mixture. Our analysis on 15 protein libraries showcased our ability to capture a wide range of global stabilities and diversity of ΔG-open distributions even for proteins with the same general topology. We derive a cooperativity metric that reflects how energies are relatively distributed across protein sequence of similar global stabilities and uncover important trends across different folds. This methodology not only demonstrates reproducibility and comparability to traditional NMR experiments but also provides new insights into the determinants of protein energy landscapes, paving the way for designing proteins with customized stability and dynamic properties.

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14 *HDX-MS studies on the interaction between Shugoshin and chromosome passenger complex (CPC)*

Nikolina Sekulić, Dario Segura-Peña, Hemanga Gogoi
University of Oslo

Shugoshins play a central role in ensuring the impartial division of chromosomes during cell division. They serve as platform proteins that localize to the centromere during cell division and recruit multiple effector proteins with enzymatic and/or structural properties that ensure uniform chromosome segregation. Interestingly, shugoshins recruit both the chromosome passenger complex (CPC) with kinase activity (important for chromosome alignment in metaphase) and protein phosphatase 2A with phosphatase activity (important for ensuring cohesion of duplicated chromosomes in metaphase). While crystal structure of shugoshin-PP2A is available, molecular details on shugoshin-CPC interaction are still missing. We have now reconstituted the localization module of CPC (consisting of survivin, borealin and INCENP subunits) and formed complex between CPC and shugoshin. Initial HDX-MS analysis shows that CPC undergoes structural rearrangement upon binding to shugoshin. The N-terminal region of the borealin subunit is protected upon binding but the C-terminal region of the borealin subunit which forms the dimerization domain shows strong deprotection with EX1 pattern. I will discuss our findings and the possible structural interpretations in the context of other biophysical experiments.

15 *Lessons from automation: optimized workflows for high-throughput HX-MS experiments*

Malvina Papanastasiou, Shaunak Raval[1], Yuqi Shi[2], Emam Hagar[3], Mahmoud Nasr[3], Rosa Viner[2], Jeff Morrow[4], Stephen Coales[4], Steven A Carr[1]; [1]Broad Institute of MIT & Harvard; [2]Thermo Scientific; [3]Harvard University; [4]Trajan Scientific & Medical
Broad Institute of MIT & Harvard

In this work, we will discuss automated workflows for the analysis of various applications such as membrane proteins, protein dynamics, protein-ligand interactions, and cell digests, by utilizing Trajan's HDX Workflow with filtration capabilities. Our methods minimize "downtime", while back-flushing capabilities available for the digestion, desalting and analytical columns eliminate carry-over and, consequently, the requirement for blank runs between samples. When using short gradients, our optimized workflows allow for the labeling and acquisition of 42 samples per day. We'll discuss important factors that affect the accuracy of D-measurements, like temperature controls that are available in all HDX compartments, randomization of samples, generation of full deuteration controls, and time-course setup. We'll further discuss recent changes to the LC flow path that enable lower flow rates and, consequently, higher MS sensitivity measurements.

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16 *HDX-MS of supra-molecular systems, why bother?*

Siavash Vahidi, Turner, Madison; Hoff, Samuel E.; Uday, Adwaith B.; Velyvis, Algirdas; Zeytuni, N.; Bonomi, Massimiliano;
University of Guelph

I developed proficiency in HDX-MS under the guidance of Lars Konermann during my PhD. Subsequently, I embarked on a transformative multidisciplinary joint postdoc with Lewis Kay and John Rubinstein, pioneers in methyl-TROSY NMR and cryo-EM structure determination, respectively. This distinctive and challenging path has given my independent research group with a comprehensive toolkit for addressing complex problems in structural biology. Our group's unique advantage lies in our ability to perform extensive wet-lab biochemistry, sample preparation, and application of various structural biology tools in-house. This enables us to identify the specific areas where HDX-MS outperforms NMR, cryo-EM, and other techniques, and equally where it falls short relative to the other tools. I will present our unpublished results on employing HDX-MS to analyze large complexes within the protein degradation machinery, with a focus on the proteasome (700 kDa) and the mitochondrial ClpXP protease (800 kDa). Our research not only reveals insights accessible through HDX-MS, insights to which NMR and cryo-EM are essentially blind, even in scenarios where sophisticated NMR data or sub 3-Å cryo-EM maps are available, but also illustrates how HDX-MS can guide these mainstream methods towards more nuanced structural analysis. This includes elucidating the action mechanisms of regulatory proteins and allosteric modulators. Overall, this talk will highlight the unique strengths of HDX-MS in structural biology and will underscore its vital role in enhancing our comprehension of intricate biological systems. I will also point out to a few areas where method development will allow HDX to take the next big step.

17 *Mapping the conformational dynamics and scaffolding regulating the Pyk2/Src kinase activation complex with H/D exchange mass spectrometry*

Alexzandrea Woudenberg, Tania M. Palhano Zanela, Hanna S. Loving, Karen G. Romero Bello, Emily Rivera, Eric S. Underbakke
Iowa State University

The post synaptic density (PSD) of neuronal cells is a signaling hub organizing a network of communication pathways that regulate synaptic plasticity. Proline rich tyrosine kinase 2 (Pyk2) is a cytosolic tyrosine kinase highly expressed in the PSD of forebrain neurons. Despite structural and sequence similarity to its ancestor, focal adhesion kinase, Pyk2 has adopted unique signaling mechanisms and sensitivity to Ca2+ flux. Our work explores the multi-layered mechanisms that regulate Pyk2 activation. Hydrogen/deuterium exchange mass spectrometry (HDX-MS) is an ideal tool to probe conformational dynamics associated with signaling regulation. We combine HDX-MS with other biophysical approaches to illuminate the molecular mechanisms leading to the assembly of the higher-order Pyk2 activation complex. Using HDX-MS, we have tracked the conformational rearrangements transitioning Pyk2 from autoinhibited state to the fully active scaffolded complex with Src kinase. The Pyk2 basal state is maintained through an autoinhibitory interaction between the FERM and kinase domains. Activation is instigated by conformational rearrangements relieving the FERM interaction to allow trans autophosphorylation of a key linker site. Scaffolding interactions with PSD-95 or SAP102 are implicated in Pyk2 clustering. After the initial linker phosphorylation, Src kinase is recruited to the complex, leading to full activation of Pyk2. Src phosphorylates the Pyk2 activation loop. HDX-MS reveals how activation loop phosphorylation organizes catalytic and substrate-binding motifs. Ultimately, mechanistic models derived from HDX data guided mutagenesis profiling to identify key regulatory residues.

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18 *Molecular Basis of Regulation of Phosphatidylinositol 4-kinase III alpha (PI4KA)*

Sushant Suresh, Alexandria Shaw, Noah J Harris, Matthew AH Parson, Meredith L Jenkins, John E Burke
University of Victoria

The type III Phosphatidylinositol 4-Kinase alpha complex (PI4KIIIα/PI4KA) is an essential lipid kinase that mediates PI4P synthesis at the plasma membrane plays a key role in membrane trafficking and organelle identity. In mammals, it is predominantly present as a heterotrimer with two accessory proteins, TTC7A/B and FAM126A/B. Misregulation of PI4KA and its binding partners have been implicated in a wide range of pathologies, highlighting the importance in understanding its regulatory mechanisms. The PI4KA complex is recruited to the plasma membrane for activity by the membrane associated protein EFR3A/B. However, the molecular mechanisms underlying the recruitment and regulation has not been elucidated. Using a synergy of Alphafold, Hydrogen Deuterium eXchange Mass Spectrometry (HDX-MS) and cryo-EM, we aim to comprehensively map the binding interactions between PI4KA and lipidated EFR3. We have shown that the evolutionarily conserved, disordered C-terminus of EFR3 binds to TTC7 and FAM126 suggesting a molecular mechanism for the recruitment and activation of PI4KA to the plasma membrane. This research furthers our knowledge on PI4KA regulation ad provides a molecular framework for future therapeutic advances.

19 *Applications of HDXMS in large molecule drug discovery: defining epitope landscape across a target*

Deepa Balasubramaniam
Eli Lillly

Use of HDXMS in drug discovery has grown over the last decade. Major advancements in instrumentation and analysis methods in addition to the use of small amounts of samples to probe proteins of any size under native conditions without the need for modifications makes HDXMS an attractive technique. It is widely used to probe antigen dynamics, characterize epitopes, paratopes and understand structure activity relationships or mechanism of action of a drug molecule. This presentation will focus on examples of how we use HDXMS in large molecule discovery and in addition will also touch upon the technological innovations that we have incorporated into the method to support a large portfolio of projects. We hope to illustrate how we use HDXMS from characterizing candidate molecule epitope to defining epitope landscape across a specified target in combination with epitope binning and incorporating epitope diversity into the molecule selection process much earlier in the discovery process.

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20 *Really Paying Attention to Your HDX Data Yields Big Dividends in Drug and Vaccine Development*

Derek Wilson, Esther Wolf, Vimanda Chow, Joe Anacleto, Cristina Lento
York University, Canada

We all know the conventional deltaHDX experiment: Look for the uptake difference between the free and bound state, summed over all labelling timepoints. And sure, that can tell you about where your drug candidate is binding, which is super-useful, but can we do MORE? Can we figure out which signals are from allostery and which ones aren't? Can we get a K_D for the interaction? Can we do everything faster so that drug companies will want to work with us even more?? Using a plethora of real-world drug/vaccine development examples from COVID, cancer and neurodegenerative disease, this talk will definitely-absolutely convince you (the choir, I know) beyond all possible doubt, that there is more in your already-beautiful HDX data than you may think there is. Information that can yield molecular Mechanism-of-Action data (drug companies still sometimes pretend they don't want this, but they do! Because the FDA does!) and even good estimates of quantitative parameters like K_D to validate those... questionably reliable... SPR measurements they already have. I don't think it's understating it to say that you will leave this talk a new person, ready to examine your HDX data with fresh eyes and thus to bring the world of true dynamic structural biology to your drug/vaccine developer partners!

21 *Pushing the Boundaries of HDX-MS for Low Affinity Fragments Interactions Studies*

Alessio Bortoluzzi, Catarina F. Malta, Joerg Bomke, Tiago M. Bandeiras, Diana O. Silva, Pedro M. F. Sousa, Daniel Schwarz, Ulrich Grädler
iBET

Characterization of protein-ligand interactions is essential for the pre-clinical development of drug candidates and Hydrogen-Deuterium Exchange-Mass Spectrometry (HDX-MS) has emerged as a valuable tool in this process. HDX-MS has been predominantly applied to high affinity compounds with only few examples of its application for weak ligand such as fragments. Nevertheless, HDX-MS usage could be instrumental in Fragment-based Drug Discovery (FBDD) programs especially when dealing with challenging targets that cannot be studied by other higher resolution structural techniques e.g. X-Ray crystallography and NMR. In this work we use the drug-target protein Cyclophilin D (CypD) as model to explore how far HDX-MS can be pushed for fragment binding characterization. We performed a systematic study on the best conditions for HDX-MS execution and found that fragments with low affinity interaction in the double-digit mM range are amenable by HDX-MS. We observed how, despite the intrinsic low resolution of HDX-MS, partially overlapping fragments binding sites can still be distinguished. Through this work we describe technical caveats in the experimental execution and data analysis that were found to be critical for obtaining genuine information from HDX-MS experiments applied to low affinity fragment interaction studies. This work contributes to assert HDX-MS as an instrumental method for FBDD and offers a methodological framework to investigate such interactions using HDX-MS.

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22 *FastHDX (ms2min HDX-MS) system applications and future commercialisation*

Lindsay Cole
Applied Photophysics Limited

Applied Photophysics has developed a prototype automated "FastHDX" (ms2min HDX-MS) system capable of HDX labelling from 50ms to multiple minutes. This complements and extends traditional hand-pipetted or liquid-handler prepared HDX samples which is limited to labelling time points in the 10s of seconds to hours. The faster time domain allows the elucidation of the structural changes of highly dynamic features of protein structure, and so provides new insights into structural dynamics of proteins including intrinsically disordered regions, protein conformational change and protein allostery. Importantly the system allows for direct transfer of methods from other HDX methods, with similar sample complexity, back exchange, sequence coverage and redundancy. With a series of publications in the last 1.5 years (see below) demonstrating the abilities and the applications of the ms2min HDX system there has been considerable interest shown by the HDX community in a commercial system. In this talk we will explain: the features of the FastHDX prototype; what new insights can be gleaned from the faster labelling data in various application areas. We want to use this introductory talk, and direct meetings at our booth to engage with potential customers of the system and better understand your application needs as we move forward to commercialisation.
References: Monika Kish, Victoria Smith, Natasha Lethbridge, Lindsay Cole, Nicholas. J. Bond and Jonathan J. Phillips. Online Fully Automated System for Hydrogen/Deuterium-Exchange Mass Spectrometry with Millisecond Time Resolution.

23 *Deciphering Lipoprotein Lipase Regulation: How metastability is utilized to control lipolysis*

Thomas J.D. Jørgensen
University of Southern Denmark

Lipoprotein lipase (LPL) plays a pivotal role in intravascular lipolysis, a process critical for the hydrolysis of triglyceride-rich lipoproteins into free fatty acids and monoacylglycerol. We have used hydrogen/deuterium exchange mass spectrometry (HDX-MS) to unravel the metastable nature of LPL and its regulatory mechanisms. The borderline stability of the LPL hydrolase domain is essential for its function and regulation, with the unfolding of this domain leading to enzyme inactivation. This work demonstrates how LPL interaction with its transport protein, GPIHBP1, and its activator protein, APOC2 protects it against inactivation. In contrast, the interaction with endogenous inhibitors (e.g., ANGPTL4) induces unfolding of the hydrolase domain. These findings offer molecular insights into the regulation of LPL activity by its metastable nature, highlighting the intricate balance of protein stability and interaction with regulatory proteins in controlling lipid metabolism. This work advances our understanding of LPL's role in lipid metabolism and its potential as a target for therapeutic intervention in dyslipidemias.

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24 *Structural basis of DNMT3A oligomeric states in acute myeloid leukemia revealed by hydrogen-deuterium exchange (HX) and cross-linking (XL) mass spectrometry*

Shaunak Raval, Emma Garcia, Keith Rivera, Maya Virshup, Brian B. Liau, Steven A. Carr, Malvina Papanastasiou
The Broad Institute of MIT and Harvard

DNMT3A is essential for regulating gene expression and its dysregulation has been implicated in cancer. The DNMT3A activity is modulated through interactions between its three domains. The WT DNMT3A forms a homotetramer via interactions between its MTase domain. Mutations along the oligomer-forming surface, notably R882H that is prevalent in myeloid malignancies, have been shown to promote larger oligomeric DNMT3A species, which reduces enzymatic activity of DNMT3A, while deletion of PWWP domain prevents the oligomerization. Therefore, understanding the interactions between DNMT3A domains is critical for determining its role in disease and developing new therapies. Although structural approaches have provided detailed mechanistic insights into DNMT3A regulation, no published structure has successfully resolved all three domains. In an effort to better understand structural determinants of oligomerization, we employ DNMT3A WT, R882H and mutants that disrupt the intra- and inter-domain oligomeric interface, forming dimers and monomers. Comparative analysis between WT and a mutant dimer revealed lower D-uptake of many overlapping peptides spanning the MTase and the PWWP domain. PWWP is missing from high-resolution structures, but has been implicated in stabilizing the WT tetramer formation. XL-MS of WT DNMT3A revealed extensive contacts between MTase and PWWP, further supporting the HX-MS data and its role in stabilizing the oligomeric interface. These preliminary findings provide a comprehensive view of intra- and inter-domain interactions between the full length WT tetramer and a mutant dimer that are difficult to obtain using other methods, laying the groundwork for future studies involving clinically relevant pathogenic mutations such as R882H.

25 *Cleavage preferences of proline-selective enzymes revisited*

Petr Man, Zuzana Kalaninova, Jasmina M Portasikova, Daniel Kavan, Petr Novak
Institute of Microbiology, Czech Acad Sci

Proteolysis plays a critical role in all structural proteomics workflows, providing spatial resolution (HDX), enabling the analysis of large, heterogeneously modified proteins, and facilitating the precise localization of modifications (FPOP, cross-linking). Although each workflow has its own "golden standard" protease, the search for new enzymes is a never-ending quest. In HDX-MS, several studies have highlighted the importance of alternative proteases such as nepenthesins, aspergillus or rhizopuspepsins, which efficiently cleave after the basic amino acids. In recent years, the utility of proline-selective enzymes such as Aspergillus niger prolylendopeptidase (AnPEP, ProAlanase) or neprosin, which mainly target proline and alanine, has been demonstrated. Here, we generated several AnPEP-based columns using a cheaper alternative to research grade ProAlanase. By testing the columns, we uncovered novel specificities not only of AnPEP (acidic serine protease) but also of neprosin (glutamate endopeptidase). We then delved deeper into cleavage preference characterization and AnPEP benchmarking, and developed a software tool called DigDig to facilitate data processing, perform rapid digest parameterization, and extract cleavage preferences from single proteins or complex mixtures. Financial support: OP JAK Photomachines, CZ.02.01.01/00/22_008/0004624

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26 *Exploiting the awesome power of hydrogen exchange to uncover hidden and transient conformations in protein folding, degradation and SARS- CoV-2 Spike.*

Susan Marqusee
UC Berkeley and National Science Foundation

The amino acid sequence of a protein encodes more than the native three-dimensional structure; it encodes the entire energy landscape – an ensemble of conformations whose energetics and dynamics are finely tuned for folding, binding and activity. Small variations in the sequence and environment modulate this landscape and can have effects that range from undetectable to pathological, even when the protein's folded structure is unchanged. I will describe our studies employing the awesome power of HDX to peak into the hidden regions of the energy landscape and investigate their importance in protein folding, degradation, and the SARS- CoV-2 Spike protein.

27 *An atlas of structural and dynamic variation throughout the HIV-1 Env glycoprotein*

Kelly Lee, Edgar A Hodge, Gajendra S Naika, Mint Laohajaratsang
University of Washington

Extraordinary sequence diversity across HIV-1 isolates is concentrated in the virally encoded surface glycoprotein, Env. At present, we have a limited understanding of the extent of structural variation and the impact on diverse Env's antigenic profile that results from the underlying sequence differences among HIV isolates. In order to understand how broadly neutralizing antibodies achieve breadth and potency, it is essential not only identify to identify the conserved aspects of their target epitopes, but to also identify how they cope with and manage the extremely variable contexts in which the conserved features are embedded. Here we use hydrogen/deuterium exchange mass spectrometry (HDX-MS) to compare structural dynamics throughout native-like Env trimers across a panel of HIV-1 isolates that is representative of the global diversity of circulating virus. The impact of epitope dynamics and stability on antibody binding and neutralization are examined. These results provide a structural dynamic atlas of variation embodied in diverse Env trimers and provide new insight into the nature of neutralization sensitive versus resistant isolates of HIV-1. Understanding the structural link between neutralization phenotype, activity of an antibody, and a given Env is anticipated to be pivotal for the development of engineered vaccines against HIV-1.

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28 *Structural dynamics and macromolecular assembly of the Ebola virus fusion glycoprotein GP*

Valeria Calvaresi, Weston Struwe
University of Oxford

The Ebola virus (EBOV) GP is a fusion glycoprotein mediating virus internalization into host cell endo-lysosomal compartment, where it undergoes proteolytic cleavage prior to receptor binding, followed by membrane fusion. Although several entry models have been proposed, the exact molecular mechanisms remain largely unknown. Here, we conducted HDX-MS and mass photometry (MP) to uncover molecular bases of GP-mediated virus entry and antibody-mediated neutralization. To understand how EBOV evolved its functionality and antigenic behaviour, we performed HDX-MS on the GP trimer of EBOV species responsible for most epidemics; Zaire EBOV (Makona and Mayinga strains) and Sudan EBOV. We developed an HDX-MS workflow with integrated glycoproteomics that enabled tracking the HDX of all 17 N-glycosylated GP sites, yielding 95% effective sequence coverage. The HDX comparison of GP at neutral and acidic pH, and the cleaved form (GPcl) at acidic pH uncovered that the receptor binding domain switches from an occluded conformation to a binding-competent exposed conformation through correlated exchange (EX1 kinetics). In GPcl, the fusion loop and HR2 in GP fusion subunit displayed increased HDX, priming GP for post-fusion transition but also expanding the local antigenic surface. By MP, we observed that neutralizing human mAbs targeting the fusion subunit can interact with GPcl at acidic pH, forming high-molecular weight crosslinked species that likely block the structural rearrangements required for fusion. Altogether, we reveal that the combination of HDX dynamic information with insight into macromolecular assembly probed by MP can elucidate the functional mechanistic aspects of fusion glycoproteins interacting with the human host.

29 *Asymmetric Packaging of Genomic RNA in RNA Viruses Promotes Egress by Integrative Cryo-EM and HDXMS*

Sean Braet, Varun Venkatakrishnan, Ganesh S Anand
Pennsylvania State University

Hydrogen/deuterium exchange mass spectrometry (HDX-MS) has Icosahedral viruses are highly symmetric structures that undergo dynamic transitions in response to environmental perturbations throughout their lifecycles. While icosahedral symmetry has been leveraged to uncover high resolution structural details by cryo-EM, viruses also contain essential asymmetric features including distinct coat protein copies and nucleic acid genomes that play a critical role in their lifecycles. The role of these asymmetric features in viral assembly/disassembly transitions and especially their roles in RNA egress into the host remain poorly understood. Here we demonstrate how amide hydrogen deuterium exchange mass spectrometry (HDXMS) is critical to offset the limitations of symmetric averaging of virus cryo-EM. These results offer a window to observe changes in RNA protein binding at the viral interior during assembly/disassembly. We find by cryo-EM that the RNA first recruits a small subset of coat proteins forming a ribonucleoprotein complex that recruits additional dimers during assembly. We further show that by HDXMS that this histone-like RNP complex is maintained in the native viral particle. Structures of disassembly intermediates reveal that the RNA genome concentrates near the viral five-fold axes prior to egress. HDXMS shows that the concentration of RNA near the fivefold axes is accompanied by the formation of additional RNP contacts and reveals an asymmetric genome egress site at a single five-fold axis. Viral nucleic acid genomes act as drivers for both viral particle self-assembly and asymmetric disassembly and are far from passively packaged entities. These results have far-reaching implications for RNA virus assemblies, packaging and gene delivery.

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30 *Benchmark Performance of the Vanquish Neo UHPLC System Integrated with Thermo ScientificTM Orbitrap ExplorisTM Mass Spectrometer for HDX-MS workflow*

Ulrik H. Mistarz, Yuqi Shi[1], Ulrik H. Mistarz[2], Christopher Pynn[3], Martin Samonig[3], Rosa Viner[11]Thermo Fisher Scientific, San Jose, CA; [2]Thermo Fisher Scientific, Allerød, Denmark; [3]Thermo Fisher Scientific, Germering, Germany
Thermo Fisher Scientific

Hydrogen/deuterium exchange mass spectrometry (HDX-MS) has emerged as a critical analytical tool to study protein conformation, conformational dynamics, and protein-protein interaction. Here we present and benchmark a fully automated next-generation HDX-MS solution that interfaces Trajan LEAP robot with a Vanquish Neo/Flex UHPLC system and Orbitrap ExplorisTM 240 MS and highlight the Vanquish Binary Pump N analytical pump capabilities at performing chromatographic separation at HDX-MS compatible conditions in both low-, medium, and high-micro flow rates with great sensitivity, reproducibility, and performance.

Phosphorylase b 15 pmol/injection was diluted, quenched, and subjected to online enzymatic digestion (proteaseXIII/pepsin, NovaBioAssays), followed by desalting (Acclaim PepMap trap) and separation. The separated peptides were analyzed with data dependent MS/MS HCD and identified using Thermo ScientificTM BioPharma Finder software.

System performance was evaluated at different gradients (3, 7, and 10-min) and flowrates (10, 40, and 80 µL/min). Over 1000 peptides were identified for 7 min and 10 min gradients at 40 µL/min with above 95% sequence coverage, and over 700 peptides at 93% sequence coverage were identified with short 3-min gradient method. For higher 80 µL/min flow rate, the system was able to identify close to 1000 peptides for both 7-min and 10-min gradient, and about 600 peptides at 3-min gradient. Moreover, Vanquish Neo separation pump was able to operate robustly at low microflow condition (10 µL/min), providing excellent sensitivity and reproducibility with close to or above 1000 peptides identified for the 7-min and 10 min gradient, and close to 700 peptides for the 3-min gradient.

31 *Mapping the Conformational Landscape of the Rabies Virus Glycoprotein*

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UC Berkeley

Rabies causes approximately 60,000 deaths per year. There is an unmet need for cost-effective, efficacious treatments that are accessible to all areas of the world. The rabies virus glycoprotein (RABV-G) is densely displayed on the surface of the virion, making it the primary target for therapeutics. RABV-G is a highly dynamic protein that is known to reversibly sample a prefusion and extended-intermediate conformation at physiological pH, making it difficult to study using traditional methods. In this work we use HDX-MS to measure the pH-dependent conformational equilibrium of RABV-G both as a soluble construct and displayed on a virus-like particle (VLP) to interrogate the importance of the environment on the conformational landscape. We measure local stability at varying pHs and find that the presence of a membrane biases the conformational landscape of RABV-G towards the prefusion conformation. Rationally designed mutations previously reported to trap RABV-G in the prefusion conformation based on viral fusion assays were found to not change the conformational landscape of RABV-G in a soluble construct. This suggests the fusion deficit may be caused by interfering with a later fusion step. Finally, by examining the effect of antibody binding with both soluble and VLP displayed protein, we can bridge the gap between in vitro binding measurements and efficacy of the antibody to inhibit infection. This illustrates how our HDX-MS method is changing our understanding of the RABV-G protein in a way that will fuel the next generation of therapeutics to combat this lethal virus.

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32 *A PARP2-specific active site alpha-helix melts upon DNA damage-induced enzymatic activation*

Ben Black, Emily S. Smith, Ramya Billur,
Marie-France Langelier, John Pascal,
University of Pennsylvania

Poly (ADP-ribose) polymerase (PARP) 1 and PARP2 recognize DNA breaks immediately upon their formation, signal their location, and are co-targeted by all current FDA-approved forms of PARP inhibitors (PARPi) used in the cancer clinic. We have used HXMS to define key aspects of PARP1 activation upon binding to a DNA break, as well as to gain insight into allosteric impacts of substrate, NAD+ (and PARPi), and its binding partner, HPF1. HXMS has been particularly complementary to more traditional structural and biochemical methodologies since it powerfully accesses information about protein dynamics. Recent evidence indicates that the same PARPi molecules impact PARP2 differently from PARP1, raising the possibility that allosteric activation may also differ. Indeed, we now find that despite an activation mechanism shared by both PARPs involving destabilization of an autoinhibitory domain, PARP2 requires further unfolding of an active site alpha-helix absent in PARP1. Only one clinical PARPi, Olaparib, stabilizes the PARP2 active site alpha-helix, representing a feature with the potential to discriminate small molecule inhibitors. Collectively, our findings reveal unanticipated differences in structure/backbone dynamics between PARP1 and PARP2 that impact their allosteric regulation.

33 *A disordered light chain allosterically enhances the protease activity of muPA*

Constanza Torres-Paris, Harriet J Song, Felipe Engelberger, César A. Ramírez-Sarmiento, Elizabeth A Komives
University of California San Diego

The urokinase-type plasminogen activator (uPA) is a serine protease involved in extracellular remodeling. This protein has an EGF-like, a Kringle, a disordered 27-residue linker, and a serine protease domains. Previous authors have shown that an antibody binding to the protease domain inhibited the proteolytic activity of murine uPA (muPA) differently when the linker was present. We hypothesized that the linker was allosterically affecting the ensemble of conformations acquired by the protease domain of muPA. To test this hypothesis, we generated two variants of muPA that consisted of the protease domain only or the linker region plus the protease domain. Their activity and dynamics were compared using activity assays, accelerated molecular dynamics and hydrogen deuterium exchange mass spectrometry (HDX-MS). Our results show that the linker enhances the protease catalytic activity by 3.7-fold. The linker is very dynamic, exchanges all its amides rapidly, and does not protect a defined binding site on the protease domain. HDX-MS revealed allosteric effects of the linker in mainly two regions: 1) the linker dampens deuterium uptake in the 140s loop; and 2) the linker changes the proportion of conformational ensembles in the 220s loop, which generally exhibits a bimodal distribution of deuterium uptake under the conditions studied here. In addition, two mutations, K4G and F(-2)A in the linker of muPA, were also tested which exhibited partially (K4G) or completely (F(-2)A) the same allosteric effects of the wild type linker. Finally, a mechanism explaining how the linker could allosterically affect the protease domain is proposed.

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34 *Asymmetry and Allostery in Pre and Post Steady-State Dynamics of Mo-dependent Nitrogenase*

Brian Bothner, Monika Tokmina-Lukaszewska, Maureen Kitheka, Luke Berry, Hayden Kallas, Qi Huang, John W. Peters, Lance C. Seefeldt, Simone Raugei
Montana State University

Mo-dependent nitrogenases overcome the high energy barrier to the reduction of atmospheric nitrogen (N2) through the coordinated action of a six subunit protein complexes. The reaction is dependent on a steady input of energy in the form of ATP and electrons that are supplied through a repeating catalytic cycle between MoFe protein (FeP) and Fe protein (FeP). A kinetics analysis of pre-steady state reactions concluded that negative cooperativity was at the heart of the FeP protein electron delivery cycle. Biophysical and Computational analyses suggest that conformational gating driven by mechanical coupling coordinates electron transfer. Here in we describe a set of in solution and in silico experiments designed to test the hypothesis that conformational change and protein dynamics are an integral component of negative cooperativity in the catalytic cycle of MoFeP. Pre-steady-state and equilibrium hydrogen-deuterium exchange mass spectrometry (HDX-MS) was used to quantify peptide-level deuterium incorporation on the millisecond time scale. This revealed distinct conformational and dynamic asymmetry of the structurally symmetric MoFeP. Our findings were then tested using course-grained computations to investigate correlated and anticorrelated motions. Together, HDX-MS and computation were used to develop a temporal and spatial model for the allosteric regulation of FeP mediated electron delivery to nitrogenase. Overall dynamics of the complex appear to be controlled by FeP with distinct pre-steady-state patterns actuated when 0, 1, or 2 FeP are bound. We show the role of protein dynamics and tuning of the conformational landscape during catalysis occur on a millisecond time scale across subunits and are consistent with classic models of negative allostery.

35 *A sequential priming mechanism of the progesterone receptor revealed by structural mass spectrometry*

Patrick Griffin, Matthew D. Mann, Min Wang,
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The Wertheim UF Scripps Institute

The progesterone receptor (PR) is a nuclear hormone receptor implicated in breast cancer. PR exists as two isoforms, A and B, where isoform B has a canonically longer N-terminal domain (NTD). Ligand-bound PR dimerizes, binds to target DNA sequences, and forms a transcription complex with oncogenic co-regulator (CoR) proteins. However, these interactions and their pertinence to cancer are unknown. Current cryo-EM structures lack atomic resolution, so we utilize structural mass spectrometry to characterize PR:CoR interactions. Crosslinking (XL) and hydrogen-deuterium exchange (HDX) mass spectrometry (MS) were performed on functionally active recombinant PRA and PRB isoforms and CoRs. Through hydrogen-deuterium exchange mass spectrometry, we find that PR isoforms differ in how they bind CoRs and that PR preferably binds activation sites in the CoR, steroid receptor coactivator 3 (SRC3). XL-MS shows that both isoforms can interact with co-regulators SRC3 and P300 in a similar manner. However, HDX-MS reveals that PR-A undergoes significant reorganization upon both PRE binding, a phenomenon not seen in PR-B. XL-MS reveals that the addition of histone acetyltransferase P300 further strengthens these interactions. We observe increased deuterium uptake in the PR-LBD after SRC3 binding and the PR-NTD after P300 binding, suggestive of CoR-mediated weakening of the PR dimer and CoR-priming mechanism for recruitment of other CoR proteins. In summary, we have identified novel interaction sites between PR and its co-regulators and we describe a new mechanism for PR transcriptional activation. Our activation model agrees with determined transcriptional responses and the non-atomic-level resolution cryo-EM structures previously published.

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ABSTRACTS - POSTERS

P-1 *Unravel the underlying mechanism of multidrug efflux pumps via HDX-MS.*

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ULB

Amidst the mounting challenges of the 21st century, bacterial antibiotic resistance stands a concrete threat. One of the key mechanisms underlying this resistance is the action of efflux pumps, which enable bacteria to expel antibiotics and other antimicrobial agents from their cells. A thorough understanding f-of these fundamental mechanisms is imperative for the development of effective solutions. Our study focus on three efflux pumps LmrP (Lactococcus lactis), NorA and QacA (Staphylococcus aureus), belonging to Major Facilitator Superfamily (MFS). These secondary transporters, energized by the proton-motive force, have partially overlapping substrates recognition profiles. Their functional and structural homology makes them good model to study the molecular basis of multidrug recognition and the investigation of conserved versus specific mechanisms. Practically, we perform differential HDX-MS (ΔHDX-MS) experiments to compare the HDX signature of the three proteins in the presence and absence of various substrates. The first outcome shows that LmrP, QacA and NorA exhibit high level of HDX versus other MFS transporters with a narrower substrate recognition profile. Moreover, presence of substrate leads to distinctive patterns. In the case of LmrP, whole helices involved in binding substrate and energy-coupling become protected in presence of substrate. For QacA, a different phenomenon is observed, most part of the protein become more exposed in presence of substrate. These observations have suggesting that a correlation between dynamics and polyscpecificity can exist. experimental replicates complemented with molecular dynamic simulations are ongoing to con- or infirm that hypothesis.

P-2 *Investigating the protein-membrane interface of the CERT START domain using HDX-MS*

Tristan Gunther, Nicolas-Frédéric Lipp, Itay Budin, Elizabeth A. Komives
University of California, San Diego

The synthesis of sphingolipids – lipids essential to the structure and function of the plasma membrane – begins at the endoplasmic reticulum and is completed in the Golgi. Ceramide transfer protein (CERT) carries out the key ER-Golgi transfer step in this pathway by transporting the sphingolipid precursor, ceramide, in the cavity of its START domain. Structures of the soluble CERT START domain have been resolved, but questions remain about the position of the protein-membrane interface and the mechanism by which ceramide enters and exits the pocket. Hydrogen deuterium exchange mass spectrometry (HDX-MS) is a promising tool for the investigation of proteins that transiently adopt soluble vs. membrane-interacting states. Examining the lipid binding surface of a lipid transfer protein such as the CERT START domain poses a challenge, however, as its low membrane binding affinity requires the presence of high quantities of lipids. Using lipids at these concentrations in a bottom-up HDX-MS workflow is associated with numerous challenges in protein digestion, liquid chromatography, and mass spectrometry analysis. Here we incorporate a simple and effective lipid filtration step using ZrO2 into a traditional HDX-MS workflow, allowing for high experimental lipid concentrations that promote a significant fraction of membrane-bound protein, even in the case of low-affinity interactions. Using our HDX-MS method, we have preliminarily identified deuterium uptake changes that point to a combination of charged and hydrophobic residues responsible for mediating the CERT START domain-membrane interaction, providing the first structural evidence of this critical lipid-protein interface.

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P-3 *Key mutations in Green->Red photoconversion of GFP probed by HDX-MS*

Mark Petersen, Michael Shavlik, Mike Harms, and Susan Marqusee
University of California Berkeley

luorescent proteins play a key role in modern biological microscopy. Ancestral Green Fluorescent Protein (ancGFP) is a beta-barrel protein with a chromophore made by the maturation of QYG. Proteins in the Kaede subclass of GFPs are able to undergo irreversible photoconversion to RFP. This photoconversion requires a key mutation of Q62H in the chromophore. A single Q62H mutation in the ancGFP background is insufficient for photoconversion, but anc15 (a green->red photoconvertible ancestor of Kaede fluorescent proteins) has fourteen additional substitutions away from ancGFP and undergoes robust photoconversion to RFP. To understand the role of key mutations in green->red photoconversion of Kaede ancestors, we performed Hydrogen-Deuterium Exchange coupled with Mass Spec (HDX-MS) on ancGFP, anc15, the single point mutants ancGFP Q62H and T69A, and the compensatory mutants anc15 H62Q and A69T. We find minor, localized differences in the HDX-MS profiles of ancGFP and the H62Q mutant which illustrate why this mutation alone is insufficient for photoconversion. The T69A mutation in ancGFP background has no detectable effect by HDX-MS, while the compensatory A69T mutation in anc15 background exhibits strong, delocalized changes by HDX-MS that underscore the importance of this mutation outside the chromophore. This result has implications for the evolution of new colors in fluorescent proteins and the importance of compensatory mutations that affect local stability to accommodate new functions in protein evolution.

P-4 *Investigating the mechanisms of p110a inhibition*

Hunter Nyvall, Jenkins ML, Burke JE
University of Victoria

Phosphoinositide 3-Kinases (PI3K) are important lipid kinases which facilitate the formation of phosphatidylinositol 3,4,5-triphosphate (PIP3) by catalyzing the addition of a phosphate group to phosphatidylinositol 4,5-bisphosphate (PIP2). PIP3 is a critical lipid signaling molecule integral to many fundamental cellular processes such as growth, survival and proliferation. PI3K*a* consists of a catalytic (p110*a*) and regulatory (p85*a*) subunit; of these p110*a* stands out as the second most frequently mutated protein identified in cancer, with gain-of-function mutations leading to the dysregulation of tightly regulated cellular pathways. For this reason, we aimed to elucidate the intricate molecular mechanisms underlying the inhibition of p110*a* with our research. Our investigation entails a comprehensive analysis of p110*a*'s interactions with an ATP competitive inhibitor (GDC-0077) andan allosteric inhibitor (WYM3) by leveraging hydrogen-deuterium exchange mass spectrometry (HDX-MS) to map the binding sites of p110*a*. We were able to identify unique binding sites and allosteric changes for the two inhibitors on p110*a*, furthering the notion that multiple strategies can be used to effectively inhibit the activity of p110*a*. By discerning the structural nuances governing p110*a* inhibition, we aim to further the development of highly targeted,mutant specific, small molecule inhibitors, offering promising avenues for the development of pharmaceutical products which can be used to treat cancer patients bearing p110*a* mutations.

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P-5 *From transporter to motor: Helix folding cooperativity in the evolution of an auditory protein*

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Mammalian cochlear amplification relies on voltage-dependent longitudinal contractions of outer hair cells, a process triggered by concerted actions of millions of the motor protein prestin. This motor function is unique to mammalian prestin with non-mammalian orthologs being anion transporters despite having high sequence and structural similarities. The molecular basis is unknown for prestin's transition from an anion transporter to a motor and how the motor couples voltage-sensing with areal expansion involving multiple helices. Using HDX-MS, we investigate how helix folding cooperativity contributes to prestin's motor function. We find that the motors have a unique fraying anion-binding site whose folding and voltage dependence are coupled to anion binding. The transporters use the same binding site but have cooperative helices forming the gate-core interface that likely promotes transitions from inward- to outward-facing states needed for anion transport. While prestin in a lipid bilayer retains most HDX patterns seen in micelles, a cooperative lipid-facing helix, TM6, is destabilized. TM6 is further destabilized under increased membrane tension which promotes prestin's areal expansion. Mutating the conserved glycine residues on TM6 tunes prestin's voltage sensitivity, suggesting TM6's helicity is regulated during prestin's transition between compact and expanded states. Overall, our results show that changes in helix folding cooperativity, specifically fraying of the anion-binding site and cooperative unfolding of a lipid-facing helix TM6, play pivotal roles in prestin's evolution from a transporter to a motor protein.

P-6 *Structure formation in KcsA folding pathway revealed using HDX pulse-labeling*

Michael Baxa, Andrew V. Molina, Kevin C. Song, Isabelle A. Gagnon, Lydia Blachowicz, Benoît Roux, Tobin R. Sosnick
University of Chicago

Potassium channels are membrane proteins critical for electrochemical regulation and function in almost all animal cells. The process of potassium channel folding and oligomerization is disrupted in many genetic misfolding diseases but remains poorly understood. Outstanding questions concern the structure of folding intermediates, the sequential events involving selectivity filter folding and pore helix insertion to produce the native tetramer, thermodynamic characterization, and the role and generalizability of a protein-dense phase. We have extensively studied the in vitro folding behavior of KcsA, a robust model system for ion channel folding for many human potassium channels, e.g. hERG and Kv1.2. We present results from HDX-MS that demonstrate the extensive stabilization of the KcsA tetramer compared to the monomer. Most notably, we adapted HDX pulse-labeling to membrane protein folding in liposomes to observe site-resolved changes in hydrogen bond formation and stability during oligomerization for the first time. We observe rapid formation of secondary structure in the transmembrane glycine zipper and the inner half of the pore helix, followed by slower folding of the selectivity filter, turret, and outer half of the pore helix on the same slow timescales of tetramer formation. In the context of our previous work, this suggests that these slow-folding structures act as an architectural "keystone" that is assembled last to stabilize the structure into its native fold.

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P-7 Optimizing membrane protein signal in complex lipid environments: characterizing full length Influenza A neuraminidase

Mason Saunders, Noelle Reimers, Vada Becker, Dr. Jacob Croft Dr. Libin Xu, Dr. Mike Guttman, Dr. Kelly Lee
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Hydrogen/deuterium-exchange mass spectrometry (HDX-MS) experiments have classically been carried out using soluble proteins. Many proteins, however, are membrane-bound or membrane-associated, and their conformation and dynamics can be influenced in significant ways by the membrane environment. Performing HDX-MS experiments in the presence of lipids poses major challenges. Lipids in membrane protein samples can dramatically reduce the quality of HDX-MS data due to overlapping lipid-peptide mass envelopes, reduced peptide ionization, and inhibition of proteolysis of the protein target. Recently, zirconium dioxide (zirconia) beads have been shown to be effective in reducing the abundance of phospholipids in relatively ideal HDX samples with simple lipid compositions. However, zirconia beads are less effective in samples containing complex lipid mixtures that are commonly found in biological membranes. Zirconia treatment of biological membranes leaves an abundance of lipid species which can impede peptide trapping and alter chromatography as well as inhibit proteolysis and overlap peptide mass envelopes. To optimize delipidation in complex environments, we are investigating full-length influenza A neuraminidase (NA) displayed on extracellular vesicles (EV) produced by mammalian cells. While NA has been thoroughly studied as a recombinant ectodomain that excludes the stalk, transmembrane domain, and cytosolic tail, these key structural elements and the presence of membrane are known to impact enzymatic activity and antigenicity. Using the NA-EV system plus other viral antigens, we are investigating a broad range of quench, digest, and chromatographic conditions with the aim of advancing HDX-MS workflow methods for analyzing membrane protein structure and dynamics in complex, near-native specimens.

P-8 Deciphering Binding Interface of SARS-CoV-2 Nsp2 and human Rap1Gds1 using Structural Proteomics

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University of California, San Francisco

Host-directed therapeutics (HDT) is an increasingly recognized and effective approach in combating infectious diseases. To optimize HDT, targeting host factors without disrupting their natural functioning is crucial. Understanding the structural dynamics of virus-host protein complexes involved in infection is essential for identifying potential drug targets. The initial analysis of the SARS-CoV-2 protein interaction network revealed that mutations in Non-structural protein 2 (Nsp2) do not interfere with its interaction with human Rap1Gds1. However, the detailed structural and functional events governing this host-pathogen interaction (Rap1dgs1-Nsp2) remain unknown. Our recent efforts have produced an atomic model for Nsp2, combining cryoEM with AlphaFold2, but only a low-resolution structure of the complex has been achieved. To gain deeper insights, additional structural biology approaches such as native mass spectrometry (nMS), hydrogen/deuterium exchange mass spectrometry (H/DX-MS), and cross-linking mass spectrometry (XL-MS) are employed. Analysis of nMS and XL-MS data reveals that Nsp2 exists in a monomer-dimer equilibrium in solution, while Nsp2-Rap1Gds1 forms a heterodimer exclusively. H/DX-MS uncovers regions of Nsp2 with EX1 kinetics, indicating conformational heterogeneity. Notably, Nsp2 exhibits increased flexibility when interacting with Rap1Gds1, as evidenced by enhanced exchange. These findings highlight the potential of combining multiple structural biology approaches to unravel the complexities of host-pathogen interactions and pave the way for targeted therapeutics development.

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P-9 *Beyond the fold: HDX-MS unveils the structural basis for the enhanced amyloidogenicity of pathogenic human transthyretin (TTR) variants*

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University of Padua

Amyloidosis is a group of rare diseases characterized by the abnormal in vivo accumulation of misfolded proteins in the form of insoluble fibrils. TTR, a homo-tetrameric plasma protein responsible for the transport of thyroid hormones, is one of the 30 proteins linked to the onset of amyloid-based diseases. Genetic TTR amyloidosis (ATTR) is associated with single amino acid mutations in the resulting protein variants, which are much more prone to aggregate and form amyloids, compared to the wild-type protein. One common feature of TTR-amyloidosis is the presence of proteolytic TTR fragments in the amyloid deposits of patients. However, the protease(s) responsible for this proteolytic processing have been not yet safely identified. Intriguingly, the crystallographic analysis of pathogenic TTR variants usually does not provide any reasonable structural explanation for their enhanced aggregation propensity. To better understand the pathway of amyloid formation, we produced five pathogenic mutants of TTR (i.e., V30M, S52P, L55P, E51S52dup, V122I), along with a protective TTR variant (T119M), and we determined their structure and dynamics, stability and susceptibility to proteolysis in comparison to the wild-type protein. Proteolysis experiments were conducted with trypsin and subtilisin, two TTR-cleaving proteases that efficiently generate amyloidogenic fragments. Continuous- and pulse-labeling HDX-MS measurements were carried out to study the dynamics and equilibrium denaturation of TTR mutants. Our results indicate that pathogenic mutations increase the conformational flexibility and reduce the stability of TTR variants, which become more prone to dissociation into monomers and even more susceptible to proteolysis while more efficiently forming amyloid fibrils.

P-10 *Structural analysis of human serum albumin at a foam surface by hydrogen/deuterium exchange mass spectrometry*

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Ryosuke Tanoue, Susumu Uchiyama
Osaka University

Proteins are amphiphilic macromolecules which have hydrophilic and hydrophobic moieties, and thus surface active. The surface activity enables proteins to be used as foaming agents in the food industry. For example, meringue made from egg white is used in the confectionery industry to give sponge cake its characteristic texture and to increase its volume. The structure of the proteins at interfaces is thought to be closely related to the foaming ability. It is also known that protein structure changes at interfaces, and proteins form film-like aggregates at the interfaces. However, the details of the structural changes of proteins at interfaces have not been fully elucidated. In particular, there are no reports on the structure of proteins at non-flat gas-liquid interfaces, such as foam state. A structural understanding of proteins at interfaces would lead to control and improvement of protein foam properties. In this study, we developed a method for structural characterization of proteins in foam state using hydrogen/deuterium exchange mass spectrometry (HDX-MS). HDX-MS of human serum albumin revealed that the N-terminus and a loop (E492-T506) could be adsorption sites, and the conformational change could occur throughout the structure. It was suggested that both structural flexibility and hydrophobicity are important factors for HSA adsorption at the air-water interface (AWI). Because the conformational changes at the AWI were reversible, the established HDX-MS can analyze protein structure in situ and effective for the structural analysis of proteins at the AWI.

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P-11 *Untangling the molecular role of protein disorder in liquid-liquid phase separation.*

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King's College London

Liquid-liquid phase separation (LLPS) has emerged as a new paradigm for cellular organisation through the formation of protein-rich membrane-less organelles. The spontaneous and reversible self-assembly of monomeric proteins into micron-sized coacervates has broad functions in homeostasis mechanisms and, in the case of dysfunction, pathogenesis of protein aggregation diseases. Spatiotemporal, high-resolution structural knowledge of proteins that phase-separate remains elusive due to their typical intrinsically disordered nature. In this work, the intrinsically disordered N-terminus of DDX4 was used as a model system to untangle the conformational dynamics of LLPS. We utilised hydrogen deuterium exchange mass spectrometry (HDX-MS) complemented with techniques including bright field microscopy and circular dichroism (CD) to probe the dependence of LLPS on ionic strength, temperature, and pH. Initial buffer screening assays established that LLPS of DDX4 increased at lower pH, ionic strength, and temperature. Using these results, HDX-MS was then used to obtain high-resolution structural information at the millisecond timescale. HDX-MS confirmed that at lower pH, DDX4 shows increased dynamics and adopts a more open conformation. Notably, charged-patterning regions showed a greater difference in exchange highlighting the importance of electrostatic interactions in stabilising coacervates. To understand protection against HDX in monomeric DDX4 compared to phase separated DDX4, thermal ramping CD was used. Results showed that phase separated DDX4 is characteristically unfolded, and upon de-mixing into the monomeric state, a peak showing beta-sheets is observed. Moreover, this structural transition is reversible. The HDX-MS workflow created studies the phenomena of LLPS with high spatiotemporal resolution.

P-12 *HDXBoxeR: R package for statistical analysis and visualization of multiple protein states in the differential HDX-MS*

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Miklos Guttman, Rachel Klevit
University of Washington

HDX-MS, a high-information-content method, presents both opportunities and challenges for researchers. While it offers extensive insights, the process involves meticulous data analysis, with each HDX-MS experiment yielding information on hundreds of peptides. With the necessity for triplicate experiments and the inclusion of multiple time points for each protein state, comparisons between various protein states, often focusing on ligand binding or mutations affecting the wild-type apo protein state, can result in datasets comprising thousands of peptides. Addressing this complexity, HDXBoxeR, a statistical framework written in the R language, is specifically designed for post-processing after HDXExaminer analysis. HDXBoxeR focuses on differential peptide characterization between multiple sets leveraging Welch's T-test and the Critical Interval statistical approach. This automated process streamlines the matching and comparison of sets, facilitating efficient identification of significantly different peptides between specified protein states, expedites data export, generates Pymol scripts, and provides calculations and outputs for streamlined publication processes.

POSTER

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P-13 *HDX-MS Reveals RNA-binding mediated changes across Type III-E Cas Effectors*

Calvin Lin, Calvin P. Lin, Harry Li, Daniel J. Brogan, Tianqi Liu, Fangying Chen, Omar S. Akbari, Elizabeth A. Komives
University of California San Diego

Cas effectors utilize a CRISPR RNA (crRNA) for recognition and cleavage of target nucleic acid sequences and have been prolifically used as research tools and more recently as a gene-editing therapy. The high utility of these proteins and prevalence in bacterial immune systems has created an entire field of Cas protein discovery. One of the more recently discovered Cas effector subtype III-E Cas7-11 exhibits several novel characteristics among Cas proteins, namely its ability to cleave ssRNA without additional non-specific collateral cleavage garnering Cas7-11 interest as a therapeutic candidate. Additionally, Cas7-11 is unique among Cas proteins in its domain composition which represents a single-chain version of a previously seen multiprotein complex. Now fused together by linkers, Cas 7-11 is composed of structurally similar Cas7 domains and a Cas11 domain each possessing different and separate functions. Little is known about its apo state as well as its insertion (INS) domain within one of the Cas7 domains which deletion mutants have shown to have no effect on activity. Cas7-11 is a 1601 amino acid protein and we achieved over 80% sequence coverage by incubating in quench for an additional minute. We compared the apo protein to the crRNA-bound state which showed a global decrease in exchange upon RNA binding. Specifically, Cas11 showed marked decreases in exchange despite being far from the crRNA binding site. This suggests domain reordering that prime substrate recognition prior to target RNA (tgRNA) binding. Additionally, bimodals at the INS domains suggest a conformation primed for tgRNA recruitment.

P-14 *Endophilin B1 recruits and activates PI3KC2B to mediate mitochondrial fission during mitophagy*

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Leibniz Forschungsinstitut für Molekulare Pharmakologie

The class II phosphoinositide 3-kinases are involved in the spatiotemporal control of nutrient sensing and degradative processes, such as the inhibition of mTORC1 at the lysosome by PI3KC2B and the generation of clathrin mediated endocytic pits at the plasma membrane by PI3KC2A. These enzymes are activated through binding of their C-terminal PX and C2 domains binding to PI(4,5)P2 positive membranes. Here we identify a novel interacting partner of PI3KC2B, the membrane remodeling BAR protein endophilin B1, which has been shown to have roles in autophagy and mitophagy. Using a compilation of HDX-MS, biochemical assays, and cellular biology, we aim to characterize the exact mechanisms of this interaction and its role in cellular processes. Knockout of PI3KC2B prevented fission of mitochondria under stress conditions, and accumulation of mitophagy markers including PINK1. Initial in-vitro pull-down assays and HDX-MS of PI3KC2B and endophilin B1 showed weak binding, however endophilin B1 that was targeted to the mitochondria was able to recruit PI3KC2B. Lipid sedimentation assays showed strong recruitment of PI3KC2B to liposomes by endophilin B1, and kinase activity assays revealed a strong activation of PI3KC2B by endophilin B1 only through liposomes, but not in the presence of soluble substrate. HDX-MS of both proteins with different lipid substrates revealed regions of PI3KC2B that undergo conformational changes only in the presence of both endophilin B1 and membrane, explaining how PI3KC2B can specifically be recruited and activated in its various cellular processes.

POSTER

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P-15 *HDX-MS of surface-bound IgM provides insight into complement cascade activation*

Charles Mundorff, Malika Hale, Adian Valdez, Lauren Carter, Neil King, Marion Pepper, Miklos Guttman
University of Washington

Immunoglobulin Ms or IgMs are a class of antibodies that are critical in activating the immune system. IgMs are pentameric or hexameric antibodies with remarkably high affinities due to their avidity, or ability to combine many binding events into one antibody-antigen interaction. The IgM class of antibody is the most potent activator of the complement cascade, a family of proteins involved in downstream immune cell recruitment and destruction of targets. However, the mechanism by which IgMs function to activate the complement cascade is not fully understood. Here we investigated IgM-antigen interactions to better understand their ability to trigger immune responses. This was accomplished with protein-based nanoparticles that display antigens in a well-controlled and precise manner to model biologically relevant surfaces. The difference in structure and complement activation between hexameric and pentameric IgM were monitored using hydrogen-deuterium exchange mass spectrometry (HDX-MS). Changes in IgM dynamics were unique to surface-bound IgM constructs compared to solution-bound IgM. Additionally, we observed changes that were dependent on the oligomeric state of the protein, some of which showed a change in response to antigen binding and some of which didn't. The comparisons highlight many of the underlying structural changes that occur during IgM-mediated complement activation.

P-16 *A Rosetta-based pipeline for molecular modelling guided by experimental HDX-MS data*

Didier Devaurs
University of Edinburgh

Data produced by HDX-MS experiments is often interpreted using a crystal structure of the studied protein, when available. However, it has been shown that the correspondence between HDX-MS data and crystal structures is usually not satisfactory. In previous work, we developed a computational pipeline based on ad-hoc software to perform protein conformational sampling guided by experimental HDX-MS data. This method generates protein conformations that fit the HDX-MS data better than any crystal structure or conformational ensemble produced with molecular dynamics. With the goal of making this method available to a broad audience, we have worked towards integrating it within the widely popular Rosetta molecular modelling platform. The result is a Rosetta-based pipeline that takes as input a protein's structural model (e.g., of the native state) and HDX-MS data collected for this protein (e.g., for a non-native state), and that can explore this protein's conformational space to produce a conformation fitting the HDX-MS data. We have applied this pipeline to two proteins to answer different research questions. First, we have generated a structural model for the apo form of a medium-sized protein, the ligand binding domain of the vitamin D receptor, for which structures were reported in the Protein Data Bank only for its holo form. Second, we have created an atomic-resolution structural model for the native state of a large protein, the complement protein iC3b, which was previously only described by medium-resolution structural models. These two examples illustrate the breadth of applications that can benefit from our pipeline.

POSTER

POSTER

P-17 *SIRT1 N-terminal domain as a novel anchor for PPARγ insulin-sensitivity deacetylation*

Caique Camargo Malospirito, Gabriel Ernesto Jara, Victor Ulian Antunes, Giovanna Blazutti Elias, Fernanda Aparecida Heleno Batista, Marieli Mariano Goncalves Dias, Paulo Sergio Lopes de Oliveira, Ana Carolina Migliorini Figueira, Miklos Guttman
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Metabolic syndrome, characterized by insulin resistance, poses a global health threat, increasing the risk of diseases like type 2 diabetes and obesity and straining healthcare systems worldwide. In recent years, targeting the deacetylase SIRT1 has emerged as a promising approach to address insulin resistance by promoting insulin sensitivity through the deacetylation of PPARγ on residues K268 and K293. This offers potential for innovative treatment strategies that involve the control of specific acetylation levels and gene expression in adiposity cells.However, the advancement of novel strategies to treat insulin resistance is impeded by the absence of structural information regarding SIRT1 deacetylation and PPARγ activation. Previous results based on binding assays and molecular modeling show that the SIRT1 N-terminal domain (NTD) acts as a novel anchor for SIRT1 substrate recognition, and that PPARγ shares a common binding interface for NTD anchoring that is independent of the deacetylated residue.To advance our knowledge about SIRT1:PPARγ, we aim to use a hydrogen-deuterium exchange mass spectroscopy (HDX-MS) approach to identify the residues and interfaces important for complex formation. The peptides and exchange rates from HDX-MS will then be input into an in-silico model to generate an in-silico model of PPARγ deacetylation through SIRT1. This study will emphasize the importance of NTD for substrate recognition, offering potential applications in future studies for drug development targeting insulin resistance treatment.

P-18 *Structural characterisation of Neurofilament light chain (NfL) by mass spectrometry approaches (MS) and hydrogen deuterium exchange (HDX) - MS*

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National Measurement Laboratory, LGC

Neurofilament light chain is an early non-specific biomarker in neurodegenerative diseases and traumatic brain injury indicating axonal damage. Measurements of NfL in biological fluids show variations in the total levels of the protein measured across different laboratories depending on the pre-analytical steps and employed techniques e.g. immunoassay or liquid chromatography mass spectrometry (LC-MS). We selected a primary calibrator to develop a reference measurement procedure (RMP) by LC-MS for the quantitation of NfL in cerebrospinal fluid (CSF) and blood derivatives traceable to the International System of Units (SI). The availability and dissemination of the RMP will ultimately enable more accurate measurements and differential diagnosis in the clinics. As part of this workflow, we characterised recombinant NfL also by peptide mapping, intact analysis, ion mobility mass spectrometry and HDX-MS. The structure of NfL is not fully understood, some subdomains however have been crystallised and homology models are available. Ion mobility revealed that higher order structure intermediates of NfL were present. Differential HDX-MS was performed in KBPS and in artificial CSF spiked with human serum albumin (HSA) serving as a surrogate matrix mimicking CSF. Interestingly, NfL displays a very different solvent accessibility pattern in artificial CSF spiked with HSA as compared to aqueous buffer (KPBS). Overall, in KPBS NfL is more solvent accessible than in the surrogate matrix, however this profile is reversed in distinct regions for example in the N-terminal, the vicinity of 99-109 and 136-142. We anticipate that these results will help understand key structural determinants of NfL folding and assembly.

POSTER

POSTER

P-19 *Cataract-prone variants of human γD-crystallin populate a conformation with a partially unfolded domain under native conditions*

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Human γD-crystallin, a monomeric protein abundant in the eye lens nucleus, must remain stably folded for an individual's entire lifetime to avoid aggregation and cataract formation. γD-crystallin contains two homologous domains, an N-terminal domain (NTD) and a C-terminal domain (CTD), which interact via a strong hydrophobic interface. Several familial NTD-destabilizing mutations are linked to early-onset cataract. One of these, V75D, is known to populate an intermediate with a folded CTD and a completely unfolded NTD under mildly denaturing conditions. However, the intermediates populated under native conditions and their role in aggregation are not known. We employed hydrogen-deuterium exchange mass spectrometry (HDX-MS) to characterize variants of γD-crystallin under both native and mild denaturing conditions. For two cataract-prone variants of γD-crystallin, V75D and W42R, we identify a conformation populated under native conditions that retains partial structure in the NTD and is thus structurally and energetically distinct from the intermediate populated under mildly denaturing conditions. The NTD-CTD interface is crucial to the formation of this new intermediate, and disruption of the interface either by mutation or by mild denaturation permits direct observation of both intermediates at the same time. This newly identified intermediate exposes a surface which is normally buried both in the full-length protein and in the protein's isolated domains, and it therefore may play an important role in cataract formation.

P-20 *Exploring EX1 kinetics in library-scale HDX-MS experiments*

Jane Thibeault, Állan Ferrari, Gabriel Rocklin
Northwestern University

Initial experiments demonstrate the feasibility of analyzing mixtures of small protein exhibiting a range of EX1/EX2 behavior using high-throughput, top-down HDX-MS. To aid in this analysis, we are refining an in-house analysis pipeline to detect resolved and unresolved EX1 kinetics by scrutinizing isotopic cluster peak width and shape, enabling simultaneous determination of unfolding kinetics for multiple protein domains. We explore the correlation between these initial experiments and kinetic stability values previously obtained for individually purified proteins throughout the literature with the aim of highlighting the reliability of our mixture-based HDX approaches as an efficient alternative to the more labor-intensive individual protein analysis. Building on this foundation, the analysis pipeline is applied to mine additional insights from existing high-throughput HDX datasets from our lab, each dataset encompassing approximately 1000 proteins, which have primarily been analyzed with the assuming of a prevailing EX2 kinetic regime thus far. This expansion enables a more nuanced exploration of protein dynamics and stability, extending the utility of the HDX technique to a broader range of kinetic behaviors. As we continue to refine our analytical methods, we aim to broaden the scope of proteins and conditions that can be analyzed, thereby enhancing our understanding of protein behavior in complex biological systems.

POSTER

POSTER

P-21 *Comprehensive HDX-MS Analysis is a Key Driver in Pharmaceutical Research*

Sarah Mundigl, Cornelia Wagner, Urs Hanke, Maximiliane König
Roche Diagnostics GmbH

In pharmaceutical research HDX-MS is a powerful technique to characterize the interaction of therapeutic proteins and their corresponding targets. During drug development epitope mapping greatly supports the selection of the lead molecule with the desired properties. Moreover HDX-MS reveals conformational changes of the therapeutic protein upon antigen interaction, after mutation or stress testing (e.g. temperature). We have established a very robust and automated workflow that makes HDX-MS our method of choice for characterizing epitopes and associated structural changes quickly and comprehensively for multiple drug candidates. The integration of the recently developed PNGase Rc column, purchased from Affiro, into the workflow enhanced sequence coverage, peptide redundancy and consequently significance of our HDX-MS data. We show examples how HDX-MS analysis enabled us to answer various project specific questions.

P-22 *HDX-MS characterization of selective peptidic ligands for ARID1B*

Dayana Argoti, Weiping Jia, Fabian Sesterhenn, Gregor Cremosnik, Mark Knapp, Jenny Tang, Lili Xie, Yannick Mesrouze, Lukas Leder, Cesar Fernandez, Cristina Nieto-Oberhuber, Giorgio G. Galli, Patrick Rudewicz
Novartis Biomedical Research

ARID1A and ARID1B are mutually exclusive subunits of the BAF variant SWI/SNF chromatin remodeling complexes. The ARID1A subunit has been found to be frequently mutated in various cancers. These ARID1A mutant cancers depend on the paralog ARID1B for cancer cell proliferation. The high sequence similarity of ARID1A to ARID1B creates a challenge for the development of ARID1B selective binders. Recent Cryo-EM studies on BAF complex revealed the structure of the C-terminal EHD2 domain of ARID1A. ARID1A-EHD2 adopts the all-helical conformation of armadillo-repeat domains. Importantly, the EHD2 domain is sufficient and necessary for complex assembly by stabilizing the entire BAF complex by engaging multiple complex subunits. Despite the high sequence similarity between ARID1A-EHD2 and ARID1B-EHD2 domains, structural information on ARID1B is lacking, limiting an assessment of its ligandability for therapeutic targeting and the identification of selective binding pockets. The study of ARID1A/B outside of the BAF complex is challenging as a large portion of ARID1A/B is intrinsically disordered making it difficult to study by both cryoEM and Xray crystallography. In this study, two ARID1B selective and reversible peptidic ligands with nM affinities were identified as tool compounds and structural mass spectrometry techniques such as native MS and HDX MS were used to characterize conformational protein dynamics and binding areas. We present in this poster the use of both native MS and HDX-MS to study the protein dynamics of ARID1B with and without peptidic binders and elucidation of areas of intrinsic disorder and engagement with proposed binding pockets.

POSTER

POSTER

P-23 *Title: Elucidating the Mechanism of Allosteric Activation and Inhibition of Liver Pyruvate Kinase*

Evan Bonnand, Evan Bonnand, Agnieszka Bogucka, Oscar Nilsson, Liliana Håversen, Jan Borén, Morten Grøtli, Marko Hyvönen, Sheena D'Arcy
The University of Texas at Dallas

Pyruvate kinase (PK) is a tetrameric enzyme which mediates the final and energetic payoff step of glycolysis. Several natural modulators including ATP, Fructose-1,6-bisphosphate (FBP), and amino acids tune PK activity and thus glycolytic flux through both competitive and allosteric means. Recently, allosteric molecules which bind at a site distinct from that of native ligands have been developed. Some of these compounds are activating while other are inhibiting, making them useful as drugs. As a key metabolic regulator with this potential to be drugged, Liver PK (PKL) is a medicinally important target. Despite crystallographic studies investigating ligand binding with PKL, the underlying mechanism of activation/inhibition remains unclear. To elucidate how both native and non-native ligands transmit allostery and influence PKL activity, we performed a large-scale hydrogen-deuterium exchange coupled with mass spectrometry (HDX) experiment. We investigated the effect of two native ligands, ATP which inhibits and FBP which activates PKL. Additionally, we looked at nine non-native allosteric modulators, including four activators and five inhibitors, which bind in a pocket formed between two PKL protomers. We compared the deuterium uptake of PKL and PKL with ligand revealing precise signatures for activators and inhibitors. Results confirm binding within the predicted pocket for ligands lacking crystallography data. We observed allosteric changes stemming from the binding site through oligomeric contacts to the active site. Here, we propose how these small molecules impact enzyme activity. Results will inform the design of better allosteric modulators with the aim of bringing new pharmaceuticals to the clinic.

P-24 *Interpretation of Hydrogen/Deuterium Exchange Mass Spectrometry*

Yoshitomo Hamuro
Johnson and Johnson

This paper sheds light on the meaning of hydrogen/deuterium exchange-mass spectrometry (HDX-MS) data. HDX-MS data provide not structural information but dynamic information of an analyte protein. First, the paper considers the reaction mechanism of backbone amide HDX reaction and tests the correlation between the parameters from an X-ray crystal structure and the protection factors of HDX reactions of Cytochrome c. The presence of H-bond in a protein structure has a strong influence on HDX rates which represent protein dynamics, while the solvent accessibility affects only weakly on the HDX rates. Second, the energy diagrams of HDX reaction at each residue in the presence and absence of perturbation are described. The free energy change upon ligand binding may be complicated due to the presence of unbound analyte protein in the protein-ligand mixture, whereas the free energy change upon mutation can be directly measured by the HDX rates. Third, the meanings of HDX and other biophysical techniques are explained using a hypothetical protein folding well. The shape of the protein folding well describes the protein dynamics and provides Boltzmann distribution of open and closed states which yield HDX protection factors, while a protein's crystal structure represents a snapshot near the bottom of the well. All biophysical data should be consistent yet provide different information because they monitor different parts of the same protein folding well.

POSTER

POSTER

P-25 *Taking HDX-MS to the next level*

Frank Sobott, Oliver Crook, Michele Stofella
University of Leeds

We will present the outline of a new community effort, guided by a white paper, in order to move HDX-MS to the "next-level". We wish to canvas the opinion of the community and initiate efforts to establish what current practices are used by different laboratories, and why. This will help to inform an update to the previously published guidelines, by suggesting diversified workflows for HDX experiments depending on their goals and intended outcomes (e.g. mapping binding sites vs. structural dynamics). HDX is a mixture of many effects, some of which remain poorly understood and difficult to control. Current practice is overwhelmingly qualitative and relative: the majority of experiments seek to highlight statistically significant protection changes by identifying differential exchange patterns between two or more states. Quantitative and "absolute" data would provide valuable inputs for modelling and machine learning, but exchange-rates need to be calibrated correctly and experimental conditions incl. buffer-effects and back-exchange well characterized. Nevertheless some HDX experiments could be much faster and automated. As part of this exercise, we identified a number of topics which are to be addressed

- Experimental planning (protein-stability tests, types of replicates, time-points and how to use them)
- Spatial resolution (digestions strategies, peptide overlaps and alternative MS/MS fragmentation)
- Temporal resolution (fast/msec approaches, pulsed HDX, non-equilibrium experiments)
- Statistical significance (sources of error, error-propagation and suitable statistical filters)
- Measuring accurate exchange (calibrating protection-factors, controlling &quantifying back exchange, interpreting EX1-kinetics)
- Modelling structural dynamics (predicting exchange-behavior, ensemble reweighting)
- Data sharing (depositories for raw/processed data, open formats, essential metadata, machine-learnable)

P-26 *Covariance analysis provides new insights into the sources of deuteration uncertainty in HX-MS*

David Weis
Bristol Myers Squibb

Detection of subtle changes in protein structure and dynamics in differential HX-MS requires a high degree of measurement precision. Improvements in precision will minimize the uncertainty and thereby decrease the threshold for detecting these changes in proteins. Efforts directed at increasing precision will be more fruitful only if the sources of uncertainty can be identified. Some potential sources of measurement-to-measurement variability include variation in the labeling time, variations in the volume fraction of deuterium under labeling conditions, variations in pH and temperature affecting both on- and off-exchange, and mass spectral noise. Covariance, and the related correlation coefficient, are measures of how much two variables "move together". In a practical sense, large, positive covariance indicates instances where deuteration is consistently higher or lower across all peptides within a given sample replicate. Such effects are attributable to experimental factors upstream of mass spectrometry, such as, for example, labeling temperature drift, variations in dispensing volumes, and variations in labeling time. PRELIMINARY RESULTS In this work, covariance analysis was used on a large data set of HX-MS measurements obtained on bovine carbonic anhydrase II during robotic liquid handler optimization (13-15 replicates). The optimization itself, guided by sample, labeling, and quench tracers (Wrigley et al., Anal. Chem., in press), was focused on minimization of volumetric variation in the liquid handling steps. Optimization resulted in a substantial decrease in deuteration variation. In comparison to the optimized method, deuteration measurements using the original operating parameters exhibited a much higher degree of positive correlation with larger magnitudes of covariance, indicating that between-replicate factors made a substantial contribution to the overall deuteration uncertainty, particularly for peptides with a high MS signal-to-noise ratio. This result indicates that the variation arises from the amount of labeling on the peptides as opposed to variation in the measurement of the label. Within the optimized results, deuteration covariances were much smaller for 30 min of labeling compared to 31 s of labeling, suggesting that further optimization of liquid handling for short labeling times is needed.

POSTER

POSTER

P-27 *Mapping Epitopes on Borrelia burgdorferi Outer Surface Proteins by Hydrogen Deuterium Exchange Mass Spectrometry (HDX-MS)*

Clint Vorauer, Beatrice Muriuki, David Vance, Michael Rudolph, Lisa Cavacini, Nicholas Mantis, Miklos Guttman
University of Washington

Lyme disease is a growing infectious disease with limited treatment and vaccination options, making it a global health concern. The Borrelia burgdorferi pathogen's outer surface proteins are promising targets for therapeutic intervention and targeted vaccine development. Here we examine the interactions of various surface proteins with known neutralizing antibodies using Hydrogen Deuterium Exchange Mass Spectrometry (HDX-MS). Our preliminary data reveal diverse neutralizing binding epitopes, suggesting intricate interplay between the bacterium and host immune systems. Immobilized, affinity enriched, HDX-MS was utilized to provide a further level of detail on these antibody interactions. This data informs vaccine efficacy by highlighting therapeutically relevant regions of outer surface proteins recognized and targeted during natural infections and vaccinations.

P-28 *Standardization of HDX-MS Conditions for Antibody Paratope Mapping using a Range of Acidic Proteases*

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Rapid Novor Inc

Understanding paratope mapping is essential to reengineer antibodies for improved selectivity and affinity. Hydrogen-Deuterium Exchange Mass Spectrometry (HDX-MS) has gained popularity with its ability to process large proteins. Efficient digestion of large proteins for optimal sequence coverage involves testing different enzymes and buffer conditions. However, the applicability of the HDX-MS method is constrained to proteases that function within the pH 2-3 range, as alkaline or weakly acidic conditions can eliminate deuterium labeling, rendering the experiment ineffective. Identifying conditions that offer comprehensive coverage for a given monoclonal antibody (mAb) is crucial. Given their structural similarity, this pursuit aims to establish conditions applicable to a wide array of mAbs. In this study, we used various acidic proteases from commercial vendors compatible with Ultra-Performance Liquid Chromatography (UPLC) to test their efficiency for antibody digestion and coverage map optimization. The experiments were carried out using the Trajan automation platform and Waters Cyclic IMS MS. The data was analyzed using DynamX 3.0 HDX Data Analysis Software. We chose 8 different commercially available immobilized acidic proteases to digest the mAb and map the identified peptides on the protein sequence. Interestingly, the peptide mapping results showed different sequence coverage for various enzymes. Our results showed that the effective digestion of mAb can be achieved, which will help standardize the paratope mapping. The insights from this study will empower researchers to streamline the paratope mapping experiments, enhancing the efficiency of mAb characterization.

POSTER

POSTER

P-29 *Exploring Variations in Deglycosylation Effectiveness Between PNGase Dj and PNGase Rc for HDXMS*

Kristin Hughes, Charles C. Mundorff; Miklos Guttman; Deepa Balasubramaniam
Eli Lilly

Hydrogen deuterium exchange coupled with mass spectrometry (HDXMS) is a technique used for epitope mapping in large molecule discovery. N-linked glycosylation is a common post-translational modification that adds glycan chains to asparagine residues and is often crucial for proper function. For HDXMS, the presence of glycosylation poses major challenges, often limiting sequence coverage. The acid-active deglycosidases, PNGase Dj and PNGase Rc, have previously been shown to remove N-linked glycans at low pH (pH 2.5), making them compatible with HDXMS quench conditions. We, in collaboration with the Griffin lab at UF Scripps, showed the application of immobilized PNGase Dj to the HDXMS workflow (O'Leary, 2023). PNGase Rc was also immobilized by the Rand Lab and used to characterize epitopes for glycan-binding mAbs (Lambert, 2023). Here we have compared immobilized PNGase Dj and Rc under HDXMS experimental conditions to evaluate peptide coverage, peptide redundancy, relative deuterium uptake, and epitope characterization between the enzymes to explore the differences between their deglycosylation effectiveness across proteins of varying lengths and frequencies of N-glycan sites. Interestingly, we find that PNGase Dj and PNGase Rc exhibit notable differences in how efficiently they cleave different type of glycan chains (complex vs. high mannose) on glycopeptides.

P-30 *Combination of Pepsin and Prolyl Endopeptidase for Enhanced Digestion Efficiency and Sequence Resolution for Protein Higher Order Structure Characterization Using HDX MS*

Chengjie Ji, Victoria Zhang; Shanshan Guan; Jun Dai
NovaBioAssays LLC

The usage of HDX-MS to study protein folding, protein conformational change due to ligand binding, protein-protein interaction and protein modifications has increased rapidly in both academic research institutes and the biopharmaceutical industry over the past decade. Recent advancements in LC/MS, automation, and informatics technologies have made HDX-MS a robust and indispensable tool. Different enzymes, such as pepsin, protease type XIII or XVIII, nepenthesin I and II, as well as prolyl endopeptidase have been reported to be non-specific but have their preferred cleavage sites. Here we present a novel digestion approach by combining pepsin and prolyl endopeptidase enzyme columns in tandem with optimized on-line digestion condition and show data with improved digestion efficiency and sequence resolution for automated HDX-MS experiments compared with single enzyme column (pepsin or prolyl endopeptidase). The immobilized enzyme columns prepared in house were evaluated using our in-house developed HDX automation platform with CTC-PAL. The resultant peptides were separated using a customized C8 column and monitored by Thermo Exploris MS. The tested proteins were denatured either in 4 M urea/0.425 M TCEP or in 2 M guanidine HCl/0.425 M TCEP (pH2.5) for 3 min, diluted inline with 0.1% FA and then loaded onto the immobilized enzyme columns. We have been able to achieve unprecedented sequence coverage and increased resolution for the protein HOS characterization studies using the combination of pepsin and prolyl endopeptidase columns in tandem. More data (not only sequence coverage but also cleavage site statistical analysis) from the studied proteins will be presented.

POSTER

POSTER

P-31 *Using HDX-MS and FPOP-MS/MS To Identify Structural Differences in Intrinsically Disordered Regions of NFkB and IκBα Upon Binding*

Luis Oliva, Elizabeth A. Komives, Lisa M. Jones
University of California San Diego

NFkB is a family of transcription factors that activate inflammatory genes. Previous structural studies on the interactions between RelA-p50 and its inhibitor, IkBa are limited because the intrinsically disordered regions (IDRs) (residues 1-66 of IkBa and residues 322-549 of RelA) were removed. We extended the mass spectrometry protein footprinting method fast photochemical oxidation of proteins (FPOP) and HDX-MS to examine the intrinsically disordered regions in full-length IkBa both free and bound to RelA-p50 containing either residues 19-321 of RelA (NFkB_RHD) or residues 19-549 of RelA (NFkB_FL). HDX-MS and FPOP-MS indicate that residues 57-78 of IkBa are more protected when bound to NFkB_FL than when bound to the truncated NFkB_RHD. IkBa 57-78 corresponds to the region that is interacting with NFkB's amino acid F309. Previous studies indicate that IkBa sequesters the nuclear localization signal (residues 302-306) of RelA. The mechanism by which IkBa does this remains unsolved. Our data infers that the IDR of RelA in NFkB_FL allows F309 to position itself into a pocket of IkBa and allosterically constraining the NLS. FPOP detects protection of TA2's side chains when bound to IkBa. The rel homology domain of NFkB and AR1-AR6 of IkBa displayed the same trends of protection under the presence and absence of RelA's IDR. To further determine if F309 is responsible for the interactions of these two proteins, we will perform in-cell FPOP on HEK293 cells. In resting cells, IkBa is always bound to NFkB in the cytosol. In vitro data will be compared to in-cell FPOP experiments.

P-32 *LemonCatcher: an engineered protein superglue for selective enrichment under HDX quench conditions*

Dietmar Hammerschmid, Anthony Keeble, Polina Heatley, Ryan Lawrence, Mark Howarth, Eamonn Reading
University of Southampton

We set out to develop technology which could address a broader variety of biological questions using the dynamic information afforded by hydrogen deuterium exchange mass spectrometry (HDX-MS). One avenue is to provide methods capable of protein enrichment after a HDX reaction has been quenched. Doing so, we propose, would enable HDX-MS to be better applied to a range of protein dynamics investigations within complex sample mixtures or even within cells. The harsh quench conditions required to sufficiently slow the inherent D-to-H back-exchange in HDX-MS, i.e. 0 °C and pH 2.5-3.0, make this possibility challenging, especially when under time pressure. Here, we present how we engineered a protein-peptide superglue to work within a range of HDX quench conditions - all alike to Lemon juice! – and so named it the LemonCatcher-LemonTag system. Demonstrating that proteins can be engineered to withstand the harsh conditions of the essential quench step to address biological questions of current concern and, as such, technology development in this area showing real promise. However, technology alone is not enough to provide new, robust methods, with analytical developments of the workflow being essential. We, therefore, also present investigations into protein capture methodology which complies with conventional HDX-MS requirements, exploring different levels of sample complexity, surface-coupling strategies, binding conditions (including anti-freeze buffer modifiers), and elution and digestion steps. We envision that this technology and method will lay the foundation for transitioning protein dynamics studies towards more complex or even cellular environments.

POSTER

POSTER

P-33 *Advancement in MALDI for Hydrogen Deuterium Exchange Mass Spectrometry: A Systematic Evaluation*

Taylor Murphree, Miklos Guttman
University of Washington

Hydrogen Deuterium Exchange Mass Spectrometry (HDX-MS) has emerged as a valuable technique for investigating protein structure and dynamics. Although Electro-Spray Ionization (ESI) has been commonly used, Matrix Assisted Laser Desorption Ionization (MALDI) represents a rapid, promising alternative due to recent advancements that minimize back-exchange. Despite its potential, MALDI is rarely used in HDX-MS studies, in part because the effects of sample preparation and ionization on deuterium retention are not fully understood. This study aims to fill this gap by systematically assessing how buffer composition, matrix components, drying protocols, and ionization conditions influence deuterium incorporation in MALDI-HDX. We utilized a series of controls, including imidazolium-based internal exchange reporters (IERs), to examine effects specific to MALDI-HDX. Our findings indicate that significant post-quench exchange occurs within the MALDI source, primarily during sampling. Moreover, back-exchange during drying mirrors what is observed in solution, while back-exchange in the MALDI source appears significantly more nuanced. These findings provide valuable insights into the challenges associated with MALDI-HDX, while also providing a robust basis for optimizing future studies.

P-34 *Development of a thiol-ene microfluidic chip for sub-zero temperature hydrogen/deuterium exchange mass spectrometry (HDX-MS)*

Kasper Rand, Anton Berg, Rasmus R. Svejdal, Drago Sticker, Jack Barrett, Jörg P. Kutter
University of Copenhagen

HDX-MS is a uniquely useful method for analysis of the native dynamic structure and interactions of proteins in solution. Nevertheless, conventional HDX-MS is currently reliant on expensive specialized chromatographic equipment limiting accessibility of the technique to non-specialized labs and furthermore a significant portion of deuterium label is commonly lost (back-exchange). Here we describe the development and application of a microfluidic chip (HDXchip) for global or local HDX-MS analysis that can be cooled to sub-zero temperatures. HDXchip comprises a sample loop, a pepsin-functionalized monolith, and a C18-packed compartment for desalting and separation. HDXchip thus integrates the hallmarks of conventional HDX-MS in a low-cost microfluidic format. The chip is fabricated entirely by thiol-ene polymer through a replica molding process, allowing for simple and cheap manufacturing. The temperature in the chip is controlled with a Peltier-based cooling system. We have demonstrated the ability of the super-cooled HDXchip to analyze the HDX of model peptides with no back-exchange, a significant improvement in back-exchange compared to conventional HDX-MS at 0°C (30-40%). Furthermore, we observe that HDXchip allowed retention of labeling of some side-chains (Arg) in model peptides. Regioselective cooling of the chip enables proteolysis to be performed at >5°C with desalting and separation performed at temperatures from -10°C to -25°C. Work on simple model proteins, indicate that efficient proteolysis (85-100% sequence coverage) and desalting (3M GndHCl) can be achieved, albeit at higher-back-exchange values (10-30%). Work is ongoing to expand and optimize the design and performance of HDXchip for analysis of more complex protein samples.

POSTER

POSTER

P-35 *Cross-Platform Standardization of Gas-Phase Hydrogen/Deuterium Exchange*

Alesi Escobedo, Abhigya Mookherjee, Sanjit S Uppal, Cristiana Meuret, Kimberly Alonge, Miklos Guttman
University of Washington

Gas-phase hydrogen/deuterium exchange (gHDX) coupled with mass spectrometry has become a valuable tool for the characterization of biological macromolecules and discrimination of isomeric analytes. A significant challenge associated with gHDX is the issue of reproducibility due to variations in pressure, temperature, ambient humidity, and instrument settings, which can drastically change the observed exchange kinetics. Prior work has introduced gHDX reference standards for cations to account for some of this variability within a single instrument, but cross-platform data standardization has yet to be explored. Here, we develop and validate a new set of gHDX reference standards for anions and test our standardization approach across two different instruments, focusing on characterizing isomeric carbohydrate analytes. The ability to standardize measurements across different platforms is a significant step in advancing gHDX as a reliable analytical tool for the broader community.

P-36 *HDX of Carbohydrates: Effects and Applications of Labeling during Electrospray Ionization*

Elyssia Gallagher, Ana V. Quintero, Jacob B. Hatvany, Darren T. Gass, Michael S. Cordes
Baylor University

Carbohydrates are isomeric, with these features playing important roles in their biological functions. H/D exchange-mass spectrometry (HDX-MS) is a valuable method for analyzing the conformations and dynamics of solvated proteins. Carbohydrates are susceptible to HDX because they contain labile hydrogens, primarily in the form of hydroxyls. However, there are significant differences in HDX analyses of carbohydrates compared to proteins. Firstly, the exchange rate of hydroxyls is on the order of microseconds to milliseconds, which is orders of magnitude faster than the backbone amides that are traditionally labeled in proteins. Thus, we perform HDX by introducing deuterating reagents (e.g. D2O) to carbohydrates during electrospray (ESI). For these labeling reactions, HDX is effectively quenched when carbohydrates desolvate and adduct to residual metal ions in the spray solvent. Secondly, in-ESI HDX is affected by the electrospray process, including the pH change that occurs as ESI droplets evaporate. Finally, ion/ion reactions using fluoranthene to localize sites of deuterium labeling in carbohydrate-metal adducts result in gas-phase HDX, with the extent of HDX dependent on both the metal forming the adduct and the structure of the carbohydrate fragment. This is distinct from electron-transfer dissociation using fluoranthene to fragment deuterium-labeled peptides, which has been shown to minimize H/D scrambling. Even though these methods are distinct from those used for protein HDX, we have observed differences in HDX for disaccharide isomers following in-ESI HDX. Thus, the continued development of methods to label carbohydrates provides new opportunities to analyze their structures.

POSTER

POSTER

P-37 *Automated Millisecond Time-Resolved HDX-MS for Intrinsically Disordered Proteins*

Lindsay Cole, Ulrik Mistarz, Jenny Ho, Yuqi Shi, Ken Cook, Rosa Viner and Jonathan J. Phillips
Applied Photophysics Limited

Intrinsically disordered proteins and regions (IDP/Rs) are a significant subset of the eukaryotic proteome. with an estimated 33% predicted to have functional intrinsically disordered regions (IDRs)a. IDPs are also notably implicated in severe diseases such as Parkinson's and Alzheimer's. However, IDP/R present a challenge to understanding structure-function relationships due to their inherent disorder. Extending the standard HDX method with a system able to carry out deuterium labelling in the millisecond to minutes range (ms2min-HDX) provides invaluable insights into the structural dynamics of IDP/Rs at the peptide and residue levels. To demonstrate the potential of automated ms2min-HDX to analyse IDP/Rs, we utilized α-synuclein, a well-studied IDP associated with Parkinson's disease, as a model. Our approach involved automated analysis of high-resolution Orbitrap MS data of protein digested by a dual enzyme (pepsin/protease XIII) column yielding an abundance of detected peptide ions. This allowed for strict automated spectral assignment without manual intervention. This was followed by automated kinetic fitting of D uptake curves to a stretched exponential. Results demonstrated that ms2min-HDX provides unprecedented insights into IDP conformational stability, previously challenging to obtain. The technique proved easily automatable in both data collection and data analysis, showcasing its potential for widespread adoption. The fully automated workflow is amenable to screening, offering a valuable tool for drug development targeting IDPs. This research also emphasizes a broader point applicable to all HDX analyses—achieving both high coverage and redundancy allows for stricter automated spectral assignment criteria, whilst retaining good net resolution, enabling fully automated data analysis.

P-38 *Epitope mapping of diagnostic anti-rabies monoclonal antibodies provide structural insight into rabies variant identification in infected wildlife*

Megan Luo, Sophie Shoemaker, John E. Pak, Susan Marqusee
UC Berkeley

Rabies virus is a member of the lyssavirus family and causes over 60,000 deaths annually. There are currently five strains of rabies virus circulating throughout the continental United States. To better control the spread of rabies virus, the Center of Disease Control monitors the prevalence and host range of different rabies virus variants (RVV) throughout the United States. This surveillance relies on a panel of monoclonal antibodies that are known to differentially bind to the nucleoprotein of RVV in a direct fluorescent antibody test (DFA). While these tests are known to accurately distinguish RVV, it is unknown where these antibodies bind to the rabies nucleoprotein. To better understand the fundamental principles of this assay, the epitopes of three monoclonal antibodies from the current panel, which are suspected to have conformational epitopes, were determined using hydrogen-deuterium mass spectrometry. We determined these antibodies bind to distinct surface-exposed epitopes of the nucleoprotein, one of which makes contacts with multiple subunits of the 11-mer ring that is formed by the nucleoprotein. After determining the epitopes, a multiple sequence alignment was examined to look at the conservation of the amino acids in each epitope to determine how these antibodies distinguish between RVV. By determining epitopes, we can now predict how viral mutations could change the specificity and/or sensitivity of a rabies DFA. This work can be used to help further develop rapid immunoassays for more economical RVV diagnostics in the field, in regions that may lack access to microscopy-based DFA diagnostics.

POSTER

POSTER

P-39 *HDX-MS identifies SARS-CoV-2 spike glycoprotein domains with changed conformational dynamics after binding to cyanovirin-N lectin*

Christopher Haynes, Theodore R. Keppel, Sarah H. Osman, Adrian R. Woolfitt, John R. Barr, and Dongxia Wang
Centers for Disease Control and Prevention

Introduction. The COVID-19 pandemic was caused by SARS-CoV-2 coronavirus, and all vaccines to date target the viral spike glycoprotein. An important strategy in the effort to find better vaccines or therapies for COVID-19 includes identification of non-antibody spike neutralizing proteins, with an example being reported spike neutralizing activity by the lectin cyanovirin-N (CV-N). Previous publications indicate that CV-N binds to high-mannose glycan groups on spike glycoprotein, influenza A hemagglutinin, and Ebola virus Gp1,2 glycoprotein. We performed hydrogen/deuterium exchange mass spectrometry (HDX-MS) analysis of Omicron BA.1 Hexapro spike bound to CV-N with varied labeling times and stoichiometric ratios. Preliminary Results. HDX-MS analysis of Omicron Hexapro spike bound to CV-N for different labeling times showed 5 protected regions and 3 de-protected regions of spike; repeating HDX-MS analysis at three different stoichiometric binding ratios and one labeling time indicated the same 8 regions of spike. PyMOL® visualization of this data on RCSB PDB models of spike revealed that the protected RBD region 303-344 was immediately adjacent to glycan N234, and the protected 630 loop was immediately adjacent to glycan N61. This suggests CV-N bound to glycans on N61 and N234 is decreasing deuteration (dynamic motion) of adjacent spike domains via protein-protein interactions. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention. Use of trade names and commercial sources in this presentation is for identification only and does not imply endorsement by the CDC.

P-40 *Breathing dynamics of exposed and buried regions of Human Papilloma Virus capsid*

Amanda Gramm, Dr. Ganesh Anand, Dr. Susan Hafenstein, and Ebere Orji
Pennsylvania State University

Human Papilloma Virus (HPV) is a member of the papillomaviridae family, consisting of almost 200 identified strains with 12 strains considered high risk as they can lead to cancer. Of these 12 strains, HPV16 is of particular interest as it contributes to approximately 50% of all cervical cancer cases. Cryo-electron microscopy (cryo-EM) has provided high-resolution snapshots of the major capsid protein, L1, but has been unable to provide any insights on the critical, disordered structural viral protein, L2, which is responsible for viral DNA trafficking into the host cell nucleus. HDXMS has been critical to probe L2 dynamics and in combination with a specific neutralizing monoclonal antibody (mAb): 7I, we have been able to map the L2 epitope. Since 7I is too large to enter HPV16, sites of interaction as revealed by HDXMS lie outside the virus particle. Interestingly, the epitope spans a known N-terminal site spanning residues 136-145 and a more C-terminal locus spanning residues (374-390) that was previously unknown. HDXMS has revealed the C-terminal antibody recognition site to be palindromic to the N-terminal epitope site used to raise 7I. These results indicate the presence of both termini outside of the major capsid in the intact virus particle. Our results also reveal allosteric effects of antibody binding across the entire virus particle, indicating conformational rearrangements accompanying antibody binding and neutralization. A combination of antibody interactions combined with HDXMS is a powerful probe of disordered structural virus capsid proteins and uncovers viral breathing and conformational changes accompanying host entry.

POSTER

POSTER

P-41 *Utilizing HDX-MS to Characterize Interactions of HIV Envelope Protein with Immune Lectin DC-SIGN*

Meghan McGrath, Kelly K Lee, Vada Becker, Sabriyah Morshed
UW Medicinal Chemistry

Human Immunodeficiency Virus (HIV) is a rapidly evolving virus that remains of significant public health concern. The trimeric envelope glycoprotein (Env), which mediates receptor binding and membrane fusion is the sole virally encoded protein on the virion surface. Env is one of the most heavily glycosylated proteins found on viruses, with N-linked glycans comprising nearly 50% of the mass of the ectodomain. We are investigating Env recognition by DC-SIGN, a lectin protein expressed on the surface of dendritic cells. DC-SIGN binds N-linked oligomannose glycans which are often found on viruses including HIV. DC-SIGN binding to Env results in HIV internalization by a dendritic cell, followed by antigen processing and presentation; this facilitates initiation of an immune response targeting Env. We hypothesize that due to the homotetrameric nature of DC-SIGN, multivalent binding of this lectin to Env trimers may perturb their integrity. Although Env from diverse HIV isolates share ~13 highly (>60%) conserved N-linked glycan sites per protomer in the trimer, the remainder of glycan positions are highly variable. HDX-MS and MS-based glycoprofiling enable us to assess how glycan patterning and differences in glycoprofile affect DC-SIGN recognition. HDX-MS enables us to assess changes in local structural ordering at the level of overall trimer integrity and local epitope dynamics. We will examine a panel of Env from diverse HIV isolates, in conjunction with biolayer interferometry measurements of antibody recognition of known epitopes across Env +/- DC-SIGN. These studies may help inform the development of Env-based vaccine antigens for improved immune responses.

P-42 *Investigating Conformational Dynamics of the Bacterial Adhesion Protein FimH through Hydrogen-Deuterium Exchange*

Pearl Magala, Lisa Tuttle, Angelo Ramos, Rachel Klevit
University of Washington

Pathogenic bacteria, like Escherichia coli (E. coli), infect the intestinal and urinary tracts by sticking to host epithelial cells through the adhesion protein FimH. Despite the constant flow conditions in these tracts, which can dislodge bacteria, adhesion strengthens in their presence to prevent elimination from the host. Positioned at the tip of bacterial pili, FimH helps bacteria stick by specifically recognizing and binding to mannose ligands in host glycoprotein receptors, marking the initial step in establishing an infection. Unlike typical biomolecular interactions that weaken under force, FimH-mannose interactions become stronger under shear force conditions. FimH has a lectin domain for binding ligands and a pilin domain anchoring it to the bacterial pili. When it binds to a ligand, FimH undergoes significant structural changes, with its domains separating, and its binding pocket closing around the ligand. However, existing crystal structures do not explain how these structural changes are linked allosterically. By using Hydrogen-Deuterium Exchange, we aim to examine FimH's conformational dynamics to uncover the pathways and intermediate states that facilitate ligand binding and shear-enhanced FimH-mediated bacterial adhesion.

POSTER

POSTER

P-43 *Mapping the Conformational Landscape of the Rabies Virus Glycoprotein*

Sophie Shoemaker, Megan Luo, Tina X. Li, Wesley Wu, John E. Pak and Susan Marqusee
UC Berkeley

Rabies causes approximately 60,000 deaths per year. There is an unmet need for cost-effective, efficacious treatments that are accessible to all areas of the world. The rabies virus glycoprotein (RABV-G) is densely displayed on the surface of the virion, making it the primary target for therapeutics. RABV-G is a highly dynamic protein that is known to reversibly sample a prefusion and extended-intermediate conformation at physiological pH, making it difficult to study using traditional methods. In this work we use HDX-MS to measure the pH-dependent conformational equilibrium of RABV-G both as a soluble construct and displayed on a virus-like particle (VLP) to interrogate the importance of the environment on the conformational landscape. We measure local stability at varying pHs and find that the presence of a membrane biases the conformational landscape of RABV-G towards the prefusion conformation. Rationally designed mutations previously reported to trap RABV-G in the prefusion conformation based on viral fusion assays were found to not change the conformational landscape of RABV-G in a soluble construct. This suggests the fusion deficit may be caused by interfering with a later fusion step. Finally, by examining the effect of antibody binding with both soluble and VLP displayed protein, we can bridge the gap between in vitro binding measurements and efficacy of the antibody to inhibit infection. This illustrates how our HDX-MS method is changing our understanding of the RABV-G protein in a way that will fuel the next generation of therapeutics to combat this lethal virus.

P-44 *HDX-MS for probing structure of adeno-associated virus capsid*

Tomohiko Ikeda, Yuki Yamaguchi, Mitsuko Fukuhara, Yasuo Tsunaka, Aoba Matsushita, Tetsuo Trisu, Susumu Uchiyama
Osaka University

Recombinant adeno-associated virus (rAAV) is used as a viral vector for in vivo human gene therapy. The rAAV capsid is assembled from 60 subunits of three viral proteins (VPs) of different lengths namely VP1, VP2, and VP3, which share their C-terminal sequences. The structure of only VP3, which is the shortest VPs, has been reported by cryo-electron microscopy and x-ray crystallography, whereas the structure of the VP1 and VP2 unique (VP1/VP2u) region has yet to be determined, despite VP1/VP2u region possessing a biologically functional part. Thus, this study aims to clarify the entire rAAV capsid dynamics by HDX-MS. Our HDX-MS analysis successfully revealed the structural dynamics of rAAV capsid, including part of the VP1/VP2u region under the D2O labeling condition at 4 and 20°C to observe both flexible and rigid regions. Furthermore, a comparison of the structure of full rAAV capsid packaging DNA with empty capsid lacking packaged DNA showed that the packaged DNA protected the hydrogen/deuterium exchange at the VP1/VP2u region as well as the channel structure of the 5-fold symmetry axis, suggesting that the DNA interacts with the specific inner site of the capsid and causes structural changes of the capsid. This study provides the first structural information of the VP1/VP2u regions in rAAV, demonstrating the potential of HDX-MS as a technique to obtain the entire rAAV capsid structural information for the development of AAV-based gene therapy.

POSTER

VENUE

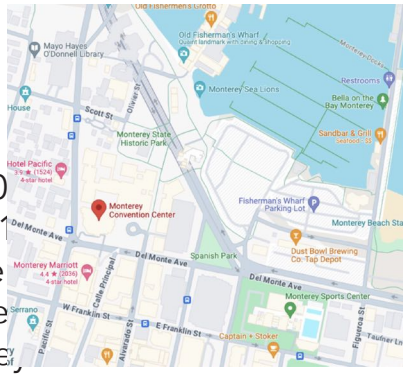
4th International Conference on Hydrogen Deuterium Exchange Mass Spectrometry

MONTEREY CONFERENCE CENTER
One Portola Plaza
Monterey, California, USA 93940

Parking

There are 900 parking spaces available in the City of Monterey's Downtown parking structures one block from the Conference Center. Downtown East located on Washington and Tyler Street between Franklin Street and Del Monte Avenue. Maximum vehicle height is 6 feet 8 inches. Downtown West is located between Tyler Street, Franklin Street and Del Monte Avenue. The first hour is free, then $1.00 per 30 minutes (or fraction thereof) with a daily max of $12.00. Daily rates reset at midnight.

Drive Times



Monterey from MRY: 10
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San Francisco to Monte
Sacramento to Montere
Los Angeles to Monterey

There are two routes to Monterey by car, both very scenic. Highway 1 which runs from San Francisco to Los Angeles, offers breathtaking views of the Pacific coastline. Highway 101 runs down the valley corridor and affords the motorist an opportunity to see the rich land that has made the Salinas Valley.

Getting to Monterey

Monterey is located on California's Central Coast, 100 miles south of San Francisco and 300 miles north of Los Angeles.

Fly direct into Monterey:

Monterey Regional Airport, only 10 minutes from Monterey Conference Center offers direct flights from eight cities: Las Vegas, Los Angeles, Phoenix, San Francisco, San Diego, Denver, Dallas/ Fort Worth and Seattle.

Closest International Airports:

San Jose International Airport is a one-hour drive north. San Francisco International Airport is a two-hour drive north.

Bus or Train:

Amtrak's Coast Starlight train passes through Salinas each day on its route between Seattle, Washington and Los Angeles, providing a scenic way to travel with packages to fit every budget. Free bus service is provided from Salinas to

Shuttle Service from San Francisco Airport OR San Jose Airport to Monterey:

GROOME Transportation
https://groometransportation.com

This company has scheduled services from both San Jose Airport and San Francisco Airport.
Average cost is approximately $62 USD per person (one way).

VENUE

Things to do:

This link will take you to the official City of Monterey website that lists all attractions and activities and that explore the area:

https://www.monterey.org/leisure/things_to_do.php

THE CITY OF MONTEREY

AIRPORT PARKING RESERVIATIONS
https://airportparkingreservations.com/shuttles/san_francisco_airport_sfo

From San Francisco Airport: This company offers several different options for shuttle services, including private sedans to scheduled bus services.

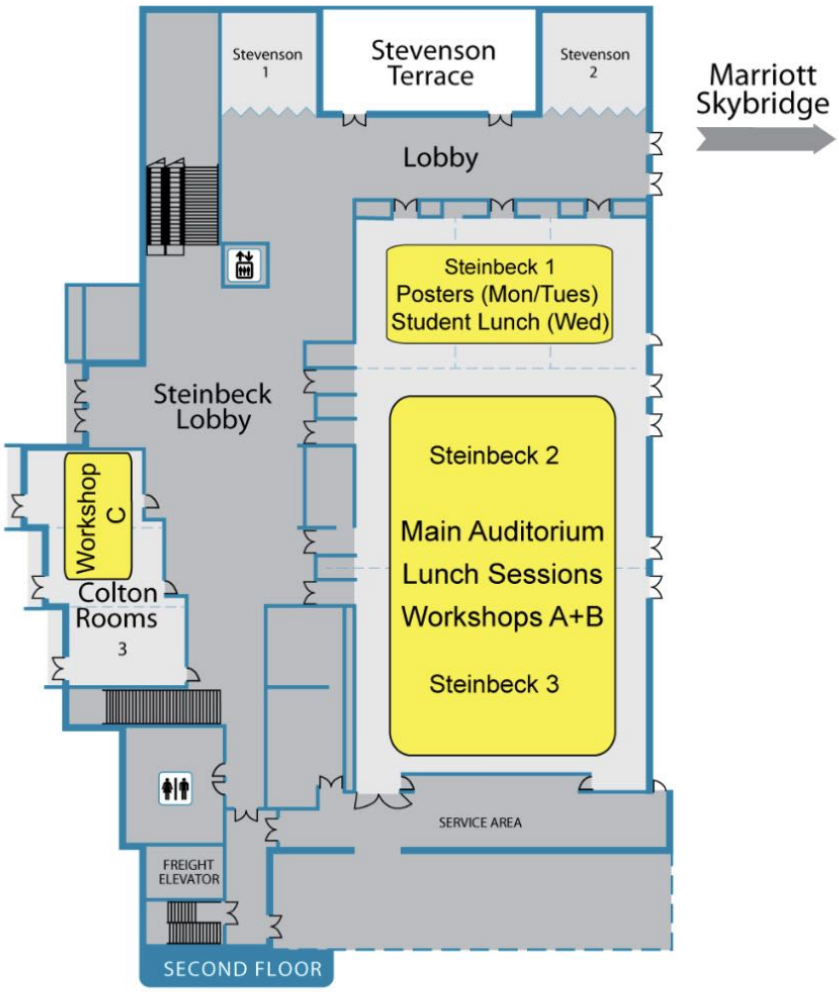
Prices are based on options selected.

Program Locations:

- The registration desk will be in the Steinbeck Lobby
- All sessions will be in Steinbeck 2 and 3.
- Posters will be displayed in Steinbeck 1.
- Workshops will be held in Steinbeck 2, Steinbeck 3, and the Colton Rooms.
- The Sunday evening reception will be in the Stevenson Terrace.
- The Monday and Tuesday Lunch Seminars will be in Steinbeck 2 and 3.
- The Wednesday Student Lunch will be in Steinbeck 1.

WIFI

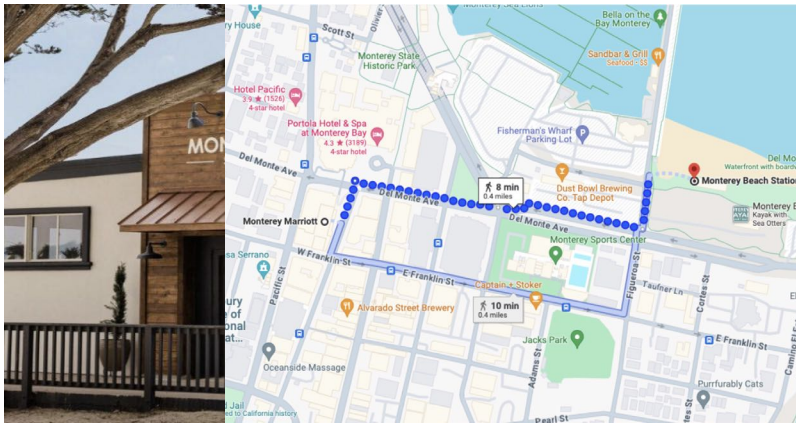
The Monterey Conference Center provides basic complimentary WiFi in all public lobby spaces with a speed of 5 Mbps.



VENUE

DINNER

DINNER, sponsored by TRAJAN
Monday, April 22, 2024 – 6:00 pm to 10:00 pm



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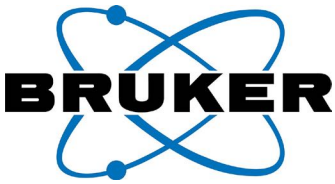


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