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PROGRAM OVERVIEW

SAT, MARCH 2 - SUN, MARCH 3	MON, MARCH 4	TUES, MARCH 5	WED, MARCH 6
	8:00 – 8:30 am Early Morning Coffee *Exhibits & Posters*	**8:00 – 8:30 am** Early Morning Coffee *Exhibits & Posters*	**8:00 – 8:30 am** Early Morning Coffee *Exhibits & Posters*
SATURDAY **9:00 am - 4:00 pm** Full-Day Course, Day 1 **Design and Analysis of Quantitative Proteomics Experiments**	**8:30 – 9:20 am** Plenary Session **Donald F. Hunt Distinguished Achievement in Proteomics Award Lecture** Jennifer Van Eyk	**8:30 – 9:20 am** Plenary Session New Investigator Award **Wilhelm Haas** Computational Proteomics: **Juergen Cox**	**8:30 – 9:20 am** Plenary Session **Lightning Talks: Tips & Tricks**
SUNDAY **9:00 am - 4:00 pm** Full-Day Course, Day 2 **Design and Analysis of Quantitative Proteomics Experiments** **9:00 am - 12:00 pm** Morning Courses **Cross-Linking Mass Spectrometry** **Glycomics and Glycoproteomics: Basics**	**9:20 – 9:50 am** Coffee Break with Exhibits & Posters	**9:20 – 9:50 am** Coffee Break with Exhibits & Posters	**9:20 – 9:50 am** Coffee Break
	9:50 – 11:10 am Parallel Sessions **Proteome Organization in Space and Time,** *Plaza Ballroom* **Immunity and the Microbiome** *Roosevelt-Madison Room*	**9:50 – 11:10 am** Parallel Sessions **Protein Interactions and Signaling**, *Plaza Ballroom* **Infectious Disease** *Roosevelt-Madison Room*	**9:50 – 11:10 am** Parallel Sessions **Posttranslational Regulation**, *Plaza Ballroom* **Glycoproteomics in Biology and Medicine**, *Roosevelt-Madison Room*
	11:10 am – 12:00 pm Plenary Session **Lightning Talks – Round I** *Plaza Ballroom*	**11:10 am – 12:00 pm** Plenary Session **Lightning Talks – Round II** *Plaza Ballroom*	**11:10 am – 12:00 pm** **Lifetime Achievement in Proteomics** **Catherine E. Costello** *Plaza Ballroom*
1:00 – 4:00 pm Afternoon Courses **Glycomics and Glycoproteomics: Advanced** **Stable and Transient Protein-Protein Interactions**	**12:15 – 1:30 pm** **Bruker Lunch Seminar**, *Regency Room*	**12:15 – 1:30 pm** **SCIEX Lunch Seminar**, *Potomac Room* **Thermo Lunch Seminar**, *Regency Room*	
	1:30 - 3:00 pm **Poster Session** *Exhibits & Posters, Atrium*	**1:30 - 3:00 pm** **Poster Sessions** *Exhibits & Posters, Atrium*	
4:30 – 5:30 pm Parallel Workshops **Interactive Career Planning Workshop** for graduate students and postdocs	**3:00 – 4:20 pm** Parallel Sessions **Multi-omics,** *Plaza Ballroom* **Metabolism and Disease** , *Roosevelt-Madison Room*	**3:00 – 4:20 pm** Parallel Sessions **Cancer Early Detection and Prevention,** *Plaza Ballroom* **Informatics: Emerging and New Approaches** *Roosevelt-Madison Room*	
6:00 – 7:00 pm Opening Plenary Lecture **Steven Gygi** *Plaza Ballroom*	**4:30 – 5:50 pm** Parallel Sessions **Advances in Technology** *Plaza Ballroom* **Structural and Chemical Proteomics** *Roosevelt-Madison Room*	**4:30 – 5:50 pm** Parallel Sessions **Protein Proteoforms in Health and Disease** *Plaza Ballroom* **Aging and Neurological Diseases** *Roosevelt-Madison Room*	
7:00 – 8:30 pm **Opening Reception** *Mixer featuring exhibits* *Atrium*	**5:50 – 6:30 pm** **Mixer** with Exhibitors *Poster-Exhibits, Atrium*	**6:00 – 7:30 pm** Workshops **Biomarkers for early detection: What should we measure?** *Roosevelt-Madison Room* **Grantwriting**, *Regency Room*	
	6:30 – 8:00 pm Workshop **NIH Roundtable** *Roosevelt-Madison Room*	**7:30 – 9:00 pm** Social Event, Light Supper *Atrium*	

GENERAL INFORMATION

VENUE. All meetings, sessions, and exhibits are at the Hilton Rockville.

PROGRAM. The abstracts for the talks and posters appear in this book and are arranged in the order of presentation. The author index references the day and type of presentation.

POSTERS AND EXHIBITORS. Posters are located in the hotel's Atrium. All posters should be mounted by Monday morning 9:30 am. Exhibit booths will be in place from Sunday evening welcome reception through Tuesday at 3:00 pm (conclusion of Poster Session on Tuesday.)

Posters are presented/attended on both Monday and Tuesday as follows:

- Odd-numbered posters 1:30-2:15 pm
- Even-numbered posters 2:15-3:00 pm

TALKS. There are two session rooms located in the hotel's meeting space area, Plaza Ballroom and the Roosevelt-Madison Room.

All speakers must appear at least 20 minutes **prior to the start of their sessions.** Speakers using their own laptops will use this time to connect laptops and assure compatibility with the projector. Speakers using the conference PC computer will use this time to load their files.

INTERNET ACCESS. WiFi Is available for conference attendees in the meeting areas. Look for walk-in slides and signage for details on network ID and password.

LUNCH SEMINARS. Free lunch seminars are hosted on Monday and Tuesday in meeting rooms (Regency and Potomac Rooms). All attendees are invited to attend, but are encouraged to RSVP at host company exhibit booths. See program schedule for details.

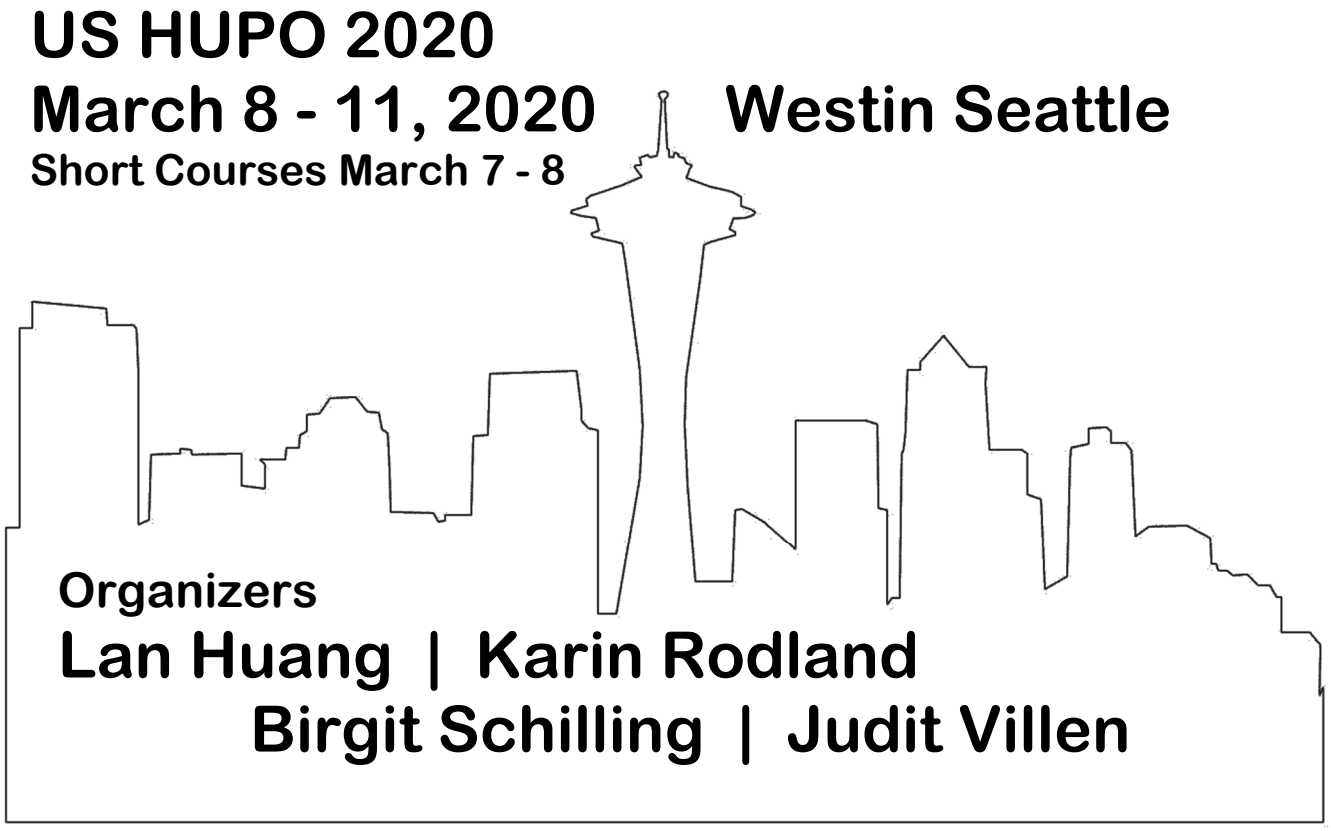
JOB BOARD. Located in Plaza foyer near Registration.

PHONES AND OTHER DEVICES. Please **TURN OFF** all devices (phones, tablets, etc) when in lecture rooms. This courtesy is requested on behalf of presenters as well as fellow audience members.

NO PHOTOGRAPHY or RECORDING of posters or talks is permitted.

YOGA – NAMASTE, Tuesday, 7:00 am, Potomac Room. Join us for an all-levels early morning yoga class. No yoga outfits required, just loose-fitting clothing. We will have towels for you to use as 'yoga mats'.

SOCIAL EVENT ON TUESDAY. Supper-style event in the Atrium featuring live jazz.



EXHIBITORS

US HUPO is pleased to acknowledge the support of conference exhibitors. Exhibit booths are located immediately outside the session rooms along with the technical posters. Sunday welcome reception, Monday-Tuesday coffee breaks, and Monday mixer will be located here with the exhibitors.

Advion

Agilent

BSi Bioinformatics Solutions Inc.

BRUKER

ESI SOURCE SOLUTIONS

EVOSEP

PBI Pressure BioSciences Inc.

MATRIX SCIENCE

MCP MOLECULAR & CELLULAR PROTEOMICS

SCIEX

SHIMADZU Excellence in Science

Silantes Stable Isotope Labeled Biomolecules

ThermoFisher SCIENTIFIC

veritomyx Accuracy matters

VENDOR LUNCH SEMINARS

The following companies offer lunch seminars to conference participants on Monday and Tuesday. Seating is limited. Please reserve a seat at company exhibit booths.

Monday, 12:15 – 1:30 pm *See program schedule for descriptions.*	**Tuesday, 12:15 – 1:30 pm** *See program schedule for descriptions.*
Bruker, *Regency Room* BRUKER	**Thermo Scientific**, *Regency Room* ThermoFisher SCIENTIFIC **SCIEX**, *Potomac* SCIEX

AWARDS

DONALD F. HUNT DISTINGUISHED CONTRIBUTION TO PROTEOMICS AWARD

Award presentation and talk, Monday 8:30 am, Plaza Ballroom

The Donald F. Hunt Distinguished Achievement in Proteomics award recognizes a focused or singular achievement in the field of proteomics. Prof. Hunt was honored as the first recipient of this award which now bears his name.

2019 Recipient: **Jennifer Van Eyk**
Cedars Sinai Medical Center

Dr. Van Eyk has a longstanding record of excellence in applying cutting-edge analytical technologies to address clinically relevant biological hypotheses and in translation into clinical therapies or diagnostics. Dr. Van Eyk is a Professor of Medicine at Cedars-Sinai Medical Center. In keeping with her research mission of bringing discovery to patient care, Dr. Van Eyk serves as Director of the Basic Science Research in the Barbra Streisand Woman's Heart Center, Director of the Advanced Clinical Biosystems Research Institute, and most recently co-director of the Cedars-Sinai Precision Health focused on in-hospital and population individualization of health care.

Dr. Van Eyk is an international leader in the area of clinical proteomics and her lab remains focused on developing technical pipelines for de novo discovery and larger scale quantitative mass spectrometry methods. These encompass data dependent acquisition- and data independent acquisition-mass spectrometry methods for discovery, as well as targeted multiple reaction monitoring (MRM, also known as SRM). Her laboratory is recognized for the rigor it applies to technical quality, control, and reproducibility of increasingly complex datasets that can address key clinical questions with improvements in throughput to enable continuous assessments of large healthy cohorts and clinical grade assays focusing on brain and cardiovascular diseases. Her list of achievements includes more than 325 published articles, over a 20 patents, and numerous research and leadership awards.

The Donald F. Hunt Distinguished Contribution to Proteomics Award is fully funded by Journal of proteome research

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LIFETIME ACHIEVEMENT IN PROTEOMICS AWARD

Award presentation and talk, Wednesday 11:10 am, Plaza Ballroom

The Lifetime Achievement in Proteomics Award recognizes a career of discovery that has made a lasting impact on the field of proteomics.

2019 Recipient: **Catherine E. Costello**
Boston University School of Medicine

Prof. Costello's research has revolutionized glycomics by enabling comprehensive structural characterization of free glycans and glycoconjugates in a sensitive and high throughput fashion. In the 1980s she was among the first to recognize the potential of combining emerging soft ionization techniques with tandem mass spectrometry for characterization of glycans. Today, her 1988 publication defining the dissociation nomenclature for glycans remains highly cited. Prof. Costello delineated the strategy of producing the greatest degree of structural detail on glycans in a single tandem mass spectrometry step. She recognized that while collisional dissociation provides useful information, its utility is limited by low abundances of key cross-ring cleavage product ions. She developed activated electron dissociation methods for glycans and glycoconjugates that are inherently more effective for producing cross-ring cleavages. This has included studies of dissociation mechanisms and influences of metal cationization. Prof. Costello and her co-workers also pioneered Fourier Transform mass spectrometry of glycans and glycoconjugates with the application of electron-based activation/dissociation methods and demonstrated these methods on a time scale compatible with on-line LC-FTICR MS/MS and LC-IM-QTOF MS/MS. Prof. Costello was the first to demonstrate top-down tandem MS analysis of intact glycoproteins. This is significant because most cell surface and secreted proteins are glycosylated and progress in biomedicine depends on the ability to characterize glycosylated proteoforms.

Prof. Costello's research activities define the present state of the art in glycan and glycoconjugate tandem mass spectrometry. These achievements address the needs in biomedicine for high throughput sequencing of glycans and glycoconjugates and for top-down analysis of intact glycoproteins. She has also explored the effects of sequence variants and post-translational modifications on the misfolding of proteins that underlie neurodegenerative and systemic amyloid diseases, the evolution of cardiovascular disease, and MHC-presentation of antigenic peptides during infection and autoimmunity.

Dr. Catherine E. Costello is a William Fairfield Warren Distinguished Professor, Departments of Biochemistry, Physiology & Biophysics, and Chemistry, Boston University, Boston, MA. She has served as President of ASMS, HUPO and the International Mass Spectrometry Foundation.

AWARDS

ROBERT J. COTTER NEW INVESTIGATOR AWARD

Award presentation and talk, Tuesday 8:30 am

The Robert J. Cotter New Investigator Award is given to an individual early in his or her career, in recognition of significant achievements in proteomics, broadly defined.

2019 Recipient: **Wilhelm Haas**
Massachusetts General Hospital Cancer Center, Harvard Medical School

Dr. Haas is an Assistant Professor of Medicine at the Massachusetts General Hospital (MGH) Cancer Center and Harvard Medical School in Boston. He started his laboratory in 2013 and his research group focuses on advancing technologies in mass spectrometry-based proteomics and their application in disease-related basic and clinical research. Born in Austria he did his undergraduate and graduate work at the University of Graz, Austria. He then worked as a postdoctoral fellow in the laboratory of Dr. Steven Gygi at Harvard Medical School, where, among other things, he worked on new technologies enabling multiplexed quantitative proteomics. His lab at the MGH Cancer Center is using quantitative proteomics for global mapping of the dynamics of interactomes to study various questions in cancer research, including the search of biomarkers and novel drug targets to improve personalized therapies for cancer.

GILBERT S. OMENN COMPUTATIONAL PROTEOMICS AWARD

Award presentation and talk, Tuesday 8:30 am

The Gilbert S. Omenn Computational Proteomics award recognizes the specific achievements of scientists that have developed bioinformatics, computational, statistical methods and/or software used by the proteomics community, broadly defined.

2019 Recipient: **Jürgen Cox**
Max Planck Institute for Biochemistry

Dr. Cox earned his Master's degree in physics from RWTH Aachen University in Germany and received his PhD from the Massachusetts Institute of Technology in theoretical particle physics. He then worked at the Basel-based bioinformatics company GeneData and, after a postdoc at the Technical University of Munich, went on to work at the Max Planck Institute of Biochemistry in Munich on problems in computational proteomics. There he heads since 2014 the research lab for computational systems biochemistry. Dr. Cox has contributed greatly to the toolset of computational proteomics by developing the software platforms MaxQuant and Perseus which are in frequent use in the proteomics community. Dr. Cox has co-authored 126 peer-reviewed journal articles.

SUNDAY, MARCH 3

Welcome to the Opening Session

Proteomics at the Frontier of Biology and Medicine

Session Chair: Ileana Cristea (Princeton University)

6:00 - 6:10 pm Opening Remarks

6:10 - 7:00 pm **Insights from Intersecting Two, Proteome-scale, Protein-Protein Interaction Networks;** Steven Gygi, *Harvard Medical School*

7:00 - 8:30 PM: **OPENING RECEPTION**, *Exhibits-Posters*

All attendees are invited to join us for food, drink, and connecting with colleagues.

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MONDAY, MARCH 4

8:00 - 8:30 AM: **EARLY MORNING COFFEE & PASTRIES**, *Exhibits-Posters*

8:30 - 9:20 AM: **Donald F. Hunt Distinguished Contribution to Proteomics Award Lecture**, *Plaza Ballroom*
Session Chair: John Yates (The Scripps Research Institute)

8:30 - 9:20 am **Proteomics Inspiring to Change Medicine**; Jennifer Van Eyk, *Cedars Sinai Medical Center*

9:20 - 9:50 AM: **COFFEE BREAK**, *Exhibits-Posters*
Refresh and visit with the exhibitors.

9:50 - 11:10 AM: PARALLEL SESSION
PROTEOME ORGANIZATION IN SPACE AND TIME, *Plaza Ballroom*
Session Chair: Steve Carr (Broad Institute)

09:50 - 10:15 am **The Spatial Organisation of the Transcriptome and Proteome**; Kathryn Lilley; *University of Cambridge, Cambridge, United Kingdom*

10:15 - 10:40 am **Systems Biochemistry of the Metaphase Spindle**; Martin Wuhr; *Princeton University, Princeton, NJ*

10:40 - 10:55 am **Microprobe CE-ESI-HRMS Reveals Proteomic Reorganization in Spatially and Temporally Developing Cell Clones Directly in Live *X. laevis* Embryos**; Camille Lombard-Banek[1]; Sally Moody[2]; Peter Nemes[1]; *[1]University of Maryland, CP, College Park, Maryland; [2]The George Washington University, Washington, DC*

10:55 - 11:10 am **Extensive Intratumor Proteogenomic Heterogeneity Revealed by Multiregion Sampling in a High-Grade Serous Ovarian Tumor Specimen**; Allison Hunt[3]; Nicholas Bateman[1, 2]; Guisong Wang[1]; Brian Hood[1]; Julie Oliver[1]; Dave Mitchell[1]; Glenn Gist[1]; Ming Zhou[3]; Brian Blanton[1]; Kelly Conrads[1]; Kathleen Darcy[1]; Craig Shriver[2]; Yovanni Casablanca[1]; G. Larry Maxwell[4]; Thomas Conrads[3]; *[1]Gynecologic Cancer Center of Excellence, Annandale, VA; [2]John P. Murtha Cancer Center, Bethesda, MD; [3]Inova Schar Cancer Institute, Falls Church, VA; [4]Department of Obstetrics and Gynecology, Inova, Falls Church, VA*

9:50 – 11:10 AM: PARALLEL SESSION
IMMUNITY AND THE MICROBIOME, *Roosevelt-Madison*
Session Chair: Aleksandra Nita-Lazar (NIAID, NIH)

09:50 - 10:15 am **Proteomic Scatomancy: Towards Stool-based Personalized Health Forecasts**; Joshua Elias; *Stanford University, Stanford, CA*

10:15 - 10:40 am **Metaproteomics to Study Drug Effects on the Gut Microbiome**; Daniel Figeys; *Ottawa Institute of Systems Biology, University of Ottawa, Ottawa, ON*

10:40 - 10:55 am **Interactome and Proteome Dynamics Uncover Immune Modulatory Associations of the Pathogen Sensing Factor cGAS**; Krystal Lum; Bokai Song; Joel Federspiel; Benjamin Diner; Timothy Howard; Ileana Cristea; *Princeton University, Princeton, NJ*

10:55 - 11:10 am **Influence of the Gut Microbiota on Histone Acetylation through Butyrate Oxidation**; Peder Lund[1]; Sarah Smith[1]; Johayra Simithy[1]; Lillian Chau[1]; Elliot Friedman[1]; Yedidya Saiman[1]; Sophie Trefely[2]; Mariana Lopes[1]; Zuo-Fei Yuan[1]; Kevin Janssen[1]; Nathaniel Snyder[2]; Gary Wu[1]; Benjamin A. Garcia[1]; *[1]University of Pennsylvania, Philadelphia, PA; [2]A.J Drexel Autism Institute, Drexel University, Philadelphia, PA*

11:10 AM – 12:00 PM: PLENARY SESSION
LIGHTNING TALKS - ROUND I, *Plaza Ballroom*
Session Chair: Laurie Parker (University of Minnesota)
High-energy (and brief) presentations selected from poster presentations. 90 seconds max or face the music!

Presentation Order

Mon 01 **Proteomic Analysis of the Developing Inner Ear in *Xenopus laevis*;** Aparna B. Baxi[1, 2]; Sally A. Moody[1]; Peter Nemes[1, 2]; *[1]George Washington University, Washington, DC; [2]University of Maryland, College Park, MD.* ***See Poster 073-ML.***

Mon 02 **cGAS is Regulated by Phosphorylation and Acetylation during Infection with Herpes Simplex Virus 1;** Bokai Song; Krystal Lum; Ileana Cristea; *Princeton University, Princeton,.* ***See Poster 099-ML.***

Mon 03 **Host-centric Stool Proteomics Reveals Latent-Phase-Expressed Host Protease Inhibitors Modulate EAE severity;** Carlos Gonzalez[1]; Stephanie Tankou[2]; Laura Cox[2]; Howard Weiner[2]; Josh Elias[1]; *[1]Stanford University, Stanford, CA; [2]BWH, Harvard School of Medicine, Boston, MA.* ***See Poster 071-ML.***

Mon 04 **Coordination between TGF-β Cellular Signaling and Epigenetic Regulation during Epithelial to Mesenchymal Transition;** Congcong Lu[1]; Simone Sidoli[1]; Katarzyna Kulej[1]; Karen Ross[2]; Cathy H Wu[2]; Benjamin A Garcia[1]; *[1]University of Pennsylvania, Philadelphia, PA; [2]University of Delaware, Newark, DE.* ***See Poster 063-ML.***

Mon 05 **Ethionine, Produced by Commensal *Lactobacillus reuteri*, Is Immunomodulatory, Proteogenic, and Leads to Ethylation of Human Proteins;** Daniel Röth[1]; Abby Chiang[1]; Gabriel Gugiu[1]; Christina Morra[2]; James Versalovic[2]; Markus Kalkum[1]; *[1]City of Hope, Duarte, CA; [2]Baylor College of Medicine, Houston, TX.* ***See Poster 101-ML.***

Mon 06 **Utilizing Parallel Reaction Monitoring for a High Throughput Diagnostic Pipeline to Establish Cardiac Troponin I phosphorylation as a Biomarker;** Daniel Soetkamp; Weston Spivia; Qin Fu; Jennifer E. Van Eyk; *Cedars-Sinai Medical Center, Beverly Hills, California.* ***See Poster 005-ML.***

Mon 07 **Glycoproteomics-based Signatures for Tumor Subtyping and Clinical Outcome in Human High-Grade Serous Ovarian Cancer;** Jianbo Pan; Yingwei Hu; Shisheng Sun; Lijun Chen; Jian-Ying Zhou; Michael Schnaubelt; Minghui Ao; Jiang Qian; Zhen Zhang; Daniel Chan; Hui Zhang; *Johns Hopkins School of Medicine, Baltimore, MD.* ***See Poster 049-ML.***

Mon 08 **Composition of the Myddosome during the Innate Immune Response;** Joseph Gillen[1]; Aleksandra Nita-Lazar[2]; *[1]NIH-NIAID, Bethesda, MD; [2]NIAID, NIH, Bethesda, MD.* ***See Poster 119-ML.***

Mon 09 **Proteomic Approaches to Study Cell Death Mechanisms in Human Stem Cell-Derived Retinal Ganglion Cells;** Joseph Mertz; Xitiz Chamling; Ah Young Lee; Xiaoli Chang; Byoung-Kyu Cho; David Clark; Cynthia Berlinicke; Hui Zhang; Donald Zack; *Johns Hopkins Medical School, Baltimore, MD.* ***See Poster 136-ML.***

Mon 10 **Systems-Wide Hijacking of Host Cells during Herpes Simplex Virus (HSV-1) Infection;** Katarzyna Kulej[1]; Ashley N. Della Fera[1]; Eui Tae Kim[1]; Matthew J. Charman[1]; Simone Sidoli[2]; Benjamin A. Garcia[2]; Matthew D. Weitzman[1]; *[1]Children's Hospital of Philadelphia, Philadelphia, PA; [2]University of Pennsylvania School of Medicine, Philadelphia, PA.* ***See Poster 153-ML.***

Mon 11 **Understanding Epigenome and Proteome Remodeling Caused by Novel Germline Histone H3.3 Mutations during Neurodevelopment ;** Khadija Wilson[1]; Geoffrey Dann[1]; Elizabeth J. Bhoj[2]; Hakon H. Hakonarson[2]; Benjamin A. Garcia[1]; *[1]University of Pennsylvania School of Medicine, Philadelphia, PA; [2]Children's Hospital of Philadelphia, Philadelphia , PA.* ***See Poster 075-ML.***

Mon 12 **Proteoform Family Identification and Quantification using Proteoform Suite;** Leah V. Schaffer[1]; Michael R. Shortreed[1]; Anthony J. Cesnik[1]; Jarred W. Rensvold[2]; Adam Jochem[2]; Trisha Tucholski[1]; Mark Scalf[1]; Brian L. Frey[1]; Ying Ge[1</; *[1]University of Wisconsin-Madison, Madison, WI; [2]Morgridge Institute for Research, Madison, WI.* ***See Poster 159-ML.***

Mon 13 **Detection of Malignancy-associated Proteome and Phosphoproteome Alterations in Human Colorectal Cancer Induced by Cell Surface Binding of Growth-Inhibitory Galectin-4;** Malwina Michalak[1]; Uwe Warnken[2]; Hans-Joachim Gabius[3]; Martina Schnölzer[2]; Jürgen Kopitz[1]; *[1]Heidelberg University Hospital, Heidelberg, Germany; [2]German Cancer Research Center (DKFZ), Heidelberg, Germany; [3]Ludwig-Maximilians-University Munich, Heidelberg, Germany.* ***See Poster 017-ML.***

Mon 14 **Lysine Benzoylation Is a Histone Mark Regulated by SIRT2.;** Mathew Perez-Neut; He Huang; Di Zhang; Yingming Zhao; *University of Chicago, Chicago, IL.* ***See Poster 109-ML.***

Mon 15 **The Nature of Phosphatidylinositol Mannosidases (PIMs) Interaction with the PPE68 protein – Revealing Novel Insights in Its Immunogenicity and Virulence;** Nagender Rameshwaram; Sangita Mukhopadhyay; *CDFD, India, Hyderabad, India.* ***See Poster 045-ML.***

Mon 16 **Tissue-Specific Protein Sulfhydrome Analysis in Mice as a Function of Age, Sex, and Diet;** Nazmin Bithi; Belinda Willard; Christopher Hine; *Cleveland Clinic Lerner Research Institute, Cleveland, OH.* ***See Poster 113-ML.***

Mon 17 **Bioinformatics Approach for Understanding the Role of Intrinsic Disordered Regions in Cancer-Related Proteins ;** Rita Hayford; Cathy Wu; Cecilia Arighi; *University of Delaware, Newark, DE.* ***See Poster 019-ML.***

Mon 18 **Uncovering the Prognostic and Therapeutic Potential of N-Acetyl-Aspartyl-Glutamate Metabolism in Cancer;** Sunag Udupa[1]; Tu Nguyen[1]; Brian Kirsch[1, 2]; Ryoichi Asaka[1]; Karim Nabi[1]; Addison Quinones[1]; Jessica Tan[1]; Marjorie Antonio[1]; Felipe Camelo[1]; Ting Li<; *[1]Johns Hopkins Medicine, Baltimore, MD; [2]Johns Hopkins Whiting School of Engineering, Baltimore, MD; [3]University of Pennsylvania Perelman SOM, Philadelphia, PA; [4]Johns Hopkins Bloomberg School of Public Health,.* ***See Poster 057-ML.***

Mon 19 **Building an Antiviral Platform: Nuclear Protein Oligomerization as a Key Contributor to Innate Immune Response;** Tim Howard; Krystal Lum; Catherina Pan; Ileana Cristea; *Princeton University, Princeton, NJ.* ***See Poster 089-ML.***

Mon 20 **Statistical Testing Using Multiple Levels of Quantitative Information in DIA Experiments;** Ting Huang[1]; Roland Bruderer[2]; Jan Muntel[2]; Olga Vitek[1]; Lukas Reiter[2]; *[1]Northeastern University, Boston, MA; [2]Biognosys AG, Schlieren, Switzerland.* ***See Poster 141-ML.***

Mon 21 **Proteomic Characterization of the Spemann Organizer in *Xenopus laevis* (frog) Embryos;** Vi Quach; Aparna Baxi; Peter Nemes; *University of Maryland, College Park, MD.* ***See Poster 149-ML.***

Mon 22 **Multiplexed Quantification Strategy for Candidate Biomarker Discovery and Verification in Alzheimer's Disease;** Xiaofang Zhong; Qinying Yu; Fengfei Ma; Dustin Frost; Lingjun Li; *University of Wisconsin-Madison, Madison, WI.* ***See Poster 001-ML.***

Mon 23 **A New Strategy for the Global Identification and Validation of Post-Translationally Spliced Peptides with Neo-Fusion;** Zach Rolfs; Stefan Solntsev; Michael Shortreed; Brian Frey; Lloyd Smith; *University of Wisconsin - Madison, Madison, WI.* ***See Poster 055-ML.***

Mon 24 **EpiProfile 2.0: A Computational Platform for Processing Epi-Proteomics Mass Spectrometry Data;** Zuofei Yuan; Simone Sidoli; Dylan M. Marchione; Johayra Simithy; Kevin A. Janssen; Mary R. Szurgot; Benjamin A. Garcia; *University of Pennsylvania, Philadelphia, PA.* ***See Poster 037-ML.***

Mon 25 **Broad Time-Dependent Proteomic and Metabolomic Effects of Atorvastatin on Hepatocytes;** Akos Vertes[1]; Albert-Baskar Arul[1]; Andrew R. Korte[1]; Peter Avar[1]; Lida Parvin[1]; Ziad J. Sahab[1]; Deborah I. Bunin[2]; Merrill Knapp[2]; Andrew Poggio[2]; Ca; *[1]Dept. of Chemistry, The George Washington Univ., Washington, DC; [2]SRI International, Menlo Park, CA; [3]GE Global Research, Niskayuna, NY.* ***See Poster 023-ML.***

Mon 26 **Early Candidate Urine Biomarkers for Detecting Alzheimer's Disease before Amyloid-β Plaque Deposition in an APP (swe)/PSEN1dE9 Transgenic Mouse Model;** Fanshuang Zhang[1]; Jing Wei[2]; Xundou Li[1]; Chao Ma[3]; Youhe Gao[2]; *[1]Basic Medicine Peking Union Medical College, Beijing, China; [2]Beijing Normal University, Beijing, China; [3]Chinese Academy of Medical Sciences, Beijing, China.* ***See Poster 077-ML***

MONDAY, MARCH 4

MONDAY LUNCH SEMINARS

Open to all attendees, but RSVP strongly encouraged (at booth or online) in advance as space is limited.

12:15 - 1:30 PM: **BRUKER**, *Deer-Elk Lake*

If you have not already registered in advance, please stop by booth Sunday night or Monday morning to RSVP.

During this informative seminar, the technology behind the timsTOF Pro, a novel QTOF instrument with a Trapped Ion Mobility Spectrometry (TIMS) front end enabling accurate CCS determination, and with 100+ Hz MSMS sequencing speeds via Parallel Accumulation Serial Fragmentation (PASEF) will be described.

12:20 - 12:35 pm **timsTOF Pro and PASEF, 120 Hz MSMS and CCS Determination Decipher Phosphopeptide Positional isomers**; Christopher Adams, Bruker Daltonics, West Coast Proteomics Manager

12:35 - 1:20 pm **Unlocking the Power of the Fourth Dimension: MaxQuant for TimsTOF Pro;** Juergen Cox, Research group leader at the Max Planck Institute of Biochemistry, Martinsried, Germany

1:30 - 3:00 PM: **POSTER SESSION**, *Posters-Exhibits*

3:00 - 4:20 PM: PARALLEL SESSION
MULTI-OMICS, *Plaza Ballroom*
Session Chair: Timothy Griffin (University of Minnesota)

3:00 - 3:25 pm **Utility of Proteogenomics in Immunotherapy**; Bing Zhang; *Baylor College of Medicine, Houston, TX*

3:25 - 3:50 pm **Informing Cancer Biology using Computational Proteogenomics**; Kelly Ruggles; *NYU School of Medicine, New York, NY*

3:50 - 4:05 pm **Post-Translationally Modified Proteins in Plasma Extracellular Vesicles as Candidate Markers for Breast Cancer Subtypes**; Hillary Andaluz[1]; I-Hsuan Chen[1]; J. Sebastian Paez[1]; Anton B. Iliuk[2]; W. Andy Tao[1]; *[1]Purdue University, West Lafayette, 0; [2]Tymora Analytical Operations, West Lafayette, IN*

4:05 - 4:20 pm **Discovery of lincRNA-encoded Peptides: An Integrated Transcriptomics, Proteomics and Bioinformatics Approach**; Chin-Rang Yang; Cameron Flower; Lihe Chen; HyunJun Jung; Viswanathan Raghuram; Mark Knepper; *NHLBI, National Institutes of Health, Bethesda, MD*

3:00 – 4:20 PM: PARALLEL SESSION
METABOLISM AND DISEASE, *Roosevelt-Madison*
Session Chair: Jennifer Van Eyk (Cedars Sinai Medical Center)

3:00 - 3:25 pm **Defining Mitochondrial Protein Function through Systems Biochemistry**; David J. Pagliarini[1, 2]; *[1]Morgridge Institute for Research, Madison, WI; [2]University of Wisconsin, Madison, WI*

3:25 - 3:50 pm **Metabolomics-based Discovery of Metabolic Aspects of Cancer and other Diseases**; Anne Le; *Johns Hopkins Medicine, Baltimore, MD*

3:50 - 4:05 pm **An Optimized Data Independent Acquisition (DIA) Method for Peptide-Centric Analysis in Metabolism Studies**; Christopher A. Barnes[1]; Lindsay K. Pino[2]; Bong J. Kim[1]; Brian C. Searle[2]; Michael J. MacCoss[2]; *[1]Novo Nordisk Research Center Seattle, Inc., Seattle, WA; [2]Department of Genome Sciences, University of Washi, Seattle, WA*

4:05 - 4:20 pm **Peroxisome Plasticity as a Metabolic Strategy for Virus Replication**; Katelyn C Cook; Pierre M Jean Beltran; Yutaka Hashimoto; Ileana M Cristea; *Princeton University, Princeton, NJ*

MONDAY, MARCH 4

4:30 – 5:50 PM: PARALLEL SESSION
ADVANCES IN TECHNOLOGY, *Plaza Ballroom*
Session Chair: David Fenyo (New York University School of Medicine)

4:30 - 4:55 pm **Capturing Site-Specific Heterogeneity with Large-Scale N-Glycoproteome Analysis**; Joshua Coon; *University of Wisconsin-Madison, Madison, WI*

4:55 - 5:20 pm **Making Quantitative Proteomics Quantitative**; Michael MacCoss, *University of Washington, Seattle, WA*

5:20 - 5:35 pm **Multiplexing Meets Automation: Medium Scale-Phosphoproteomics Assay Increases Sample Throughput and Allows for Quantification in Primary Neurons**; Katherine DeRuff; Alvaro Sebastian Vaca Jacome; Karen Perez de Arce; Malvina Papanastasiou; James Mullahoo; Deborah Dele-Oni; Steven A. Carr; Jeffrey R. Cottrell; Jacob D. Jaffe; *Broad Institute, Cambridge, MA*

5:35 - 5:50 pm **Electro-Elution Chromatography of RNA Oligonucleotides: A Novel Paradigm in RNA Analysis by nanoLC-MS/MS**; Richard Lauman; Hee Jong Kim; Sam Wein; Kevin Janssen; Benjamin A. Garcia; *University of Pennsylvania, Philadelphia, PA*

4:30 – 5:50 PM: PARALLEL SESSION
STRUCTURAL AND CHEMICAL PROTEOMICS, *Roosevelt-Madison*
Session Chair: Salvatore Sechi (NIDDK, NIH)

4:30 - 4:55 pm **Cross-linking Mass Spectrometry Strategies to Define Protein-Protein Interactions**; Lan Huang; *UC Irvine, Irvine, CA*

4:55 - 5:20 pm **In-Cell Protein Footprinting Coupled with Mass Spectrometry for Proteome-Wide Structural Biology**; Lisa Jones; *University of Maryland, Baltimore, MD*

5:20 - 5:35 pm **Understanding the Role of H2A Proteolysis during Stem Cell Differentiation**; Mariel Coradin; Kelly Karch; Simone Sidoli; Benjamin A Garcia; *University of Pennsylvania School of Medicine, Philadelphia, PA*

5:35 - 5:50 pm **Towards Protein Structure Determination in Living Cells using a New Translationally Incorporated Crosslinker that Improves Mass Spectrometric Detection**; Bjorn-Erik Wulff; Josh Elias; Pehr Harbury; *Stanford University, Stanford, CA*

5:50 - 6:30 PM: **INFORMAL MIXER**, *Exhibits-Posters*

Join exhibitors for snacks and drinks *before the evening workshop.*

6:30 - 8:00 PM: EVENING WORKSHOP, *Roosevelt-Madison*

ROUNDTABLE DISCUSSION ON PROTEOMICS WITH NIH

Co-Moderators: Ileana Cristea, Salvatore Sechi, Sudhir Srivastava, John Yates

Additional panelists may be added.

NIH Panelists
Jacob Kagan (NCI), Tina Gatlin (NHGRI), Aleksandra Nita-Lazar (NIAID),
Pamela Marino (NIGMS), Henry Rodriguez (NCI), Doug Sheeley (NIDCR)

Academic Panelists
Catherine E. Costello (Boston University School of Medicine), David Fenyo (NYU School of Medicine),
David Muddiman (North Carolina State University), Jennifer Van Eyk (Cedars Sinai Medical Center),
Mike Snyder (Stanford University)

A lively discussion about the future of proteomics from the perspective of different NIH institutes. What proteomic efforts are particularly supported by different institutes? How we can move this field forward effectively? What areas of investigation are promising and should be considered priorities?

TUESDAY, MARCH 5

7:00 AM: Namaste – Yoga, *Potomac Room*
Join us in the Potomac Room (located off the Atrium) for an early morning yoga class. Absolutely all levels. Yoga togs not required, just loose-fitting clothing. We will have towels to use as mats.

8:00 - 8:30 AM: **EARLY MORNING COFFEE & PASTRIES**, *Exhibits-Posters*

8:30 - 9:20 AM: PLENARY SESSION
AWARD PRESENTATIONS AND LECTURES, *Plaza Ballroom*
Session Chair: Gil Omenn (University of Michigan)

8:30 - 8:50 am **Robert J. Cotter New Investigator Award: Wilhelm Haas**, *Mass General, Harvard Medical School* Award presentation followed by 20 minute talk.

8:50 - 9:20 am **Gilbert S. Omenn Computational Proteomics Award: Jürgen Cox**, *Max-Planck Institute for Biochemistry*. Award presentation followed by 20 minute talk.

9:20 - 9:50 AM: **COFFEE BREAK**, *Exhibits-Posters*
Coffee and pastries with the exhibitors.

9:50 - 11:10 AM: PARALLEL SESSION
PROTEIN INTERACTIONS AND SIGNALING, *Plaza Ballroom*
Session Chair: Michael Washburn (Stower Institute)

09:50 - 10:15 am **Spatial Proteomics and Transcriptomics with Promiscuous Labeling Enzymes**; Alice Ting; *Stanford University, Stanford, CA*

10:15 - 10:40 am **Functional Proteomics for Mechanistic and Translational Insight in GBM Clinical Trials**; Forest White; *MIT, Cambridge, MA*

10:40 - 10:55 am **Biochemical Reduction of the Topology of the Diverse WDR76 Interactome**; Dayebgadoh Gerald[1]; Mihaela E. Sardiu[1]; Laurence Florens[1]; Michael P. Washburn[1, 2]; *[1]Stowers Institute for Med Res, Kansas City, MO ; [2]The University of Kansas Medical Center, Kansas City, KS*

10:55 - 11:10 am **An Antibody-Free Method for Identification of Protein Complexes using RNA Aptamers**; Angela Kruse[1]; Judhajeet Ray[1]; Abdullah Ozer[1]; Richard Johnson[2]; Michael MacCoss[2]; John Lis[1]; Michelle Heck[1, 3]; *[1]Cornell University, Ithaca, NY; [2]University of Washington, Seattle, WA; [3]USDA ARS, Ithaca, NY*

9:50 - 11:10 AM: PARALLEL SESSION
INFECTIOUS DISEASES, *Roosevelt-Madison*
Session Chair: Alison McBride (NIAID, NIH

09:50 - 10:15 am **Bernd Wollscheid**, ETH Zurich

10:15 - 10:40 am **Identifying Ways that Viruses Manipulate the Cellular Proteome**; Matthew Weitzman[1, 2]; *[1]UPenn Perelman School of Medicine, Philadelphia, PA; [2]Children's Hospital of Philadelphia, Phildelphia, PA*

10:40 - 10:55 am **Inhibition of HIV Maturation via Selective Unfolding and Crosslinking of Gag Polyprotein by a Mercaptobenzamide Acetylator**; Lisa M. Jenkins[1]; Elliott L. Paine[1]; Lalit Deshmukh[2]; Herman Nikolayevskiy[2]; Gaelyn C. Lyons[1]; Michael T. Scerba[2]; Kara George Rosenker[2]; John M. Louis[2]; Elena Chertova[3]; Rob Gorelick[3]; David E. Ott[3]; G. Marius Clore[2]; Daniel H. Appella[2]; *[1]National Cancer Institute, NIH, Bethesda, MD; [2]NIDDK, NIH, Bethesda, MD; [3]Frederick National Lab for Cancer Research, Frederick, MD*

10:55 - 11:10 am **Blood Protein Biomarkers that Diagnose and Classify Individuals with Lyme Disease**; Yong Zhou[1]; Shizhen Qin[1]; Mingjuan Sun[1, 2]; Li Tang[1]; Xiaowei Yan[1]; Taek-Kyun Kim[1]; Juan Caballero[3]; Gustavo Glusman[1]; Mary E. Brunkow[1]; Mark J. Soloski[4]; Alison W. Rebman[4]; Gilbert Omenn[1, 5]; Robert L. Moritz[1]; John N. Aucott[4]; Leroy Hood[1]; *[1]Institute for Systems Biology, Seattle, WA; [2]Second Military Medical University, Shanghai, China; [3]Molecular and Developmental Complexity Lab, Langeb, Irapuato, Guanajuato, Mexico; [4]Johns Hopkins University School of Medicine, Baltimore, MD; [5]University of Michigan, Ann Arbor, MI*

11:10 AM - 12:00 PM: PLENARY SESSION
LIGHTNING TALKS - ROUND 2, *Plaza Ballroom*
High-energy (and brief) presentations selected from poster presentations. 90 seconds max or face the music!

Presentation Order

Tues 01 **A Novel Method of Quantifying Protein Methylation Utilizing DIA-MS;** Aaron Robinson[1]; Ronald Holewinski[1]; Vidya Venkatraman[1]; Jose Mato[2]; Shelly Lu[1]; Jennifer Van Eyk[1]; *[1]Cedars Sinai Medical Center, Los Angeles, CA; [2]CIC bioGUNE, Bizkaia, Spain.* ***See Poster 102-TL.***

Tues 02 **Measuring Parkison's Disease Mitochondrial Protein Turnover Rates in Human IPSC-Derived Organoids by Mass Spectrometry;** Anthony Duchesne; Nguyen-Vi Mohamed; Wei Yi; Jean Francois Trempe; *McGill University, Montréal, Canada.* ***See Poster 076-TL.***

Tues 03 **Systems-Level Identification of PKA-Independent Vasopressin Signaling in Renal Epithelial Cells;** Arnab Datta; Chin-rang Yang; Raghuram Viswanathan; Mark A. Knepper; *NHLBI, NIH, Bethesda,.* ***See Poster 114-TL.***

Tues 04 **Antiviral Function of Mitochondrial Sirtuin 4 during Human Cytomegalovirus Infection;** Cora Betsinger; Elizabeth Rowland; Ileana Cristea; *Princeton University, Princeton, NJ.* ***See Poster 092-TL.***

Tues 05 **Quantitative Top-Down Proteomics in Complex Samples using Protein-Level Tandem Mass Tag (TMT) Labeling;** Dahang Yu[1]; Zhe Wang[1]; Qiang Kou[2]; Kenneth Smith[3]; Xiaowen Liu[2]; Si Wu[1]; *[1]University of Oklahoma, Norman, OK; [2]Indiana University-Purdue University Indianapolis, Indianapolis, IN; [3]Oklahoma Medical Research Foundation, Oklahoma City, OK.* ***See Poster 148-TL.***

Tues 06 **Comparing Personalized Profiles of host-expressed proteins and microbes in Human Stool Reveals Complementary Inter-Subject Distinction;** Ellen Casavant; Les Dethlefsen; Kris Sankaran; Daniel Sprockett; Susan Holmes; David Relman; Joshua Elias; *Stanford University, Stanford,.* ***See Poster 116-TL.***

Tues 07 **Towards Elucidation of Muscle-Specific Receptor Tyrosine Kinase (MuSK) Signaling Pathway by Differential Agonists;** Hanna Budayeva; Arundhati Sengupta Ghosh; Lilian Phu; Donald Kirkpatrick; *Genentech Inc., South San Francisco, CA.* ***See Poster 154-TL.***

Tues 08 **Bringing KINATEST-ID to Everyone: A Pipeline for Studying Tyrosine Kinases;** John Blankenhorn; James Johnson; Laurie Parker; *UMN, Minneapolis, MN.* ***See Poster 144-TL.***

Tues 09 **Quantitative Proteomics Reveals Host Protein SLFN5 as a target of HSV ICP0-mediated Ubiquitination and Degradation;** Joseph M. Dybas[1]; Eui Tae Kim[1]; Emigdio D. Reyes[1, 2]; Katarzyna Kulej[1]; Jennifer C. Liddle[1, 2]; Benjamin A. Garcia[2]; Matthew D. Weitzman[1, 2]; *[1]Children's Hospital of Philadelphia, Philadelphia, PA; [2]Perelman School of Med, University of Pennsylvania, Philadelphia, PA.* ***See Poster 096-TL.***

Tues 10 **Signatures of Ambient Exposure to Benzene and other Air Pollutants in the Human Serum Albumin Cys^{34} Adductome;** Joshua Smith[1]; Robert O'Meally[1]; Derek Ng[1]; Thomas Kensler[1, 2]; Robert Cole[1]; John Groopman[1]; *[1]Johns Hopkins University, Baltimore, MD; [2]Fred Hutchinson Cancer Research Center, Seattle, WA.* ***See Poster 008-TL.***

Tues 11 **Acetylation of the lamina Promotes the Integrity of the Nuclear Periphery and Inhibits Virus Production;** Laura Murray; Xinlei Sheng; Ileana Cristea; *Princeton University, Princeton, NJ.* ***See Poster 112-TL.***

Tues 12 **Using Protein Stability as a Matric of Protein Quality in Protein Homeostasis;** Lavender Hsien-Jung Lin; Nathan Zuniga; Joseph Creery; Marcus Hadfield; John Price; *Brigham Young University, Provo, UT.* ***See Poster 134-TL.***

Tues 13 **Multifaceted Proteomic Evaluation of Lysosome Dysfunction in Neurodegeneration via Human iPSC-Derived Neurons;** Ling Hao[1]; Ryan Prestil[1, 2]; Michael Fernandopulle[1, 2]; Stewart Humble[1]; Daniel Lee[3]; Saadia Hasan[1]; Maia Parsadanian[1]; Richard Youle[1]; Michael Ward[1]; *[1]National Inst of Neurological Disorders and Stroke, Bethesda, MD; [2]University of Cambridge, Cambridge, UK; [3]Cornell University, Ithaca, NY.* ***See Poster 128-TL.***

Tues 14 **Quantitative Interactomics to Determine Protein Quality Control Mechanisms Dictating Thyroglobulin Secretion;** Madison Wright; Lars Plate; *Vanderbilt University, Nashville, TN.* ***See Poster 124-TL.***

Tues 15 **Global Immunoproteomic Profiling of Endotoxin-Stimulated Macrophages Uncovers Specifics of TLR4- and Caspase11- Mediated Recognition;** Orna Rabinovich Ernst[1]; Mohd M. Khan[2]; Benjamin Oyler[2]; Jing Sun[1]; Nathan Manes[1]; Iain Fraser[1]; Aleksandra Nita-Lazar[1]; David Goodlett[2]; *[1]National Institutes of Health, Bethesda, MD; [2]University of Maryland, Baltimore, MD.* ***See Poster 156-TL.***

Tues 16 **Improved Protein Inference from Multiple Protease Bottom-Up Mass Spectrometry Data with MetaMorpheus;** Rachel Miller[1]; Rob Millikin[1]; Connor Hoffman[1]; Stefan Solntsev[1]; Gloria Sheynkman[2]; Michael Shortreed[1]; Lloyd Smith[1]; *[1]University Wisconsin-Madison, Madison, WI; [2]Dana-Farber Cancer Institute, Boston, MA.* ***See Poster 036-TL.***

Tues 17 **ELTA-MS: Labeling, Enrichment and Identification of ADP-Ribosylated Peptides by Mass Spectrometry;** Robert Lyle Mcpherson; Anthony Kar Lun Leung; *Johns Hopkins University, Baltimore, MD.* ***See Poster 104-TL.***

Tues 18 **Developing and Characterizing FLIM Probes to Detect Subcellular Tyrosine Kinase Activity;** Sampreeti Jena; Oscar Bastidas; Erica Pratt; Scout Allendorf; Blanche Cizubu; Laurie L. Parker; *University of Minnesota Twin Cities, Minneapolis, MN.* ***See Poster 051-TL.***

Tues 19 **An Integrated Multi-Omic Analysis in iPSC-derived Motor Neurons from C9ORF72 ALS Patients;** Victoria Dardov[1]; Ryan Lim[2]; Vidya Venkatraman[1]; Jie Wu[2]; NeuroLINCS Consortium[3]; Leslie Thompson[2]; Clive Svendsen[1]; Jennifer Van Eyk[1]; *[1]Cedars Sinai Medical Center, Los Angeles, CA; [2]University of California, Irvine, Irvine, CA; [3]NIH, Bethesda, MD.* ***See Poster 070-TL.***

Tues 20 **Dynamic Regulation of Mitochondria Morphology, Composition, Acetylation, and Function during Viral Infection;** Xinlei Sheng; Laura Murray; Ileana Cristea; *Princeton University, Princeton, NJ.* ***See Poster 094-TL.***

Tues 21 **P4Ha1 Hydroxylation of Bradykinin Allows Blood-based Characterization of Tumor Hypoxia;** Yang Liu; Christopher Lyon; Jia Fan; Tony Hu; *Arizona State University, Tempe, AZ.* ***See Poster 004-TL.***

Tues 22 **Integrated Glycoproteomic Characterization of Human High-Grade Serous Ovarian Cancer;** Yingwei Hu; Jianbo Pan; Punit Shah; Minghui Ao; Stefani Thomas; Yang Liu; Lijun Chen; Michael Schnaubelt; David Clark; Qing Li; Jiang Qian; Zhen Zhang; Daniel Chan; Hui Zhang; *Johns Hopkins University, Baltimore,.* ***See Poster 046-TL.***

Tues 23 **SILAC-based Quantitative Proteogenomics Unveil Altered MHC-associated Peptidome in Osimertinib Resistant Human Lung Adenocarcinoma;** Yue Qi; Tapan Maity; Meriam Bahta; Khoa Dang Nguyen; Constance Cultraro; Xu Zhang; Udanya Guha; *Thoracic and GI Malignancies Branch, CCR, NCI, NIH, Bethesda, MD.* ***See Poster 056-TL.***

Tues 24 **Mechanical Stimulation Induces Rapid Phosphorylation-dependent Signaling in *Xenopus* embryos;** Yutaka Hashimoto[1, 2]; Noriyuki Kinoshita[2]; Todd Greco[1]; Joel Federspiel[1]; Pierre Jean Beltran[1]; Naoto Ueno[2]; Ileana Cristea[1]; *[1]Princeton University, Princeton, NJ; [2]National Institute for Basic Biology, Okazaki, Japan.* ***See Poster 152-TL.***

Tues 25 **Top-Down Proteogenomics Analysis of Serum Autoantibody Repertoire for the Discovery of Biomarker of Systemic Lupus Erythematosus;** Zhe Wang[1]; Xiaowen Liu[2]; Kenneth Smith[3]; Si Wu[1]; *[1]University of Oklahoma, Norman, OK; [2]School of Informatics and Computing, IUPUI, Indianapolis, IN; [3]Oklahoma Medical Research Foundation, Oklahoma City, OK.* ***See Poster 006-TL.***

Tues 26 **Optimized Cross-linking Mass Spectrometry for *in situ* Interaction Proteomics;** Zheng Ser[1, 2]; Paolo Cifani[1]; Alex Kentsis[1, 2]; *[1]Sloan Kettering Institute, New York, NY; [2]Tri-Institutional PhD Program in Chemical Biology, New York, NY.* ***See Poster 040-TL.***

Tues 27 **Global Quantification of Proteome and Phosphoproteome Revealed Novel Cellular Signaling Mechanisms Responsive to Hypoxia and Iron Deficiency;** Luke Erber; Yao Gong; Maolin Tu; Phu Tran; Yue Chen; *University of Minnesota, Minneapolis,.MN* ***See Poster 146-TL.***

Tues 28 **Location, Location, Location: Using Spatial Proteomics to Uncover Functional Protein Translocations during Viral Infection;** Michelle Kennedy; Ileana Cristea; *Princeton University, Princeton, NJ.* ***See Poster 090-TL.***

TUESDAY, MARCH 5

TUESDAY LUNCH SEMINARS

Open to all attendees, but RSVP strongly encouraged (at booth or online) in advance as space is limited.

12:15 - 1:30 PM: **THERMO FISHER SCIENTIFIC**, *Regency Room*

Join us for our seminar. Lunch will be served. RSVP not required.

A Multi-omic Targeted Assay for Detecting Alterations in Signaling; Emily Chen, Ph.D., Thermo Fisher Scientific

Multiplex IP Assays Combined with Targeted Mass Spectrometry Reveals Signaling Pathway Changes in Lung Tumor Patient-derived Xenograft Models; Jonathan Krieger, Ph.D.,SPARC Biocentre at the Hospital for Sick Children

12:15 - 1:30 PM: **SCIEX**, *Potomac Room*

If you have not already registered in advance, please stop by booth Sunday night or Monday morning to RSVP.

Fast-Track your Quantitative Proteomics with Microflow SWATH® Acquisition; Christie Hunter, SCIEX Proteomics has typically been done using nanoflow LC for sensitivity but with slow time to results. Increasingly, labs are using microflow LC for some applications when sufficient sample is available, to accelerate throughput. The ability to utilize both nanoflow and microflow rates enables maximum functionality. Therefore easy switching between flow regimes is important for full workflow flexibility. The new OptiFlow™ Source and Interface with the NanoLC™ 400 system allows for easy switching in minutes between flow regimes on the TripleTOF® 6600 system. One key quantitative workflow that has proven to work extremely well with microflow is SWATH® Acquisition. An extensive evaluation of impact on the gradient length vs proteins quantified has been performed using gradients as short as 5 minutes and results will be reported. Impact on quantitative measurements will be presented as well as results from biological studies.

1:30 - 3:00 PM: **POSTER SESSION**, *Posters-Exhibits*

3:00 - 4:20 PM: PARALLEL SESSION
CANCER EARLY DETECTION AND PREVENTION, *Plaza Ballroom*
Session Chair: Henry Rodrguez (NCI, NIH)

3:00 - 3:25 pm	**Translational Applications of Mass Spectrometry to Early Cancer Detection and Cancer Treatment**; Amanda Paulovich; *FHCRC, Seattle, WA*
3:25 - 3:50 pm	**Helping Bad Biomarkers Fail Fast: Strategies for Prioritizing Candidate Biomarkers**; Karin Rodland; *Pacific Northwest National Laboratory, Richland, WA*
3:50 - 4:05 pm	**Digitizing the Proteomes From Big Tissue Biobanks**; Jan Muntel[3]; Nick Morrice[2]; Christie Hunter[1]; Roland Bruderer[3]; Nicholas Dupuis[3]; Lukas Reiter[3]; *[1]SCIEX, Redwood City, CA; [2]SCIEX, Warrington, UK; [3]Biognosys, Schlieren, Switzerland*
4:05 - 4:20 pm	**Can AI Find A Tree in the Woods? Issues and Considerations for Hypothesis Free Discovery in Large Clinical Proteomics Datasets**; David Bramwell; Will Dracup; *Biosignatures Ltd, Newcastle, United Kingdom*

3:00 – 4:20 PM: PARALLEL SESSION
INFORMATICS: EMERGING AND NEW APPROACHES, *Roosevelt-Madison*
Session Chair: Alexey Nesvizhskii (NCI, NIH)

3:00 - 3:25 pm **Semantic Computing for Protein Knowledge Network Discovery**; Cathy H Wu; *University of Delaware, Newark, DE*

3:25 - 3:50 pm **Algorithms and databases for mining insight from quantitative mass spectrometry experiments of post-translational modifications**; Kristen Naegle; *University of Virginia, Charlottesville, VA*

3:50 - 4:05 pm **Topoliogiical Scoring of Protein Interaction Networks**; Michael Washburn; *Stowers Institute for Medical Research, Kansas City, MO*

4:05 - 4:20 pm **High-Throughput Identification of MS-Cleavable and Non-cleavable Chemically Crosslinked Peptides with MetaMorpheus**; Lei Lu; Michael R. Shortreed; Robert J. Millikin; Lloyd M. Smith; *University of Wisconsin, Madison, WI*

4:30 – 5:50 PM: PARALLEL SESSION
PROTEIN PROTEOFORMS IN HEALTH AND DISEASE, *Plaza Ballroom*
Session Chair: Peter Nemes (University of Maryland, College Park)

4:30 - 4:55 pm **A PTM Code for Membrane Protein Maturation**; John R. Yates[1]; Sandra Pankow[1]; Casimir Bamberger[1]; Diego Calzolari[2]; Salvador Martínez-Bartolomé[1]; Mathieu Lavallée-Adam[3]; *[1]The Scripps Research Institute, La Jolla, CA; [2]Qualcomm, San Diego, CA; [3]University of Ottawa, Ottawa, Canada*

4:55 - 5:20 pm **Polycomb Loss Mediated Reprogamming of the Epigenome Drives Oncogenesis in Malignant Peripheral Nerve Sheath Tumors**; John B. Wojcik; Dylan M. Marchione; Simone Sidoli; Benjamin A. Garcia; *University of Pennsylvania School of Medicine, Philadelphia, PA*

5:20 - 5:35 pm **Deciphering the Human Heart Proteoform Landscape in Cardiac Disease and Regeneration**; Trisha Tucholski[1]; Ling Gao[2]; Zachery Gregorich[1]; Wenxuan Cai[1]; Kyle Brown[1]; Yanlong Zhu[1]; Bifan Chen[1]; Samantha Knott[1]; Andrew Alpert[3]; Jianyi Zhang[2]; Ying Ge[1]; *[1]University of Wisconsin - Madison, Madison, Wisconsin ; [2]University of Alabama at Birmingham, Birmingham, AL; [3]PolyLC Inc., Columbia , MD*

5:35 - 5:50 pm **Cell Type-Resolved Analysis of Blood Proteoforms by Large-Scale Top-Down Proteomics**; Paul Thomas[1]; R. Vince Gerbasi[1]; Rafael Melani[1]; Jacek Sikora[1]; Timothy Toby[1]; Kristina Srzentic[1]; Luca Fornelli[1, 2]; Richard LeDuc[1]; Josiah Hutton[1, 3]; Ryan Fellers[1]; Joseph Greer[1]; Jeannie Camarillo[1]; Lissa Anderson[4]; Chris Hendrickson[4]; Neil Kelleher[1]; *[1]Northwestern University, Evanston, IL; [2]University of Oklahoma, Norman, OK; [3]Princeton University, Princeton, NJ; [4]NHMFL, Tallahassee, FL*

4:30 - 5:50 PM: PARALLEL SESSION
AGING AND NEUROLOGICAL DISEASES, *Roosevelt-Madison*
Session Chair: Birgit Schilling (The Buck Institute)

4:30 - 4:55 pm **Integrating MS-based Proteomics and Lipidomcs Studies in the Cerebellar Degeneration Disorder, Niemann-Pick Type C1**; Stephanie Cologna; *Univ of Illinois at Chicago, Chicago, IL*

4:55 - 5:20 pm **The Role of Senescence in Aging and Age-related Diseases – Proteomics as Tool to Decipher Mechanisms**; Nathan Basisty[1]; Abhijit Kale[1]; Okhee Jeon[1]; Christopher D. Wiley[1]; Su Liu[1]; Chisaka Kuehnemann[1]; Anja Holtz[1]; Julie Anderson[1]; Pankaj Kapahi[1]; Luigi Ferrucci[2]; Judith Campisi[1]; Birgit Schilling[1]; *[1]Buck Institute for Research on Aging, Novato, CA; [2]National Institute on Aging, NIH, Rockville, MD*

5:20 - 5:35 pm **A Quantitative Perspective of the Dynamics of Huntingtin Protein Interactions Provides Insight into Huntington's Disease Pathogenesis**; Todd Greco; Joel Federspiel; Ileana Cristea; *Princeton University, Princeton, NJ*

5:35 - 5:50 pm **Mapping the Brain Proteome of HIV-associated Neurocognitive Decline**; Saima Ahmed[1, 2]; Amanda Guise[1, 2]; Hendrik Wesseling[1, 2]; Judith Steen[1, 2]; Hanno Steen[1, 2]; *[1]Boston Children's Hospital, Boston, MA; [2]Harvard Medical School, Boston, MA*

TUESDAY, MARCH 5

6:00 - 7:30 PM: EVENING WORKSHOP, *Roosevelt-Madison*
BIOMARKERS FOR EARLY DETECTION: WHAT SHOULD WE MEASURE?
Organizer: Karin Rodland (PNNL)
Panelists: Udayan Guha (NCI, NIH), Jacob Kagan (NCI, NIH), Amanda Paulovich (Fred Hutchinson), Richard Semba (Johns Hopkins University), and Sudhir Srivastava (NCI, NIH)

Biomarker science is changing. New instruments are enabling new types of measurements, with greater sensitivity and higher throughput. Clinical practice has begun to reveal which biomarkers have clinical utility – and which create new problems. Precision medicine has changed the rules for selecting therapies, and created new opportunities for biomarkers. In this session experienced practitioners of biomarker science will discuss what constitutes a good biomarker, the challenges encountered in translating biomarkers to the clinic, and the future of biomarkers in a world of precision medicine.

6:00 - 7:30 PM: EVENING WORKSHOP, *Regency Room*
GRANTWRITING WORKSHOP
Organizers-Presenters: Peipei Ping (UCLA) and Oleg Barski (NIGMS, NIH)

In the current competitive landscape for grant funding developing a strategy for successful grant writing is critical. Early career academics will learn techniques and gain insight into what is needed to submit a successful grant. Even those with past experience will benefit from fresh perspectives to bring to their next application.

7:30 - 9:00 PM: **SOCIAL EVENT**
Atrium

This special event is sponsored by Pressure BioSciences

Join all attendees for a supper style event featuring live jazz!

PBI Pressure BioSciences Inc.

8:00 - 8:30 AM: **EARLY MORNING COFFEE & PASTRIES**, *Plaza Ballroom Foyer*

8:30 - 9:20 AM: PLENARY SESSION
ANNOUNCEMENT OF BEST STUDENT AND POST-DOC POSTER AWARD WINNERS

TIPS & TRICKS (TECHNOLOGY FOCUS) LIGHTNING SESSION
Plaza Ballroom
Four-minute presentations selected from poster presentations.
Focus is on work-arounds, hacks, and new technology. Posters will be on display in foyer during the coffee break.

Presentation Order

Wed 01 **Integrating Kinetic and Quantitative Proteomics to Investigate Autophagy Substrates in Tumors;** Monique Speirs; John Price; *Brigham Young University, Provo, UT.*

Wed 02 **Spray-Capillary: An Electrohydrodynamic Spray Assisted Device for Quantitative Ultra-Low Volume Extraction;** Lushuang Huang; Zhe Wang; Si Wu; *University of Oklahoma, Norman, OK.*

Wed 03 ***Burkholderia* Rewires its Proteome to Lower Antibiotics Sensitivities and to Support Biofilm Formation;** Mohd M. Khan[1, 5]; Supaksorn Chattagul[2]; Bao Q. Tran[3, 6]; Jeffrey A. Freiberg[4]; Aleksandra Nita-Lazar[5]; Mark E. Shirtliff[4]; Rasana W. Sermswan[2]; Robert K. Ernst[4]; David R. Goodlett[3]; *[1]University of Maryland School of Medicine, Baltimore, MD; [2]Melioidosis Research Center, Khon Kaen University, Khon Kaen, Thailand; [3]University of Maryland School of Pharmacy, Baltimore, MD; [4]University of Maryland School of Dentistry, Baltimore, MD; [5]LISB, NIAID, National Institutes of Health (NIH), Bethesda, MD; [6]U.S. Army Edgewood Chemical Biological Center, Gunpowder, MD.*

Wed 04 **Total Solubilization of FFPE Samples for High Throughput Clinical Proteomics;** John P. Wilson[1]; Ilyana Ilieva[2]; Darryl J. Pappin[1, 3]; John B. Wojcik[2]; *[1]ProtiFi, LLC, Farmingdale, NY; [2]University of Pennsylvania, Philadelphia, PA; [3]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.*

Wed 05 **30 Second Analysis of Histone Post-Translational Modifications by Direct Infusion Mass Spectrometry;** Yekaterina Kori[1]; Simone Sidoli[1]; Mariana Lopes[2]; Zuo-Fei Yuan[1]; Hee Jong Kim[1]; Katarzyna Kulej[1]; Kevin Janssen[1]; Laura Agosto[1]; Julia Pinheiro Chagas da Cunha[2]; Benjamin A. Garcia[1]; *[1]University of Pennsylvania, Philadelphia, PA; [2]Instituto Butantan, Sao Paulo, Brazil.*

Wed 06 **Kinetics of Acetone Precipitation: Optimizing Conditions to Efficiently Concentrate and Purify Protein Samples in Minutes with the ProTrap XG;** Jessica Nickerson; Alan A. Doucette; *Dalhousie University, Halifax, Canada.*

Wed 07 **Rapid Qualitative and Absolute Quantification of Plasma based proteins using a Novel Scanning Quadrupole DIA Acquisition Method;** Roy Martin[1]; Lee Gethings[2]; *[1]Waters, Beverly, MA; [2]Waters, Wilmslow, UK*

9:50 - 11:10 AM: PARALLEL SESSION
POSTTRANSLATIONAL REGULATION, *Plaza Ballroom*
Session Chair: Benjamin Garcia (University of Pennsylvania School of Medicine)

09:50 - 10:15 am **The Lysine Acylation Pathways as a Bridge between Metabolism and Epigenetic Mechanisms**; Yingming Zhao; *University of Chicago, Chicago, IL*

10:15 - 10:40 am **High-Throughput Quantitative Top-down Proteomics for Deep Characterization of Intact Proteoforms with Post-Translational Modifications**; Si Wu; *University of Oklahoma, Norman, OK*

10:40 - 10:55 am **The LPS-Responsive ADP-Ribosylated Proteome in Primary Human Immune Cells**; Casey Daniels; Arthur Nuccio; Aleksandra Nita-Lazar; *NIAID, NIH, Bethesda, MD*

10:55 - 11:10 am **Pathway-scale Targeted Mass Spectrometry for High-Resolution Functional Profiling of Cell Signaling**; Paolo Cifani; Alex Kentsis; *Sloan-Kettering Institute, New York, NY*

9:50 - 11:10 AM: PARALLEL SESSION
GLYCOPROTEOMICS IN BIOLOGY AND MEDICINE, *Roosevelt-Madison*
Session Chair: Christopher A. Barnes (Novo Nordisk Research Center, Seattle)

09:50 - 10:15 am **Nutrient Regulation of Transcription & Signaling by O-GlcNAc**; Gerald Hart; *CCRC, University of Georgia, Athens, GA*

10:15 - 10:40 am **Innovations in Chemistry and Mass Spectrometry Platform Technologies for Quantitative Glycomics**; David Muddiman[1]; Jaclyn Gowen[1]; James Dodds[1]; Erin Baker[1]; James Petitte[1]; Alison Motsinger-Reif[1]; Michael MacCoss[2]; Thomas Montine[3]; *[1]North Carolina State University, Raleigh, NC; [2]University of Washington, Seattle, WA; [3]Stanford University, Palo Alto, CA*

10:40 - 10:55 am **Advancing Cardiac Glycomics: Protein Glycosylation in Primary and Stem Cell-derived Human Cardiomyocytes**; Christopher Ashwood[1]; Matthew Waas[1]; Ranjuna Weerasekera[1]; Rebekah L. Gundry[1, 2]; *[1]Medical College of Wisconsin, Milwaukee, Wisconsin; [2]Center for Biomedical Mass Spectrometry, MCW, Milwaukee, WI*

10:55 - 11:10 am **Mapping the O-glycoproteome using Site-Specific Extraction of O-linked Glycopeptides (EXoO)**; Weiming Yang; Minghui Ao; Yingwei Hu; Qing Kay Li; Hui Zhang; *Johns Hopkins University, Baltimore, MD*

11:10 AM - 12:00 PM: **LIFETIME ACHIEVEMENT IN PROTEOMICS AWARD,** *Plaza Ballroom*
Session Chair: Ileana Cristea (Princeton University)

11:10 am - 12:00 pm **Adventures in the Garden of Proteomics**; Catherine E. Costello; *Boston University School of Medicine, Boston, MA*

MONDAY 9:50 – 11:10 AM
PROTEOME ORGANIZATION IN SPACE AND TIME,
Plaza Ballroom

Mon Talk 09:50 - 10:15 am: The Spatial Organisation of the Transcriptome and Proteome

Kathryn Lilley
University of Cambridge, Cambridge, United Kingdom

In biological systems, genome size does not correlate with organismal complexity. Indeed, the proteome of higher organisms is arguably too small for the complex functions that it is has to perform. Proteome functionality is enhanced by post translation modification, spatial restriction and the interactions proteins make with other biomolecules. The location of protein synthesis also plays a role in protein function, and the aberrant translation of proteins in the wrong location underpins multiple disease states. The relationship between the transcriptome and proteome both in terms of physical interaction and spatial location is therefore of great importance.

Here, we present emerging methodologies that capture the spatial relationship between the proteome and transcriptome.

Firstly, in order to characterise intracellular RNA location, efficient extraction of RNA species and their coordinating protein binding partners (RBPs) is essential. To date RBP capture has centred round UV crosslinking RNA to protein and enrichment with oligo(dT)-coupled beads. This approach is limited to RBPs that coordinate polyadenylated RNA. Here describe the orthogonal organic phase separation (OOPS), a highly efficient method, that enables reproducible recovery of RBPs or protein-bound RNA (PBR), compatible with downstream proteomics and RNA sequencing and independent of polyadenylation status of RNA (1). We demonstrate its application to a number of different studies revealing some surprising roles as RBPs for a variety of different metabolic proteins.

Secondly, we introduce a series of new spatial proteomics works workflows including, LOPIT-DC (2), a straightforward high-resolution spatial proteomics approach that uses differential centrifugation coupled with machine learning approaches to efficiently interrogate data

Finally, we show how modification of subcellular fractionation methods are necessary for compatibility with OOPS, to generate spatial information for not only proteins but also RNA species on a cell wide scale.

1. Queiroz (2019) Nature Biotechnology – doi:10.1038/s41587-018-0001-2
2. Geladaki (2019) Nature Communications - doi:10.1038/s41467-018-08191-w

Mon Talk 10:15 - 10:40 am: Systems Biochemistry of the Metaphase Spindle

Martin Wuhr
Princeton University, Princeton, NJ

The metaphase spindle is composed of chromosomes, microtubules, and an unknown number of proteins. Spindle microtubules are dynamic with an average lifetime of ~20 seconds making isolation of native spindles extremely challenging. We developed methods to isolate metaphase spindles in less than 5 seconds from undiluted Xenopus egg extract via a rapid filtration approach. Using quantitative multiplexed proteomics, we determined the partitioning between spindle and cytoplasm for ~5,500 proteins. We observed over 100 new spindle proteins and confirmed the localization for a subset via microscopy. We were able to globally measure the spindle's proteome turn-over by adding cell lysate from a different frog species and follow the equilibration of these proteins with quantitative proteomics. Lastly, we determined the absolute amount of each protein bound to the spindle. This systems level measurement allowed us to compare the concentration of spindle microtubules with bound microtubule associated proteins (MAPs). In contrast to standard textbook cartoons, microtubules seem to be saturated with MAPs. We demonstrate that MAPs are competing for microtubule binding sites in the spindle suggesting a simple model how spindle composition and morphology could adapt to the drastically changing cell sized in early embryonic development. Thus, we present the first measurement of the composition of a metaphase spindle with endogenous dynamics at molecular resolution. This generated new insight into spindle architecture and might provide a framework to understand how the spindle can adapt its size for different developmental contexts.

Mon Talk 10:40 - 10:55 am: Microprobe CE-ESI-HRMS Reveals Proteomic Reorganization in Spatially and Temporally Developing Cell Clones Directly in Live *X. laevis* Embryos

Camille Lombard-Banek[1]; Sally Moody[2]; Peter Nemes[1]
[1]University of Maryland, CP, College Park, MD; [2]The George Washington University, Washington, DC

Establishment of cell-to-cell differences (single-cell heterogeneity) is critical to normal embryonic development and tissue formation. Quantitative proteomics with single-cell resolution has the potential to deepen our understanding of cell type specific gene expression during embryonic development. We here realize this potential by developing an approach to enable the proteomic analysis as an identified cell gives rise to a cell clone directly in the early developing vertebrate embryo. Our strategy integrated optically guided capillary microsampling, capillary electrophoresis (CE) nanoelectrospray ionization (nanoESI), and high-resolution tandem mass spectrometry (Q-Exactive +). The optimized technology accomplished 700 zmol lower limit of detection for model peptides. Moreover, it enabled the identification of ~750–800 protein groups (PGs, <1% FDR) from only 5 ng of protein digest extracted from *Xenopus laevis* (frog) embryos. We validated the approach by identifying proteomic differences between the animal and vegetal poles of the zygote, in which molecular heterogeneity is known in space. The approach allowed us to reproducibly quantify ~460 PGs as the dorsal-animal cell (D11 cell) divided to give rise to its neural-fated clone in the 16-, 32-, 64-, and 128-cell embryo. Hierarchical cluster analysis (HCA) of quantitative proteomic data revealed reorganization of the single-cell proteome, revealing ~90 proteins with significant dysregulation (fold change > 1.3, $p < 0.05$, Student's t-test). Our microanalytical single-cell proteomic approach opens new opportunities to study molecular mechanisms of cell differentiation directly in spatially and temporally evolving tissues in live vertebrate embryos and other biological models.

Mon Talk 10:55 - 11:10 am: Extensive Intratumor Proteogenomic Heterogeneity Revealed by Multiregion Sampling in a High-Grade Serous Ovarian Tumor Specimen

Allison Hunt[3]; Nicholas Bateman[1, 2]; Guisong Wang[1]; Brian Hood[1]; Julie Oliver[1]; Dave Mitchell[1]; Glenn Gist[1]; Ming Zhou[3]; Brian Blanton[1]; Kelly Conrads[1]; Kathleen Darcy[1]; Craig Shriver[2]; Yovanni Casablanca[1]; G. Larry Maxwell[4]; Thomas Conrads[3]
[1]Gynecologic Cancer Center of Excellence, Annandale, VA; [2]John P. Murtha Cancer Center, Bethesda, MD; [3]Inova Schar Cancer Institute, Falls Church, VA; [4]Department of Obstetrics and Gynecology, Inova, Falls Church, VA

We generated 200 consecutive thin sections from a single high-grade serous ovarian carcinoma (HGSOC) tumor and laser microdissected (LMD) four spatially separated tumor "core" regions throughout the depth of the tissue block to examine proteogenomic intratumor heterogeneity (ITH). Tumor epithelium and stroma were LMD enriched at 150µm intervals and mixed epithelial and stromal (e.g. whole tumor) samples were harvested from adjacent thin sections; the remaining tissue was cryopulverized. Mass spectrometry-based proteomics quantified 6,053 proteins and 4,225 phosphosites and RNA sequencing mapped to 20,785 transcripts. Unsupervised hierarchical

cluster analysis of 1,018 and 584 differentially proteins and transcripts demonstrated distinct sub-clusters of tumor cores and enriched tumor epithelium versus enriched stroma and whole tumor samples, with the cryopulverized proteome clustering independently of these sample groups. Comparison of protein and transcripts with historic prognostic molecular subtypes for HGSOC showed that enriched stroma, but not tumor epithelium collections was positively correlated with mesenchymal molecular subtype, which is associated with poor disease outcome. Further analyses revealed prognostic biomarkers predicting risk of suboptimal surgical debulking were also anti-correlated between enriched stroma and tumor epithelium. While there was concordance between protein and transcript abundance for each LMD collection type, transcript abundance from enriched stroma was most strongly correlated with cognate protein products from the mixed epithelial/stromal collection rather than enriched stroma, potentially explainable by the secretory nature of ovarian stromal cells and inclusion of extracellular matrix proteins in mixed LMD collections. Proteins measured from the cryopulverized tissue were overall negatively correlated with enriched tumor epithelium, and notably had limited detection of the ovarian tumor cell biomarker CA-125. This proteogenomic analysis reveals stark molecular heterogeneity in the cellular admixture of the HGSOC tumor microenvironment and underscores the need to account for compartmental ITH in molecular profiling analyses of cancer.

MONDAY 9:50 – 11:10 AM
IMMUNITY AND THE MICROBIOME, *Roosevelt-Madison*

Mon Talk 09:50 - 10:15 am: Proteomic Scatomancy: Towards Stool-based Personalized Health Forecasts

Joshua Elias
Stanford University, Stanford, CA

The intestinal microbiome profoundly influences immune responses within the gut, and can have far-reaching effects throughout the body. Complex, dynamic interactions between host, microbe and diet underlie these effects, but are only partially reflected by 16S and metagenomic sequencing which remain foundations of microbiome research. Towards building a more mechanistic understanding of how our resident microbes influence health and disease, our “host-centric proteomics of stool” method directly measures proteins secreted or shed into the gut in response to changing intestinal ecosystems. Where microbe sequencing data can correlate certain taxa with possible health effects, well-annotated host proteins more directly implicate specific host processes in health maintenance and disease. These kinds of pathway-level observations can be important for assessing health before the onset of symptoms, when interventions may be most effective. In this talk, I will describe our recent efforts in understanding pre-symptomatic changes to host innate immune responses in a mouse model of multiple sclerosis, and with an exploration of natural variation in self-reported healthy human subjects. I will also touch upon how our unconstrained database search software (TagGraph) can incorporate metaproteomic information into these assessments -- even when protein sequence databases are incomplete. With it, we can characterize dynamic host, microbial and dietary interactions directly from stool. This is a fundamental step towards understanding the forces that shape the gut microbiome, and how it in turn can shift the balance between health and disease.

Mon Talk 10:15 - 10:40 am: Metaproteomics to Study Drug Effects on the Gut Microbiome

Daniel Figeys
Ottawa Institute of Systems Biology, University of Ottawa, ON

We are interested in understanding the role of the microbiome in pediatric inflammatory bowel disease. In particular, we will present some of our recent results on the changes in the metaproteome occuring in the microbiome during the disease.

We are also interested in the development of in vitro assays to rapidly assess the effects of compounds on individual microbiome. We will also present recent development of an in vitro microbiome assay coupled to metaproteomics to understand the effects of different compounds on individual human microbiomes.

Mon Talk 10:40 - 10:55 am: Interactome and Proteome Dynamics Uncover Immune Modulatory Associations of the Pathogen Sensing Factor cGAS

Krystal Lum; Bokai Song; Joel Federspiel; Benjamin Diner; Timothy Howard; Ileana Cristea
Princeton University, Princeton, NJ

Detection of virus DNA genomes is an essential component of the mammalian innate immune response. One prominent host defense factor is the DNA sensor cyclic-GMP-AMP synthase (cGAS). Upon direct binding to viral DNA, cGAS catalyzes the generation of cyclic dinucleotides, which activate the adaptor protein STING to induce expression of type I interferons. Our lab further established that cGAS additionally induces apoptosis in a STING-dependent manner under certain infection conditions with herpes simplex virus 1 (HSV-1). Despite the critical role for cGAS in sensing viral DNA, little is known about how cGAS activity is regulated upon infection. How cGAS is homeostatically maintained in an inactive state and whether these rely on certain protein interactions remain unclear. Additionally, our understanding of the global impact of HSV-1 infection on the cellular proteome is limited. Here, we provide the first unbiased characterization of cytoplasmic cGAS protein interactions during cellular states of active immune signaling via infection with HSV-1 in primary human fibroblasts. We place cGAS interactions in the context of temporal proteome alterations using isobaric-labeling (TMT) mass spectrometry (MS). We compare several HSV-1 strains that induce varying cytokine responses and apoptosis, presenting the most in-depth characterization of proteomes during HSV-1 replication. Via domain construction, CRISPR-mediated knockouts, antiviral cytokine measurements, and targeted MS (PRM) quantification, we establish a functional interaction between cGAS and an RNA pathogen-sensing factor, 2'-5'-oligoadenylate synthase-like protein OASL. We demonstrate that this interaction occurs in different types of cells. We further establish that the OAS-like domain of OASL interacts with the cGAS Mab21 domain, while the OASL ubiquitin-like domain inhibits cGAS-mediated interferon response. Therefore, we uncover a regulatory crosstalk between the traditional RNA and DNA sensing pathways. Our findings explain how cGAS may be inactively maintained in cellular homeostasis, with OASL functioning as a negative feedback loop for cytokine induction.

Mon Talk 10:55 - 11:10 am: Influence of the Gut Microbiota on Histone Acetylation through Butyrate Oxidation

Peder Lund[1]; Sarah Smith[1]; Johayra Simithy[1]; Lillian Chau[1]; Elliot Friedman[1]; Yedidya Saiman[1]; Sophie Trefely[2]; Mariana Lopes[1]; Zuo-Fei Yuan[1]; Kevin Janssen[1]; Nathaniel Snyder[2]; Gary Wu[1]; Benjamin A. Garcia[1]
[1]University of Pennsylvania, Philadelphia, PA; [2]A.J Drexel Autism Institute, Drexel University, Philadelphia, PA

The gut microbiota is a diverse microbial community, residing primarily in the human colon, that accomplishes functions related to host defense, digestion, and immunoregulation. Given that disruptions to the microbiota are associated with pathological conditions, including inflammatory bowel disease (IBD), investigating the molecular interactions between the microbiota and host is important for understanding how the microbiota impacts host health. One mode of interaction involves small molecules, such as butyrate. Butyrate is a product of bacterial fermentation and has long been known to inhibit histone deacetylases (HDACs), which represent one class of

chromatin-modifying enzymes that regulate gene expression by controlling histone post-translational modifications. Thus, the microbiota has the potential to broadly modulate host cell epigenetics and gene expression. Accordingly, using quantitative mass spectrometry, we have observed that germ-free mice, which lack a microbiota, have reduced levels of histone H4 acetylation in the colon. Sequencing analyses suggest that the loss of acetylation occurs across the genome rather than at specific loci, though transcription start sites appear protected. While the reduced acetylation in germ-free mice may stem from elevated HDAC activity in the absence of butyrate, an alternative possibility is that germ-free mice have lower histone acetyltransferase activity because of less oxidation of butyrate to acetyl-CoA, the necessary cofactor for histone acetylation. Indeed, through isotope tracing analyses performed in cell culture and in mice, we have demonstrated that butyrate and inulin, a fermentable plant polysaccharide, provide carbon for histone acetylation reactions. Ongoing work is focused on determining the relative contribution of butyrate to histone acetylation as a source of acetyl-CoA versus an HDAC inhibitor. Overall, our findings will advance insight into how the microbiota influences host cell epigenetics and gene expression programs, which may be relevant to the pathogenesis of inflammatory disorders, such as IBD.

MONDAY 3:00 – 4:20 pm
MULTI-OMICS, *Plaza Ballroom*

Mon Talk 3:00 - 3:25 pm: Utility of Proteogenomics in Immunotherapy

Bing Zhang
Baylor College of Medicine, Houston, TX

Using proteogenomics data from the Clinical Proteomic Tumor Analysis Consortium (CPTAC), I will present a few examples on how proeogenomic integration can expand our knowledge on cancer genes, prioritize cancer drivers, clarify puzzling genomic observations, and correct misinterpreted gene functions. I will also discuss our recent works on using proteogenomics to identify tumor antigens and understand immune evasion mechanisms.

Mon Talk 3:25 - 3:50 pm: Informing Cancer Biology using Computational Proteogenomics

Kelly Ruggles
NYU School of Medicine, New York, NY

Cancer has been well established as a disease of the genome, with a subset of somatic mutations frequently acting as drivers of tumor progression, and thereby influencing diagnosis, prognosis and treatment. The integration of cancer genomics with mass spectrometry-based proteomics and phosphoproteomics can be used to supplement genomic information, determining the effect of genomic aberrations at the protein level, guiding biomarker development and predicting effective drug combinations for treatment. We have applied informatics methods focused on cancer proteogenomics to a number of diverse tumor types to identify novel peptides, aberrant kinase gene expression and phosphorylation status and clinically relevant druggability based on altered signaling pathways.

Mon Talk 3:50 - 4:05 pm: Post-translationally Modified Proteins in Plasma Extracellular Vesicles as Candidate Markers for Breast Cancer Subtypes

Hillary Andaluz[1]; I-Hsuan Chen[1]; J. Sebastian Paez[1]; Anton B. Iliuk[2]; W. Andy Tao[1]
[1]Purdue University, West Lafayette, 0; [2]Tymora Analytical Operations, West Lafayette, IN

Breast cancer is a complex disease that can be majorly classified into four molecular subtypes, Luminal A/B, Her 2 positive and triple negative. With a wide variety of pathological features and biological behaviors, the diagnosis or prognosis of specific subtypes is critical to assign treatment. Here, we present a novel strategy for developing serial PTM-omics in plasma-derived extracellular vesicles (EVs) as biomarkers to differentiate among breast cancer subtypes. Our study identified 11824, 192, 1259 and 805 of unique pS/T, pY phosphorylation, N-glycosylation and acetylation peptides respectively in EVs, isolated from plasma samples. Using label-free quantitative PTM-omics, several PTM sites showed significantly different increases across certain subtypes, and PCA further confirms that the expression profile of each PTM is also different. In addition, several targets were verified in each subtype by using parallel reaction monitoring (PRM) approach. Together, this study demonstrates the great potential of this strategy for developing the biomarkers for different subtypes in breast cancer.

Mon Talk 4:05 - 4:20 pm: Discovery of lincRNA-encoded Peptides: An Integrated Transcriptomics, Proteomics and Bioinformatics Approach

Chin-Rang Yang; Cameron Flower; Lihe Chen; HyunJun Jung; Viswanathan Raghuram; Mark Knepper
NHLBI, National Institutes of Health, Bethesda,MD

Long non-coding RNA (lncRNA) refers to the family of RNA transcripts that cannot encode a protein and are more than two-hundred nucleotides in length. However, it has been shown that a subset of lncRNA transcripts do in fact contain open reading frames (ORFs), that have the potential to encode short peptides and show significant functional roles within the cell. Many of these peptides remain unannotated and uncharacterized due to relatively low molecular weight, low abundance, and tissue specificity. This study presents an integrated workflow combining proteomics, transcriptomics and bioinformatics to enable comprehensive profiling a subset of lncRNA transcripts called “lincRNA” (long intergenic noncoding RNAs that do not overlap with known genes) - encoded peptide. We test this workflow on the mouse kidney inner medulla (IM), a region that contains the collecting duct system responsible for regulated water transport. In brief, short peptides of molecular weights between 2 and 20 kDa were enriched by tricine protein gel, in-gel trypsinized into peptides, and then analyzed using high resolution mass spectrometry. However, identification of peptide sequences from the resulting fragmentation spectra requires a reference protein sequence database which must be generated *de novo* in the tissue of interest. To do this, an RNA-Seq based workflow was implemented to translate identified expressed lincRNA transcripts in mouse IM (using five biological replicates of strand-specific RNA-Seq experiments) into predicted ORFs. Candidates were further evaluated using several quality control criteria and bioinformatics tools. We will present three novel peptides that passed all criteria and conserved in rat and human may have potential roles in water transport in this conference. This workflow can be applied to other cell or tissue types to discover more novel lincRNA-encoded peptides.

MONDAY 3:00 – 4:20 pm
METABOLISM AND DISEASE, *Roosevelt-Madison*

Mon Talk 3:00 - 3:25 pm: Defining Mitochondrial Protein Function through Systems Biochemistry

David J. Pagliarini[1, 2]
[1]Morgridge Institute for Research, Madison, WI; [2]University of Wisconsin, Madison, WI

Despite their position as the iconic powerhouses of cellular biology, many aspects of mitochondria remain remarkably obscure—a fact that contributes to our poor ability to address mitochondrial dysfunction therapeutically. Such dysfunction contributes to a vast array of human diseases through distinct means. For instance, aberrant mitochondrial biogenesis can fail to properly set cellular mitochondrial content; dysregulated signaling processes can fail to calibrate mitochondrial activity to changing cellular needs; and malfunctioning proteins can render core bioenergetic processes ineffectual. A major bottleneck to

understanding—and ultimately addressing—these processes is that the proteins driving them are often undefined. Concurrently, the functions of hundreds of mitochondrial proteins that may fulfill these roles are not known, or at best are poorly understood. Thus, the high-level goal of my research program is to help achieve a more complete, systems-level understanding of mitochondrial biology by systematically establishing the functions of orphan mitochondrial proteins and their roles within disease-related processes. We do so by first devising multi-dimensional analyses designed to make new connections between these proteins and established pathways and processes. We then employ mechanistic and structural approaches to define the functions of select proteins at biochemical depth. This 'systems biochemistry' strategy is helping us address three outstanding biological questions: Which orphan mitochondrial proteins fulfill the missing steps in classic mitochondrial processes, including the biosynthesis of coenzyme Q and other aspects of respiratory chain function? What proteins assist in the orchestrated assembly of lipids, metabolites, and proteins (from two genomes) to ensure proper mitochondrial biogenesis? And, which resident signaling proteins direct the post-translational regulation of mitochondrial activities? In answering these questions, we aim to help transform the mitochondrial proteome from a component list into a metabolic circuitry of connected functions, and to elucidate the biochemical underpinnings of mitochondrial dysfunction in human disease.

Mon Talk 3:25 - 3:50 pm: Metabolomics-Based Discovery of Metabolic Aspects of Cancer and other Diseases

Anne Le
Johns Hopkins Medicine, Baltimore, MD

Dr. Anne Le's research primarily focuses on cancer metabolism. Using metabolomics technologies, her work has led to breakthrough discoveries revealing several characteristic features of the metabolism of cancer. In her talk, Dr. Le will discuss the complexity and diversity of cancer cell metabolism within the same tissue of origin, and even within individual tumor cell populations. Importantly, she will illustrate how to take advantage of metabolomics technologies for the discovery of metabolic aspects of cancer and other diseases.

Mon Talk 3:50 - 4:05 pm: An Optimized Data Independent Acquisition (DIA) Method for Peptide-Centric Analysis in Metabolism Studies

Christopher A. Barnes[1]; Lindsay K. Pino[2]; Bong J. Kim[1]; Brian C. Searle[2]; Michael J. MacCoss[2]
[1]Novo Nordisk Research Center Seattle, Inc., Seattle, WA; [2]Department of Genome Sciences, University of Washi, Seattle, WA

Small, bioactive polypeptides including those derived from posttranslational prepropeptide hormone processing of longer translated gene products are often integral bioactive factors that control metabolic processes. Very well known examples such as insulin and glucagon are both short, processed polypeptides that act temporally to regulate glucose homeostasis. Even after decades studying these and other peptide hormones, there is still much unknown about the other peptide fragments that can be liberated from the same proprotein precursors. In the case of the proglucagon precursor, the polypeptide chain can be specifically cleaved into numerous bioactive peptides including both glucagon and the also therapeutically important glucagon-like peptide 1 (GLP-1). LC-MS/MS-based proteomics approaches using data-independent acquisition (DIA) are peptide-centric by nature and potentially suitable for this sort of novel peptide discovery. Using the recently published DIA-based chromatogram-library approach, we demonstrate optimization of precursor mass window size on the Thermo Lumos platform for both DIA-based peptide identifications and profiling of different biological samples. We show that relative quantitation is feasible with overlapping 8 m/z ("mzol") windows allowing for an effective 4 m/z precursor range. In a comparison of liver proteomes generated from fasted, fed, and refed (after fasting) mice, we show that this DIA approach yielded a slight increase in quantified proteins compared to tandem mass tagging (TMT). Analysis of the same sample set with both techniques generating believable proteome changes in well-known proteins associated with fasting such as the observed increased levels of phosphoenolpyruvate carboxykinase (PCK1) or the observed decreased levels of fatty acid synthase (FASN) in fasted mice. We conclude that both TMT and DIA using on-column chromatogram libraries can yield high quality biologically-meaningful results with DIA offering improved modularity and a higher attention to individual peptide reproducibility that will be more easily ported to the peptide-centric nature of future discovery experiments.

Mon Talk 4:05 - 4:20 pm: Peroxisome Plasticity as a Metabolic Strategy for Virus Replication

Katelyn C Cook; Pierre M Jean Beltran; Yutaka Hashimoto; Ileana M Cristea
Princeton University, Princeton, NJ

Peroxisomes are cellular organelles with essential functions in human health, such as lipid production, fatty acid oxidation, and detoxification. However, peroxisomes are among the least studied organelles in biological contexts that rely on organelle remodeling, such as virus infections. Viruses cause broad alterations in organelle composition, structure, and localization in order to facilitate virus replication and spread. Here, we uncover a previously unrecognized function for peroxisomes in the replication of enveloped viruses. We initially examined human cytomegalovirus (HCMV), a beta-herpesvirus with nearly 90% worldwide seroprevalence and a significant health concern for pregnant women and immunocompromised patients. Using quantitative targeted mass spectrometry (parallel reaction monitoring, PRM), we found that peroxisome proteins increased in abundance as infection progressed, notably including biogenesis and lipid synthesis proteins. With mathematical modeling and microscopy structural analyses, we showed that infection triggers peroxisome growth and fission and the translocation of key host proteins to peroxisome membranes, interfering with peroxisome structure and increasing peroxisome biogenesis by nearly 4-fold. To determine the functional relevance of these changes, we generated a series of CRISPR knockouts in primary human fibroblasts and used pharmacological treatments to perturb peroxisome abundance and morphology. This analysis demonstrated that HCMV hijacks peroxisome metabolic pathways to facilitate virus production, which we further examined by lipidomics mass spectrometry. We discovered that infection enhances the synthesis of plasmalogen phospholipids at peroxisomes, and this is required for the assembly of new viruses. Moreover, by comparing other virus infections and analyzing samples from patients with genetic peroxisome disorders, we found this mechanism to be conserved across replication cycles of enveloped viruses, and likely relevant for a range of critical human diseases. Our integrative study illustrates the ability of human pathogens to manipulate subcellular organization for their replication and spread, and defines peroxisome regulation as a key aspect of virus replication.

MONDAY 4:30 – 5:50 pm
ADVANCES IN TECHNOLOGY, *Plaza Ballroom*

Mon Talk 4:30 - 4:55 pm: Capturing Site-Specific Heterogeneity with Large-Scale N-Glycoproteome Analysis

Joshua Coon
University of Wisconsin-Madison, Madison, WI

Protein glycosylation is a highly important, yet poorly understood protein post-translational modification. Thousands of possible glycan structures and compositions create potential for tremendous site heterogeneity. A lack of suitable analytical methods for large-scale analyses of intact glycopeptides has limited our abilities to both address the degree of heterogeneity across the glycoproteome and to

understand how it contributes biologically to complex systems. Here we show that N-glycoproteome site-specific microheterogeneity can be captured via large-scale glycopeptide profiling methods enabled by activated ion electron transfer dissociation (AI-ETD), ultimately characterizing 1,545 N-glycosites (>5,600 unique N-glycopeptides) from mouse brain tissue. Our data reveal that N-glycosylation profiles can differ between subcellular regions and structural domains and that N-glycosite heterogeneity manifests in several different forms, including dramatic differences in glycosites on the same protein. Moreover, we use this large-scale glycoproteomic dataset to develop several visualizations that will prove useful for analyzing intact glycopeptides in future studies.

Mon Talk 4:55 – 5:20 pm: Making Quantitative Proteomics Quantitative

Michael MacCoss
University of Washington, Seattle, WA

Our goal is to develop generally applicable high throughput methods for sampling peptides with a mass spectrometer that can be used as a robust measure of molecular phenotyping to characterize the state of the cell. To do this we would like a tandem mass spectrometry (MS/MS) method that can comprehensively sample all peptides in a sample continuously throughout the chromatographic elution. MS/MS offers significant advantages in terms of selectivity, sensitivity, and dynamic range over a single stage of mass analysis. Modern mass spectrometers now make this data independent acquisition sampling feasible in a way that wasn't possible before. With our ability to sample peptides systematically and on a chromatographic time-scale, we are now focused on the development of strategies that enable us to compare signal intensities between acquisition batches, between laboratories, and even between instrument platforms. Furthermore, we are working on proteomics strategies that can assess individually for any and all of the 1000s of measured peptides if the measured signal reflects the change in quantity for a given sample matrix. By bringing modern assay validation strategies to proteomics measurements, we should improve and enable reproducible research within our community.

Mon Talk 5:20 - 5:35 pm: Multiplexing Meets Automation: Medium Scale-Phosphoproteomics Assay Increases Sample Throughput and Allows for Quantification in Primary Neurons

Katherine DeRuff; Alvaro Sebastian Vaca Jacome; Karen Perez de Arce; Malvina Papanastasiou; James Mullahoo; Deborah Dele-Oni; Steven A. Carr; Jeffrey R. Cottrell; Jacob D. Jaffe
Broad Institute, Cambridge, MA

Phosphoproteomics offers deep insights into cellular signaling and processes, but often requires large amounts of material for standard analyses. In this work, we sought to balance lower phosphoproteomic coverage with increased throughput and reduced sample input requirements, which would allow us to analyze rare cell types and tissues, such as neurons. Expanding upon automation protocols we have previously developed (Abelin et al. 2016), we have now produced a fully-automated, discovery proteomics workflow that leverages Tandem-Mass-Tag labeling and single-shot LC-MS/MS analysis that provides biologically relevant phosphosite information from sample amounts as low as 10 ugd. As a proof-of-principle, we employed mouse and rat cortical neurons and perturbed them with compounds that are known to affect synaptic plasticity for various time points up to 48 hours. The sensitivity of the assay allowed us to analyze 80 samples in triplicates using a total amount of 40 ug per sample. We were able to characterize >5000 phosphopeptides that constitute ~40% of phosphopeptide identifications using traditional phosphoproteomics workflows (Mertins et al. 2018). Mapping of these peptides onto KEGG pathways demonstrates that adequate coverage is achieved to produce a snapshot of important signaling activity at a fraction of the cost and time of traditional phosphoproteomics. The work presented here illustrates the value of the technology to samples with limited amounts.

Mon Talk 5:35 - 5:50 pm: Electro-Elution Chromatography of RNA Oligonucleotides: A Novel Paradigm in RNA Analysis by nanoLC-MS/MS

Richard Lauman; Hee Jong Kim; Sam Wein; Kevin Janssen; Benjamin A. Garcia
University of Pennsylvania, Philadelphia, PA

RNA post-transcriptional modifications are ubiquitous and have been associated with splicing events, oxidative stress and in the structure of tRNA and ribosomal RNA. Current nanoLC-MS/MS RNA for post transcriptional modifications methods are limited by the length of the RNA oligo or by ion pairing reagents: losing valuable context in the sequence and from loss of signal due to ion pairing reagent suppression. In this newly designed method, we use a previously known technique that has been so far limited to small molecules to retain and release RNA oligonucleotides selectively by using a novel automated 2D gradient and electro-elution. Using a standard solvent nanoflow LC, a voltage was applied to the porous graphite column over a range to retain and release the RNA molecules. The Electrochemically Modulated Reverse Phase (EMRP) nanoLC-MS/MS method is designed to retain and elute numerous RNA oligomers, solely dependent on their size and charge state. The release of the oligos is dependent on the voltage switch to the column and subsequently the polarity switch of the column. Using synthetic RNA, we determined a limit of detection (LODs) in the range of 100-200 attomole/uL, dependent on sequence of RNA. This detection limit is well within the possible concentrations in cells and even serum. MS^1 and MS^2 data has been processed with the use of a novel program developed in house (NucleicAcidSearchEngine, NASE), which can discern the higher charge states and determine sequences of the RNA oligonucleotides with an applied FDR. Combined with this computational software, we now have complete platform to detect smaller sequences of RNA, such as microRNAs or RNA digested with nuclease from any cellular source, removing much of the issues surrounding PCR amplification.

MONDAY 4:30 – 5:50 pm
STRUCTURAL AND CHEMICAL PROTEOMICS, *Roosevelt-Madison*

Mon Talk 4:30 - 4:55 pm: Cross-linking Mass Spectrometry Strategies to Define Protein-Protein Interactions

Lan Huang
UC Irvine, Irvine, CA

Protein-protein interactions (PPIs) are fundamental to the formation of protein complexes and crucial for regulating various cellular activities. Cross-linking mass spectrometry (XL-MS) have become an emergent technology for mapping PPIs at the systems-level and elucidating architectures of large protein complexes. In comparison to standard structural methods, XL-MS approaches offer distinct advantages due to speed, accuracy, sensitivity and versatility, especially for the study of heterogeneous and dynamic protein complexes. Despite its great potential, XL-MS analysis remains challenging due to the difficulty in effective detection and identification of cross-linked peptides. Here, we will describe the development of sulfoxide-containing MS-cleavable cross-linking reagents (e.g. disuccinimidyl sulfoxide (DSSO)) for advancing MS analysis and identification of cross-linked peptides1,2. In addition, their applications in defining PPIs and structural dynamics of protein complexes will be presented. The analytical methods presented here can be directly applied to study PPIs in other biological systems. Ref 1. Kao, A. et, al. MCP, 2011; 2. Yu, C. et al, Anal. Chem. 2018.

Mon Talk 4:55 - 5:20 pm: In-Cell Protein Footprinting Coupled with Mass Spectrometry for Proteome-Wide Structural Biology

Lisa Jones
University of Maryland, Baltimore, MD

In recent years, protein footprinting coupled with mass spectrometry has been used to identify protein-protein interaction sites and regions of conformational change through modification of solvent accessible sites in proteins. The footprinting method, fast photochemical oxidation of proteins (FPOP), utilizes hydroxyl radicals to modify solvent accessible sites in proteins. To date, FPOP has been used *in vitro* on relatively pure protein systems. We have further extended the FPOP method for both in cell and *in vivo* analysis of proteins. This will allow for study of proteins in their native cellular environment and be especially useful for the study of membrane proteins which can be difficult to purify for *in vitro* studies. We have designed and built a single cell flow system to enable uniform access of cells to the laser. Results demonstrate that in cell FPOP (IC-FPOP) can oxidatively modify over 1300 proteins in various cellular compartments. Owing to the high number of proteins that can be modified by IC-FPOP, we can use the method for proteome-wide structural biology. By comparing breast cancer cells treated with vehicle or with the anti-cancer drug Gleevec, we can identify on and off targets of the drug. We have further extended the method for *in vivo* analysis in *C. elegans*. We have demonstrated that with using an optimized flow system and a higher laser frequency we can modify over 500 proteins in various organisms within the worms. This demonstrates that both in-cell and *in vivo* FPOP can be used to study proteins in their native cellular environment.

Mon Talk 5:20 - 5:35 pm: Understanding the Role of H2A Proteolysis during Stem Cell Differentiation

Mariel Coradin; Kelly Karch; Simone Sidoli; Benjamin A Garcia
University of Pennsylvania School of Medicine, Philadelphia, PA

Histone proteolysis is a poorly understood process by which the N-terminal tails get irreversible cleaved (clipped). This process has been described in cellular senescence, inflammation and stem cell differentiation, where its role remains unclear. In this study we combined Top-down MS, transcriptomics, and structural proteomics to interrogate the functional role of clipped H2A (cH2A) during stem cell differentiation and assess its consequences on nucleosome stability. Our data showed that H2A is cleaved during mouse embryonic stem cells (mESCs) differentiation by the lysosomal protease Cathepsin L. Using Top-Down MS, we were able to map the major cleavages sites to be at L23 and G44 (cH2A). Cells treated with Cathepsin L inhibitors showed lower levels of cH2A, indicating that Cathepsin L also serves as H2A protease *in vivo*. Using RNA-sequencing, we found that inhibition of this enzyme leads to upregulation of genes involved in endoderm formation. We also assessed the modification landscape of H2A in mESCs. Using bottom-up proteomics, we found that the N-terminal tail is dynamically modified during this process. Similar to H3 and H4, acetylation levels of H2A increase in early stages of development, and as lineage commitment continues, acetylation levels drop significantly. We have also identified members of SWI/SNF chromatin remodeler complex (BRG1, SMARCC2) as binding acetylated H2A. Proteins in this complex are essential in embryonic cell self-renewal, highlighting a potential role of acetylated H2A in early development. Finally, we sought to probe the structural consequences of cH2A, which lacks the alpha-1 helix. Using *in vitro* reconstituted dimers, we compared the hydrogen-deuterium exchange rate of full-length H2A and cH2A containing complexes. Our findings reveal that cH2A/H2B dimers are less stable than full-length H2A dimers. Taken together, our data suggest that histone proteolysis could be a novel mechanism for nucleosome eviction during mammalian development.

Mon Talk 5:35 - 5:50 pm: Towards Protein Structure Determination in Living Cells using a New Translationally Incorporated Crosslinker that Improves Mass Spectrometric Detection

Bjorn-Erik Wulff; Josh Elias; Pehr Harbury
Stanford University, Stanford, CA

Atomic-resolution protein structures have been critical for biological discovery of life's molecular basis. A major current limitation, however, is that protein structures cannot be determined inside cells, where proteins exist in their native environment. This deficiency particularly impacts membrane proteins, cytoskeletal proteins, protein super-complexes, intrinsically disordered proteins and proteins that undergo phase separation *in vivo*. High-throughput crosslinking mass spectrometry (XLMS) is an emerging technology with potential to provide the missing information. Analogously to NMR structure determination, XLMS can read out large numbers of amino acid proximity constraints that define the three-dimensional protein fold. Importantly, the crosslinks must be short and distributed throughout the protein core, necessitating translational incorporation of a crosslinker that is typically light-activatable. However, such crosslinkers are devilishly difficult to detect by mass spectrometry.

We synthesized and tested a new family of ultra-short and light-activatable crosslinkers. We show that these can be translationally incorporated in yeast, and we present a path to incorporating them in other organisms. Two advances improve detection of the resulting crosslinks. First, we created a technique that depletes a trypsinate of peptides with only a single N-terminus. This removes the excess of linear peptides and leaves concentrated crosslinked peptides, which have two N-termini. This technique is agnostic to the details of the crosslink and the protease used. Second, our amino acid analogs incorporate a thioether bond that can be broken by very gentle CID that does not affect other bonds. It separates the crosslinked peptides from each other and any background species at the MS2 level and sequences them individually at the MS3 level. The 'mass scars' left by the crosslinker identify the individual amino acids that formed the crosslink. We show that custom control software for the mass spectrometer, which searches for and exploits this behavior, achieves speed and accuracy with great sensitivity.

TUESDAY 9:50 – 11:10 am
PROTEIN INTERACTIONS AND SIGNALING, *Plaza Ballroom*

Tues Talk 10:15 - 10:40 am: Functional Proteomics for Mechanistic and Translational Insight in GBM Clinical Trials

Forest White
MIT, Cambridge, MA

Glioblastoma (GBM) is the most common and devastating form of brain cancer, affecting ~100,000 people worldwide, with limited therapeutic options and poor 5-year survival rates. Over the past twenty years several hundred clinical trials for glioblastoma have been performed, yet almost all of these trials have been unsuccessful, potentially due to poor target selection, poor drug delivery, or adaptive response / resistance of the tumor cells. To gain better insight into clinical trials in GBM and other diseases, we have developed a technology platform combining functional proteomics and mass spectrometry-based imaging, toward the goal of monitoring drug distribution and drug efficacy with high spatial resolution from patient biopsies. Here we describe the initial results from application of this platform to AZD-1775, a wee1 kinase inhibitor, in patient-derived xenograft (PDX) tumors and biopsies from human clinical specimens in a neo-adjuvant setting.

Tues Talk 10:40 - 10:55 am: Biochemical Reduction of the Topology of the Diverse WDR76 Interactome

Dayebgadoh Gerald[1]; Mihaela E. Sardiu[1]; Laurence Florens[1]; Michael P. Washburn[1, 2]

[1]Stowers Institute for Med Res, Kansas City, ; [2]The University of Kansas Medical Center, Kansas City, KS

WD40 repeat proteins form a diverse protein family constituting about 1% of the protein coding genome with functions ranging from DNA damage repair, cell cycle progression, apoptosis, and autophagy. Thus, characterizing novel WD40 repeat proteins is of great interest. Here we rigorously carry out a series of Affinity-purification coupled to mass spectrometry (AP-MS) to reduce the topology of the WDR76 interactome using different biochemical conditions. Our high confidence results uncovered macromolecular diversity and the stability of WDR76 interactions. Our data links human WDR76 to multiple biological processes: protein folding, gene silencing, DNA damage, mitotic cell cycle, and metabolism. For example, we connect WDR76 to GAN, HELLS, and SIRT1 and show that these interactions are outside of the WD40 repeat domain of WDR76. Taken together, GAN, HELLS, and SIRT1 are possible hubs that confer WDR76 its biological specificity.

Tues Talk 10:55 - 11:10 am: An Antibody-free Method for Identification of Protein Complexes using RNA Aptamers

Angela Kruse[1]; Judhajeet Ray[1]; Abdullah Ozer[1]; Richard Johnson[2]; Michael MacCoss[2]; John Lis[1]; Michelle Heck[1, 3]

[1]Cornell University, Ithaca, NY; [2]University of Washington, Seattle, WA; [3]USDA ARS, Ithaca, NY

Affinity purification mass spectrometry (AP-MS) has revolutionized the study of protein interactions in vivo. A major challenge in AP-MS experiments is the high abundance of peptides derived from the antibodies used for protein complex isolation. We present a method to use RNA aptamers in place of antibodies to pull down target proteins and protein complexes from living cells. RNA aptamers are short oligonucleotides developed using an *in vitro* process called Systematic Evolution of Ligands by Exponential Enrichment (SELEX), and have affinity for proteins, small molecules, and even carbohydrates that is comparable to antibodies. Aptamers are small and easily penetrate animal tissues. To test the hypothesis that RNA aptamers could be used in protein complex isolation, we performed five replicate isolations of two different protein complex isolations from human cells: heat shock factor-1 (HSF1) fused to GFP and negative elongation factor E (NELF E) using GFP and NELF E aptamers, respectively. Protein complex isolations were analyzed using 1-D gels and high resolution mass spectrometry. Gel analysis revealed clean and reproducible banding patterns enriched for the target proteins. Mascot and Scaffold were used for protein identification and spectral counting. Significance Analysis of Interactomes (SAINT) was used to identify members of these protein complexes. The use of multiple aptamers allowed us to make a database of common contaminant RNA binding proteins useful for aptamer pulldown experiments. We confidently identified many known HSF1-interacting proteins and all of the known NELF E-interacting proteins using this method. New proteins assigned to these protein complexes may represent novel discoveries in the molecular regulation of these proteins. This method will improve the development and testing of RNA aptamers for medical and industrial applications, and offers an alternative to antibody-based pulldown methods that improves the identification of low-abundance binding partners in protein complexes without the need for antibody development.

TUESDAY 9:50 – 11:10 am
INFECTIOUS DISEASES, *Roosevelt-Madison*

Tues Talk 10:15 - 10:40 am: Identifying Ways that Viruses Manipulate the Cellular Proteome

Matthew Weitzman[1, 2]

[1]UPenn Perelman School of Medicine, Philadelphia, PA; [2]Children's Hospital of Philadelphia, Phildelphia, PA

Viruses are obligate intracellular parasites that manipulate cellular environments to achieve efficient viral gene expression, DNA replication and packaging of viral genomes. Viral DNA genomes replicating in the nucleus of infected cells encounter a myriad of host factors that can facilitate or hinder viral replication. The battle between virus and host generates a genomic conflict as the host attempts to limit viral infection and protect its genome. Viral proteins expressed early during infection modulate cellular proteins interacting with viral genomes, recruiting factors to promote viral replication while limiting access to antiviral repressors. We have employed a number of proteomic approaches to study virus-host interactions and identify the cellular factors that are harnessed or inactivated as viruses hijack cellular machinery. We have identified viral tactics deployed to eliminate, evade or exploit intrinsic cellular defense. Many of these involve changes to post-translational modifications, such as harnessing the cellular ubiquitin machinery to degrade or modify function of host proteins. Studying the dynamic interactions on viral and host genomes has revealed insights into viral replication, and has identified intrinsic cellular defenses against viral infection. Proteomic approaches provide a global view of viral and host proteomes during infection, helping to explain how viruses takeover cellular processes and also suggest targets for antiviral therapies.

Tues Talk 10:40 - 10:55 am: Inhibition of HIV Maturation via Selective Unfolding and Crosslinking of Gag Polyprotein by a Mercaptobenzamide Acetylator

Lisa M. Jenkins[1]; Elliott L. Paine[1]; Lalit Deshmukh[2]; Herman Nikolayevskiy[2]; Gaelyn C. Lyons[1]; Michael T. Scerba[2]; Kara George Rosenker[2]; John M. Louis[2]; Elena Chertova[3]; Rob Gorelick[3]; David E. Ott[3]; G. Marius Clore[2]; Daniel H. Appella[2]

[1]National Cancer Institute, NIH, Bethesda, MD; [2]NIDDK, NIH, Bethesda, MD; [3]Frederick National Lab for Cancer Research, Frederick, MD

Although antiretroviral therapy has advanced, there remains a critical need for new therapeutics, especially those targeted at resistant strains of HIV. The Gag polyprotein, and in particular its constituent nucleocapsid protein, NC, represents a prime target for antiretroviral inhibition. NC is composed of two highly conserved zinc-binding domains and plays multiple roles throughout the virus replication cycle. We have developed a class of small molecule inhibitors of NC, based upon an S-acyl-2-mercaptobenzamide thioester (SAMT) scaffold, that covalently modify the zinc-binding domains of NCp7 *in vitro* and in cells. These compounds display potent antiviral activity without evidence of cytotoxicity in cell models, *ex vivo* cervical explants, and in animal models. We investigated the mechanism of viral inactivation of the Gag polyprotein both in vitro and in virions released from cells. Mass spectrometry experiments identified multiple sites of covalent modification throughout the Gag polyprotein due to SAMT reaction. The earliest reaction occur within matrix and NC, with additional sites observed at later times. These covalent reactions inhibit maturation and prevent formation of infectious viral particles. Targeting multiple residues in HIV Gag and Gag-Pol greatly limits the potential of the emergence of virus escape mutations, making mercaptobenzamide antiviral compounds a strong starting point for developing a new therapeutic agent against HIV.

Tues Talk 10:55 - 11:10 am: Blood Protein Biomarkers that Diagnose and Classify Individuals with Lyme Disease

Yong Zhou[1]; Shizhen Qin[1]; Mingjuan Sun[1, 2]; Li Tang[1]; Xiaowei Yan[1]; Taek-Kyun Kim[1]; Juan Caballero[3]; Gustavo Glusman[1]; Mary E. Brunkow[1]; Mark J. Soloski[4]; Alison W. Rebman[4]; Gilbert Omenn[1, 5]; Robert L. Moritz[1]; John N. Aucott[4]; Leroy Hood[1]

[1]Institute for Systems Biology, Seattle, WA; [2]Second Military Medical University, Shanghai, China; [3]Molecular and Developmental Complexity Lab, Langeb, Irapuato, Guanajuato, Mexico; [4]Johns

Hopkins University School of Medicine, Baltimore, MD; [5]University of Michigan, Ann Arbor, MI

BACKGROUND: Lyme disease, a tick-borne illness caused by the spirochete *Borrelia burgdorferi*, is the most popular and widely spread infectious disease in USA, with an CDC estimated ~300K new cases annually. Current laboratory diagnosis of Lyme disease suffers with low sensitivity, ineffective for early detection of infection and no predictive value for the risk of post-treatment Lyme disease syndrome, in ~20-30% of patients. Here we adapted an alternative approach to target proteins that may signal an exposure to *B. burgdorferi* in humans by investigating blood proteins that are expressed predominantly in human organs affected by the infection, e.g., brain, heart, liver and skin, as well as acute phase and innate immune response proteins. The main aim of this study is to discover surrogate biomarkers that support early diagnosis of Lyme disease and/or that predict a patient's risk of progression to PTLDS after the acute phase.

METHODS: In this study, depleted serum samples from a longitudinal cohort were investigated by selected reaction monitoring, which includes 40 Lyme disease patients and 20 matched controls, with samples collected from time of diagnosis to 4-6 years post antibiotic treatment.

RESULTS: We identified 16 proteins—mainly acute phase / innate immune system response proteins and proteins highly enriched in *B. burgdorferi* affected organs—that may serve as biomarkers for early diagnosis of Lyme disease. Five of these 16 proteins may possess predictive value to distinguish patients who later developed PTLDS from those who returned to health after treatment.

TUESDAY 3:00 – 4:20 pm
CANCER EARLY DETECTION AND PREVENTION, *Plaza Ballroom*

Tues Talk 3:25 - 3:50 pm: Helping Bad Biomarkers Fail Fast: Strategies for Prioritizing Candidate Biomarkers

Karin Rodland
Pacific Northwest National Laboratory, Richland, WA

Biomarker discovery strategies often feature heavy reliance on lists of differentially abundant transcripts or proteins derived from unbiased comparisons of diseased versus healthy tissues or biofluids, often with an emphasis on depth of coverage at the expense of statistical power. Translation of candidate biomarkers into clinically approved assays requires relatively high throughput testing on a large number of patients from one or more independent cohorts – and the logistics of doing this with tens to hundreds of markers on many hundreds of patients is daunting, particularly when considering that the typical biomarker failure rate is at least 85%. Here we will describe a strategy based on targeted mass spectrometry, potentially augmented with pathway-specific information, for rapidly and efficiently disqualifying biomarker candidates that are not sufficiently robust for clinical applications.

Tues Talk 3:50 - 4:05 pm: Digitizing the Proteomes from Big Tissue Biobanks

Jan Muntel[3]; Nick Morrice[2]; Christie Hunter[1]; Roland Bruderer[3]; Nicholas Dupuis[3]; Lukas Reiter[3]
[1]SCIEX, Redwood City, CA; [2]SCIEX, Warrington, UK; [3]Biognosys, Schlieren, Switzerland

Tissue biopsies have been preserved and stored in biobanks for more than a century in the hope that their future analysis will provide a better understanding of health and disease. These samples are often very well characterized by classical pathological methods and provide great potential for precision medicine and the discovery of new diagnostic/stratification markers and therapeutic targets. A powerful way to take advantage of this repository is to quantify large numbers of proteins across all the samples so that correlations can be made with respect to various health and disease states. Such an endeavor would require highly reproducible sample preparation, a robust analytical platform for high throughput sample analysis, as well as robust data analysis.

Here, microflow SWATH Acquisition was used to generate quantitative proteomics data on a cohort of colon cancer samples from a biobank. This study demonstrates how high throughput proteomics can be used to interrogate these precious samples from biobanks and how this research can pave the way to a better understanding of health and disease. 105 colon cancer samples were analyzed in ~5 days with high analytical depth (~4500 proteins quantified across the healthy and disease sample types). Using Spectronaut for data analysis, quantitative results were quickly generated, revealing 3 cancer subtypes and some interesting protein clusters.

Tues Talk 4:05 - 4:20 pm: Can AI Find a Tree in the Woods? Issues and Considerations for Hypothesis Free Discovery in Large Clinical Proteomics Datasets

David Bramwell; Will Dracup
Biosignatures Ltd, Newcastle, United Kingdom

It is a common paradigm in 'omics sciences to make many complex measures of a sample and to use AI or statistical analysis to find 'biomarkers' that separate specified groups. This is usually termed 'hypothesis free discovery' as little or no additional information is used to pre-select candidate analytes.

It is still typical to see 'biomarker discovery' experiments that take tens of disease cases and compare them to a matched set of controls. In general, questions like this are 'ill posed' i.e. there are far more analytes than case examples to learn from. Without rebalancing the data via analyte pre-selection, many algorithms will be prone to 'over-learning' and will not produce an answer that validates on new samples. An additional issue is that diagnosis information is usually binary or ordinal with few cases, which limits the pre-selection tools that can be used.

Clinical prognostics, diagnostics and screens are areas of great need and intense research interest. This presentation enumerates some of these issues on a large clinical proteomics data set. We ask a question that is asked surprisingly infrequently; 'Can we use our algorithms build a model to measure something that we know is present in the data set?' To give an example, will the AI systems be able to select the right combination of analytes to measure A1AT from the proteomics data set if we give them the clinical immunoassay A1AT measures to learn from alongside the proteomics data?

Data for several clinical analytes will be presented, some known to be in the proteomics measures and some not expected to be within the measurement limits. The quantifiable issues will be shown and discussed in terms of what these results imply for hypothesis free discovery of disease biomarkers.

TUESDAY 3:00 – 4:20 pm
INFORMATICS: EMERGING AND NEW APPROACHES,
Roosevelt-Madison

Tues Talk 3:00 - 3:25 pm: Semantic Computing for Protein Knowledge Network Discovery

Cathy H Wu
University of Delaware, Newark, DE

To realize the value of genome-scale data for disease understanding we have develop a semantic computing framework that connects text mining, data mining and biomedical ontology for protein knowledge discovery. We have employed natural language processing and machine learning approaches with linguistic generalization to develop text mining tools applicable to various types of entities and relations, including post-translational modification (PTM) enzyme-substrate-site relationships and their protein-protein interactions, as well as associations with diseases, genomic anomalies and drug responses. To foster large-scale text analytics across documents, we have further

developed iTextMine with an automated workflow. To support protein-centric semantic integration of biomedical data of increasing volume and complexity, we have developed the Protein Ontology as a reference ontology in the Open Biological and Biomedical Ontologies Foundry, allowing both human understanding and computational reasoning of proteins in biological contexts. Through federated SPARQL queries of multiple ontologies and knowledge sources, including PTM-proteoform sites specific functional annotation in PRO, we proposed mechanisms connecting PTMs, variants, and cancer. The rich PTM knowledge is being integrated in iPTMnet to support exploration of PTM enzyme-substrate relationships, regulation of PTM enzymes, cross-talk, and conservation across species. Our tools are FAIR (Findable, Accessible, Interoperable, Reusable), accessible programmatically via RESTful API, and dockerized and available from project websites, public repositories and cloud-based environment. This talk will highlight projects involving PTM enzyme (kinase) enrichment analysis and the use of LINCS (Library of Integrated Network-based Cellular Signatures) for interpretation of large-scale proteomic data to identify the upstream signaling pathways that are responsible for the observed PTM state of the cell and further our understanding of the impact of kinase inhibitor drugs on signaling pathways in cancer therapy.

Tues Talk 3:25 - 3:50 pm: Algorithms and databases for Mining Insight from Quantitative Mass Spectrometry Experiments of Post-Translational Modifications

Kristen Naegle
University of Virginia, Charlottesville, VA

My research lab is deeply interested in predicting and testing the function of tyrosine phosphorylation in proteins and protein networks. In the pursuit of this work, we have developed new algorithms and resources for identifying, analyzing, and inferring meaning about post-translational modifications. In this talk, I will share methods for ensemble clustering, which have identified new protein-protein interactions from quantitative mass spectrometry data and share information about ProteomeScout, our resource for improving accessibility and analysis of quantitative experiments and whole proteomes-level information about post-translational modifications.

Tues Talk 3:50 - 4:05 pm: Topoliogiical Scoring of Protein Interaction Networks

Michael Washburn
Stowers Institute for Medical Research, Kansas City, MO

It remains a significant challenge to define individual protein associations within networks where an individual protein can directly interact with other proteins and/or be part of large complexes, which contain functional modules. Here we demonstrate the topological scoring (TopS) algorithm for the analysis of quantitative proteomic analyses of affinity purifications. Data is analyzed in a parallel fashion where a bait protein is scored in an individual affinity purification by aggregating information from the entire dataset. A broad range of scores is obtained which indicate the enrichment of an individual protein in every bait protein analyzed. TopS was applied to interaction networks derived from human DNA repair proteins and yeast chromatin remodeling complexes. TopS captured direct protein interactions and modules within complexes. TopS is a rapid method for the efficient and informative computational analysis of datasets, is complementary to existing analysis pipelines, and provides new insights into protein interaction networks.

Tues Talk 4:05 - 4:20 pm: High-Throughput Identification of MS-Cleavable and Non-cleavable Chemically Crosslinked Peptides with MetaMorpheus

Lei Lu; Michael R. Shortreed; Robert J. Millikin; Lloyd M. Smith
University of Wisconsin, Madison, WI

Protein chemical cross-linking combined with mass spectrometry has become an important technique for the analysis of protein structure and protein–protein interactions. Reliable, rapid, and user-friendly tools for large-scale analysis of cross-linked proteins, however, are still needed. MetaMorpheus has recently been updated to identify both MS-cleavable and noncleavable cross-linked peptides. MetaMorpheus crosslink search does not require the presence of signature fragment ions, a major advantage compared with similar programs. One complication associated with the need for signature ions from cleavable cross-linkers such as DSSO (disuccinimidyl sulfoxide) is the requirement for multiple fragmentation types and energy combinations, which is not necessary for MetaMorpheus. MetaMorpheus can, however, search fragmentation from multiple dissociation types for the same precursor (e.g. CID & ETD, CID & HCD) and also MS2/MS3 data for those users desirous of such information. Another significant advantage of MetaMorpheus is the ability to perform proteome-wide analysis. MetaMorpheus is also faster than other currently available MS-cleavable cross-link search software programs. Finally, MetaMorpheus provides immediate and straightforward MS2 annotation of each assignment in a format that can be exported in portable data format (.pdf). This feature enables users to manually validate identifications.

TUESDAY 4:30 – 5:50 pm
PROTEIN PROTEOFORMS IN HEALTH AND DISEASE, *Plaza Ballroom*

Tues Talk 4:30 - 4:55 pm: A PTM Code for Membrane Protein Maturation

John R. Yates[1]; Sandra Pankow[1]; Casimir Bamberger[1]; Diego Calzolari[2]; Salvador Martínez-Bartolomé[1]; Mathieu Lavallée-Adam[3]
[1]The Scripps Research Institute, La Jolla, CA; [2]Qualcomm, San Diego, CA; [3]University of Ottawa, Ottawa, Canada

A component to understanding biological processes involves identifying the proteins expressed in cells as well as their modifications and the dynamics of processes. This process has benefited from the sequencing of genomes, although this information Is not uncovered from DNA sequencing. Mass spectrometry together with informatic tools can uncover the type of modification and it's a location in a peptide sequence. Advances in multi-dimensional separations as well as mass spectrometry have improved the scale of experiments for protein identification. This has improved the analysis of protein complexes, and more complicated protein mixtures. Quantitative mass spectrometry has also helped to determine the role of modifications in regulating biological processes. Using the loss of function mutant form of the Cystic Fibrosis Transport Regulator (DF508) as it progresses through the folding pathway as a model to understand the regulation of protein maturation, we have discovered a post translational modification code that regulates the maturation of CFTR. This has provided a better understanding of the loss of function associated with mutation.1,2We are now exploring if this code is general for membrane protein maturation.

1Pankow et al Nature2015, 528, 510-6., 2Pankow et al Science Signaling 2019, 12(562) eaan7984.

Tues Talk 4:55 - 5:20 pm: Polycomb Loss Mediated Reprogamming of the Epigenome Drives Oncogenesis in Malignant Peripheral Nerve Sheath Tumors

John B. Wojcik; Dylan M. Marchione; Simone Sidoli; Benjamin A. Garcia
University of Pennsylvania School of Medicine, Philadelphia, PA

Malignant peripheral nerve sheath tumor (MPNST) is an aggressive sarcoma with recurrent loss of function alterations in polycomb-repressive complex 2 (PRC2), a histone-modifying complex involved in transcriptional silencing. To understand the role of PRC2 loss in pathogenesis and identify therapeutic targets, we conducted parallel global epigenomic and proteomic analysis of archival formalin-fixed, paraffin-embedded human MPNSTs with and without PRC2 loss

($MPNST_{LOSS}$ vs. $MPNST_{RET}$). In $MPNST_{LOSS}$, histone post-translational modifications (PTMs) associated with active transcription, most notably H3K27Ac and H3K36me2, were increased and polycomb-mediated repressive H3K27 di- and trimethylation (H3K27me2/3) were globally lost, without a compensatory gain in other repressive PTMs. Instead, DNA methylation was globally increased. Epigenomic changes were associated with upregulation of proteins in growth pathways and reduction in interferon signaling and antigen presentation, suggesting a role for epigenomic changes tumor progression and immune evasion, respectively. The epigenomic changes also resulted in therapeutic vulnerabilities. Knockdown of NSD2, the methyltransferase responsible for H3K36me2, restored MHC expression and induced interferon pathway expression in a similar manner to polycomb restoration. $MPNST_{LOSS}$ were also highly sensitive to DNA methyltransferase inhibitors and HDAC inhibitors, suggesting that global loss of polycomb-mediated repression renders $MPNST_{LOSS}$ differentially dependent on DNA methylation to maintain transcriptional integrity and thus highly sensitive to therapeutics that promote aberrant transcription initiation.

Tues Talk 5:20 - 5:35 pm: Deciphering the Human Heart Proteoform Landscape in Cardiac Disease and Regeneration

Trisha Tucholski[1]; Ling Gao[2]; Zachery Gregorich[1]; Wenxuan Cai[1]; Kyle Brown[1]; Yanlong Zhu[1]; Bifan Chen[1]; Samantha Knott[1]; Andrew Alpert[3]; Jianyi Zhang[2]; Ying Ge[1]

[1]University of Wisconsin, Madison, WI; [2]University of Alabama at Birmingham, Birmingham, AL; [3]PolyLC Inc., Columbia , MD

Heart diseases remain the leading cause of death in developed countries for both men and women. Altered post-translational modifications (PTMs) and variations in amino acid sequence for cardiac sarcomere proteins have been implicated as causative factors for cardiovascular diseases. Nevertheless, the disease mechanisms are highly heterogeneous and poorly understood. To begin to understand the molecular biology underlying human heart disease and cardiac regeneration, we must obtain a global qualitative and quantitative view of proteoform landscape with critical knowledge on the combinatorial PTM-amino acid sequence variants. Mass spectrometry (MS)-based top-down proteomics (TDP) is the most powerful technology for deciphering PTM codes together with amino acid sequence variations, providing essential insight into the structure and function of proteoforms, the effectors of all biological processes. Herein, we seek to develop and implement novel TDP tools to qualitatively and quantitatively characterize human heart proteoforms to deepen our understanding of heart health and disease. Using a proteomics platform, which combines serial size-exclusion chromatography with high-resolution top-down MS, we have gained access to the high-molecular weight portion of the human heart proteome and identified previously unknown phosphoproteins in the cardiac sarcomere. Using quantitative top-down proteomics, we have unveiled a reversal of deleterious PTM changes following cardiac injury and treatment with an induced pluripotent stem cell (iPSC) – derived patch. Additionally, a novel quantitative proteomics platform developed in our lab has allowed us to use top-down LC-MS to assess expression-level changes of proteins in the cardiac sarcomere following iPSC-patch therapy. Combined with global label-free proteomics, we uncover the proteome-level changes in a swine model for iPSC-patch therapy and cardiac regeneration.

Tues Talk 5:35 - 5:50 pm: Cell Type-Resolved Analysis of Blood Proteoforms by Large-Scale Top-Down Proteomics

Paul Thomas[1]; R. Vince Gerbasi[1]; Rafael Melani[1]; Jacek Sikora[1]; Timothy Toby[1]; Kristina Srzentic[1]; Luca Fornelli[1, 2]; Richard LeDuc[1]; Josiah Hutton[1, 3]; Ryan Fellers[1]; Joseph Greer[1]; Jeannie Camarillo[1]; Lissa Anderson[4]; Chris Hendrickson[4]; Neil Kelleher[1]

[1]Northwestern University, Evanston, IL; [2]University of Oklahoma, Norman, OK; [3]Princeton University, Princeton, NJ; [4]NHMFL, Tallahassee, FL

Proteoforms have emerged as a new unit of measurement in proteomics that is strongly connected to protein function and organismal phenotypes. While many protein catalogs have been created over the years, few contain and report on the proteoforms present. Here, we will explore the rationale for cataloging proteoforms in health and disease and describe challenges and solutions that arise when performing top-down proteomics “at-scale.”

Blood is a suspension of cells in plasma that circulates throughout the human body. It is responsible for transport of proteins and metabolites as well as mounting immune responses to external threats. Techniques such as fluorescence activated cell sorting (FACS) and immunocapture can be used to isolate specific subsets of cells for further analysis. Here, we used FACS and positive and negative immunopurification to isolate cell populations. Next, we extract and analyze intact proteoforms from these multiple, diverse cell types from both the lymphoid and myeloid lineage to examine their proteoform content and establish cell-type signatures.

Top-Down proteomics is a technique that allows users to catalog full, intact proteoforms by omitting the protease digestion used in standard proteomics workflow. To this point, most top-down proteomics experiments have been limited in scope, largely due to the many challenges encountered in the acquisition and analysis of very large top-down datasets (>100s of files). This project required the development of novel solutions to these challenges including robust sample preparation workflows, cloud-based solutions for high-throughput analysis of top-down proteomics data, and algorithms for accurate false-discovery rate estimation. With these challenges met, robust, reproducible sample-to-answer workflows can be established for top-down proteomics. At full scale, an atlas of proteoforms from healthy blood will help researchers in the future to understand health and disease at proteoform-level resolution.

TUESDAY 4:30 – 5:50 pm
AGING AND NEUROLOGICAL DISEASES, *Roosevelt-Madison*

Tues Talk 4:30 - 4:55 pm: Integrating MS-Based Proteomics and Lipidomcs Studies in the Cerebellar Degeneration Disorder, Niemann-Pick Type C1

Stephanie Cologna

Univ of Illinois at Chicago, Chicago, IL

Advances in mass spectrometry technology have enabled large-scale differential analysis of proteins and lipids to investigate altered pathways in human diseases. Our laboratory studies Niemann-Pick Disease, Type C1 (NPC1) a lysosomal storage disorder with visceral involvement and progressive cerebellar neurodegeneration. Using a combination of differential proteomics, lipidomics and mass spectrometry imaging, we have identified new candidate biomarkers in a mouse model of NPC1. These alterations now provide a basis for evaluation in NPC1 patients and also can be used for therapeutic development. Several examples will be presented.

Tues Talk 4:55 - 5:20 pm: The Role of Senescence in Aging and Age-related Diseases – Proteomics as Tool to Decipher Mechanisms

Nathan Basisty[1]; Abhijit Kale[1]; Okhee Jeon[1]; Christopher D. Wiley[1]; Su Liu[1]; Chisaka Kuehnemann[1]; Anja Holtz[1]; Julie Anderson[1]; Pankaj Kapahi[1]; Luigi Ferrucci[2]; Judith Campisi[1]; Birgit Schilling[1]

[1]Buck Institute for Research on Aging, Novato, CA; [2]National Institute on Aging, NIH, Rockville, MD

Cellular senescence is a striking example of a prime driver of aging phenotypes and pathologies across multiple tissues. This complex stress response causes an essentially irreversible arrest of cell proliferation and the development of a multi-component senescence-associated secretory phenotype (SASP). We hypothesize that, via the SASP, senescent cells exert cell non-autonomous effects that can disrupt cells and tissues locally and at a distance and contribute to

neurodegeneration, thrombosis, and multiple age-related pathologies. Using modern proteomic technologies has allowed us to start to better understand the role of secreted proteins and SASP. Using proteomic technologies and quantitative SILAC as well as more comprehensive data-independent acquisitions approaches, we have assessed the composition and functions of the SASP in aging and disease contexts. As anticipated, the human SASP is incredibly diverse and heterogeneous, depending on different cell types, tissue source and cause of senescence burden. Recently, our proteomic screens have identified a novel role for senescent cells and SASP in hemostasis and blood coagulation. Since senescent cells accumulate with age, it is tempting to speculate that the SASP is at least partly responsible for thrombotic events that increase with age. The general role of senescent cells as driver of age-related diseases has moved forward potential strategies and therapeutics (senolytics) to remove senescent cells to improve health span. Overall, it will be of key importance to identify senescence markers, both as biomarkers for aging and age-related diseases, in order to monitor therapeutic interventions to eliminate senescent cells, such as by using senolytics in human trials.

Tues Talk 5:20 - 5:35 pm: A Quantitative Perspective of the Dynamics of Huntingtin Protein Interactions Provides Insight into Huntington's Disease Pathogenesis

Todd Greco; Joel Federspiel; Ileana Cristea
Princeton University, Princeton, NJ

Huntington's disease is a monogenic disorder, with only one known causative gene, huntingtin (Htt). Expansion of a trinucleotide (CAG) repeat within the Htt gene results in the Htt protein containing polyglutamine (polyQ) stretches, which through yet uncharacterized mechanisms, is the main driver of neuronal degeneration in the brain, predominantly in the striatum and cortex. To understand the role of polyQ in disease pathogenesis, the protein interactions of wild-type and mutant Htt have been an active area of investigation. While numerous putative interactions have been proposed and a subset found altered under pathological conditions, there are still open questions about which interactions are the most proximal to disease progression. To identify these interactions, we studied the dynamics of Htt protein interactions in the striatum of mice with wild-type (Q20) and mutant (Q140) Htt at pre- and post-symptomatic ages using complementary IP-MS approaches. Label-free IP-MS was used to determine the specificity and abundance of the interactions. Isotope-labeled IP-MS using the I-DIRT approach was employed to identify stable versus transient interactions. We found that Htt interaction abundances were predominantly increased and driven by polyQ length, but in different subset of interactions at 2 and 10 months. Functional analysis suggested these early interaction changes involve proteins that function in synaptic transmission and phagocytosis, while later interactions mediate changes in synapse morphogenesis and impact the actin cytoskeletal network. Combining these abundance changes with I-DIRT IP-MS, we found that interaction stability was increased in a polyQ-dependent manner at both 2 and 10 months, largely represented by proteins that regulate actin filament polymerization. We also observed a subset of proteins with decreased stability at 10 versus 2 months, which included components of phosphatase and kinase signaling networks. Overall, these experiments have identified the most dynamic components of interaction networks driven by mutant Htt at different disease stages.

Tues Talk 5:35 - 5:50 pm: Mapping the Brain Proteome of HIV-associated Neurocognitive Decline

Saima Ahmed[1, 2]; Amanda Guise[1, 2]; Hendrik Wesseling[1, 2]; Judith Steen[1, 2]; Hanno Steen[1, 2]
[1]Boston Children's Hospital, Boston, MA; [2]Harvard Medical School, Boston, MA

The advancement of antiretroviral therapies has significantly improved health outcomes for HIV (Human Immunodeficiency Virus) infected patients. Despite these improvements, the problem of HIV infection-associated neurological complications remains unresolved. A spectrum of neurocognitive impairment associated with HIV infection known as HAND (HIV associated neurocognitive decline) include asymptomatic neurocognitive decline (ANI), minor cognitive and motor disorder (MCMD), and HIV-associated dementia (HAD). Here, we present a proteomic investigation of human post mortem brain specimens from HIV-infected patients with various stages of HAND and HIV positive neurocognitive normals. Using sarkosyl fractionation approach coupled with our house developed high throughput MStern blotting strategy (Berger et al. Mol Cell Proteomics. 2015; 14:2814), our analysis revealed many significantly changed proteins and pathways differentiating various stages of HAND. Apart from several pathways known to be dysregulated in HAND, this first of its kind neuroproteomic study also revealed several proteins associated with HAND that hasn't been described before. The pathway analysis is reflective of the well described dysregulation of small molecule metabolic processes and small GTPase mediated signal transduction (both: GO-BP), as well as downregulation of glucose metabolism in ANI and MCMD compared the neurocognitive normals. In addition, we observed inflammation-related dysregulation of the protein folding, proteasomal and ubiquitination machinery proteins accompanying changes in proteins associated with T cell activation, antiviral signaling, and neuronal injury proteins.

In addition to the mapping the proteomes, we also investigated Tau, a signature protein of dementia. The observed striking differences in the composition of the proteoforms and modifications of Tau in both soluble and insoluble fractions of various brain regions in HAND vs. other tauopathies were confirmed using antibody-based methods.

In summary, our results can be used to further elucidate the underlying pathogenesis of HIV infection induced neurological complications to facilitate the development of definitive markers and a better approach to treatment.

WEDNESDAY 9:50 – 11:10 am
POSTTRANSLATIONAL REGULATION, *Plaza Ballroom*

Wed Talk 09:50 - 10:15 am: The Lysine Acylation Pathways as a Bridge between Metabolism and Epigenetic Mechanisms

Yingming Zhao
University Of Chicago, Chicago, IL

There are tens of thousands metabolites in mammalian cells. However, less than 1% of these metabolites' non-metabolism functions have been characterized, representing a major knowledge gap in biology. In this presentation, we will report a new family of histone modifications that can be induced by short-chain lipid metabolites. Using mass spectrometry and biochemical methods, we detected and comprehensively validated nine types of lysine short-chain, acylation pathways: propionylation, butyrylation, crotonylation, malonylation, succinylation, glutarylation, 2-hydroxyisobutyrylation, 3-hydroxybutyrylation, and benzoylation. These acylation pathways use short-chain CoAs as cofactors for their modification reactions that can be stimulated by their corresponding short-chain lipids. We identified ~500 histone marks bearing the new PTMs. Using mass spectrometry-based proteomics approaches, we identified and quantified protein substrates bearing these modifications in core histones and non-histone proteins. We subsequently characterized the new PTM pathways by identifying their binding proteins and regulatory enzymes. Interestingly, some HDACs (e.g., SIRT5) have high activities to these new lysine acylations but not the widely studied lysine acetylation, suggesting that some of HDACs were mistakenly classified as deacetylases. These new PTM pathways are associated with gene expression, cellular physiology and diverse metabolic diseases.

Wed Talk 10:15 - 10:40 am: High-throughput Quantitative Top-down Proteomics for Deep Characterization of Intact Proteoforms with Post-Translational Modifications

Si Wu
University of Oklahoma, Norman, OK

The isobaric chemical tag labeling coupled with 2D pH RP/RPLC separation has been widely applied for the identification and quantification of peptides and proteins in bottom-up proteomics. However, until recently, successfully applying these approaches to top-down proteomics has been limited. Here we reported a high-throughput quantitative top-down platform with the following innovations: (1) developing a first proteome-level TMT top-down MS platform for quantifying intact proteoforms with the molecular weight (MW) of less than 35 kDa; (2) optimizing an offiline 2D pH RP/RPLC separation for top-down MS; (3) establishing an online 2D nano-UPLC system for small quantity sample analysis. Applying this platform, we successfully identified 1400+ proteoforms in unlabeled E. coli cell lysate (5 μg total protein), and quantified 1000+ intact proteoforms in 15 μg TMT labeled proteins (i.e., E. coli and HeLa cell lysate). We observed that the detected signal to noise ratios were enhanced using the online 2DLC platform, which facilitate the identification of low abundance proteoforms with PTMs. To summarize, we have developed a highly effective technique for deep proteoform characterization and quantification in complex protein samples.

Wed Talk 10:40 - 10:55 am: The LPS-responsive ADP-Ribosylated Proteome in Primary Human Immune Cells

Casey Daniels; Arthur Nuccio; Aleksandra Nita-Lazar
NIAID, NIH, Bethesda,MD

Protein ADP-ribosylation is known to be an important regulatory element in DNA repair and cell death, cellular processes critical to cancer development. As such, PARP inhibitors have successfully been developed as chemotherapeutic agents and are now being used in the clinic to treat a growing range of cancers. These clinical applications have revealed that PARP inhibitors modulate the human immune system, and today PARP inhibitors are being combined with immunotherapy in human patients. This exciting new work is happening in the absence of a basic understanding of how ADP-ribosylation and PARP inhibition affects immune cells, a gap which we seek to address here using mass spectrometry-based proteomics. Our two databases reveal changes in the ADP-ribosylated proteome during activation of the innate immune response as modeled by the exposure of macrophages to lipopolysaccharide (LPS), a highly immunogenic molecule found on the outside of gram-negative bacteria. The first database analyzes human primary, mouse primary, and cell-line derived macrophages and describes LPS-responsive ADP-ribosylation events on over 1000 proteins. The second database gives a detailed view of the changes in primary human monocyte-derived macrophages, wherein over 3,000 sites from 1,400 proteins are robustly quantified during immune activation using isotopic labeling. Analysis of this data has revealed LPS-sensitive ADP-ribosylation of proteins known to regulate the MAP kinase cascade and NFκB signaling. We share our mechanistic studies related to these findings and discuss the implications of this work in our understanding of PAR, PARP and PARP inhibitor biology. This research was supported by the Intramural Research Program of NIAID, NIH.

Wed Talk 10:55 - 11:10 am: Pathway-scale Targeted Mass Spectrometry for High-Resolution Functional Profiling of Cell Signaling

Paolo Cifani; Alex Kentsis
Sloan-Kettering Institute, New York, NY

In spite of extensive studies of cellular signaling, many fundamental processes such as pathway integration, cross-talk and feedback remain poorly understood. In addition, most observed oncogenic mutations are of unknown significance and precise and comprehensive measurements of cancer cell signaling remain elusive. To measure differential regulation of cellular biochemical activities, thus enabling precision medicine and cell biology, we have developed the Quantitative Cell Proteomics Atlas (QCPA, http://qcpa.mskcc.org). QCPA consists of panels of targeted mass spectrometry (MS) assays to determine abundance and stoichiometry of regulatory post-translational modifications of proteins covering most recurrently mutated and functionally relevant pathogenic pathways in human cells. QCPA currently profiles 1,944 peptides from 467 effectors of cell surface signaling, apoptosis, stress response, gene expression, quiescence, and proliferation. For each protein, QCPA includes peptides covering known post-translational regulatory sites to determine their stoichiometry, and independent unmodified peptides to measure protein abundance. These measurements are then used as surrogates of regulation and biochemical activities. We implemented QCPA using the recently developed accumulated ion monitoring (AIM), to achieve enrichment-free near-zeptomolar sensitivity of targeted MS detection for phosphorylated and unmodified peptides. This enabled precise and accurate quantitation of functionally modified proteins from clinically-accessible, microgram minute patient specimens, or as few as 10,000 cells. As a result, we were able to measure integrated multi-parametric signaling profiles, such as those induced by growth factor stimulation, serum starvation, and drug treatment. In particular, we determined oncogenic signaling induced by mutant FLT3 receptor tyrosine kinase in acute myeloid leukemia cells, and defined the mechanisms of susceptibility and resistance to new selective kinase inhibitors. The ability to precisely and accurately deploy thousands of targeted chemoproteomic assays per experiment enables a paradigm shift for comprehensive analysis of cell signaling. In addition, precision and robustness of this method are well suited for functional proteomics of clinical specimens.

WEDNESDAY 9:50 – 11:10 am
GLYCOPROTEOMICS IN BIOLOGY AND MEDICINE, *Roosevelt-Madison*

Wed Talk 09:50 - 10:15 am: Nutrient Regulation of Transcription & Signaling by O-GlcNAc

Gerald Hart
CCRC, University of Georgia, Athens, GA

O-GlcNAcylation cycles on and off thousands of nucleocytoplasmic proteins and has extensive crosstalk with protein phosphorylation. O-GlcNAc is abundant on nearly all proteins involved in transcription, where it regulates gene expression in response to nutrients. O-GlcNAc also regulates the cycling of the TATA-binding (TBP) protein on and off DNA during the transcription cycle.

Targeted deletion of the O-GlcNAc Transferase in excitatory neurons of adult mice results in a morbidly obese mouse with a satiety defect. Thus, O-GlcNAcylation not only serves as a nutrient sensor in all cells, but also regulates appetite. More than eighty-percent of all human protein kinases are modified by O-GlcNAc, and all kinases that have been tested to-date are indeed regulated by the sugar. Abnormal O-GlcNAcylation of CAMKII contributes directly to diabetic cardiomyopathy and to arrhythmias associated with diabetes. Prolonged elevation of O-GlcNAc, as occurs in diabetes, contributes directly to diabetic complications and is a major mechanism of glucose toxicity. Targeted over-expression of OGT to the heart causes severe heart failure in mice, which is reversed when they are crossed with mice having OGA over-expressed in their hearts. Drugs that elevate O-GlcNAcylation in the brain, which prevents hyperphosphorylation, appear to be of benefit for the treatment of Alzheimer's disease in animal models. To date, all cancers have elevated O-GlcNAc cycling, which plays a key role regulating the metabolism of cancer cells. Supported by NIH P01HL107153, R01GM116891, and R01DK61671.

Dr. Hart receives a share of royalty received by the university on sales of the CTD 110.6 antibody, which are managed by JHU.

Wed Talk 10:15 - 10:40 am: Innovations in Chemistry and Mass Spectrometry Platform Technologies for Quantitative Glycomics

David Muddiman[1]; Jaclyn Gowen[1]; James Dodds[1]; Erin Baker[1]; James Petitte[1]; Alison Motsinger-Reif[1]; Michael MacCoss[2]; Thomas Montine[3]

[1]North Carolina State University, Raleigh, NC; [2]University of Washington, Seattle, WA; [3]Stanford University, Palo Alto, CA

Mass spectrometry offers the most robust platform to discover and characterize new diagnostic, prognostic, and therapeutic targets for disease. A detailed characterization of glycans provides mechanistic insights into disease by understanding the role of aberrant glycosylation in disease. To this end, we have developed bioanalytical tools to characterize structurally challenging analytes that are critical to a systems-level analysis. To increase the electrospray response of *N*- and *O*-linked glycans, perform stable-isotope relative quantification, and semi-automated data analysis, we synthesized novel hydrophobic tagging reagents (INLIGHT™). While this chemistry is robust and yielded deep glycome coverage, fundamentally we should be able to further increase the ionization of glycans by over 2 orders of magnitude. Moreover, isomers are also challenging in glycomics (aka the isomer barrier); while difficult to resolve chromatographically, ion mobility spectrometry (gas-phase separations) offers an avenue to effectively address this challenge. We demonstrate this approach can be realized using nanoLC-IM-TOF-MS of INLIGHT labeled glycans in model and complex samples. Finally, we are developing new algorithms to analyze these complex data sets and integrate the information with glycopeptide datasets, including the analysis of IM-TOF-MS data.

Wed Talk 10:40 - 10:55 am: Advancing Cardiac Glycomics: Protein Glycosylation in Primary and Stem Cell-derived Human Cardiomyocytes

Christopher Ashwood[1]; Matthew Waas[1]; Ranjuna Weerasekera[1]; Rebekah L. Gundry[1, 2]

[1]Medical College of Wisconsin, Milwaukee, Wisconsin; [2]Center for Biomedical Mass Spectrometry, MCW, Milwaukee, WI

Protein glycosylation plays an integral role in cardiomyocyte function, by modulating ion channel localization and function. However, our current view of the human cardiomyocyte glycome is limited. To address this, we applied an optimized PGC-LC-ESI-MS/MS method to characterize and quantify *N*- and *O*- glycan structures in primary human heart tissue, primary isolated cardiomyocytes, and human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs; 17 timepoints across days 20-100 of differentiation). These novel libraries, composed of 200 glycan structures quantified over three orders of magnitude, provide coverage of all major *N*-glycan classes and are expected to benefit future functional analyses of these glycans as well as glycoproteomic analyses.

In the primary tissue homogenate and isolated cardiomyocytes, 103 *N*-glycan structures were identified. Of the 15 structures which were significantly increased in the isolated cardiomyocytes, high mannose *N*-glycans were predominant, suggesting their potential cell-type specific expression within the myocardium. In the analysis of hiPSC-CM differentiation, 183 *N*-glycan structures were identified, including 37 that increase and 51 that decrease over time, demonstrating that glycosylation is dynamic throughout maturation. Specifically, 2 glycan structures that increased exponentially with time were also identified in the primary adult cardiomyocytes. Thus, they may represent novel markers of myocyte maturation. Finally, in addition to the similarities observed between primary cells and hiPSC-CMs, 32 structures were unique to primary cardiomyocytes. This suggests that glycomic analyses will be an important complement to other 'omic and functional approaches when assessing the accuracy of hiPSC-CMs for disease modeling and drug testing.

Altogether, these data provide the most comprehensive glycomic analysis of primary human cardiomyocytes, providing the first step towards generating a cell-type and chamber-resolved glycomic map of the human heart. Moreover, the discrepancies between primary cardiomyocytes and hiPSC-CMs provide direction for future stem cell engineering efforts to generate hiPSC-CMs that more closely represent primary cardiomyocytes.

Wed Talk 10:55 - 11:10 am: Mapping the O-Glycoproteome using Site-Specific Extraction of O-linked Glycopeptides (EXoO)

Weiming Yang; Minghui Ao; Yingwei Hu; Qing Kay Li; Hui Zhang

Johns Hopkins University, Baltimore, MD

Protein glycosylation is one of the most abundant post-translational modifications. However, detailed analysis of O-linked glycosylation, a major type of protein glycosylation, has been severely impeded by the scarcity of suitable methodologies. Here, a chemoenzymatic method is introduced for the site-specific extraction of O-linked glycopeptides (EXoO), which enabled the mapping of over 3,000 O-linked glycosylation sites and definition of their glycans on over 1,000 proteins in human kidney tissues, T cells, and serum. This large-scale localization of O-linked glycosylation sites demonstrated that EXoO is an effective method for defining the site-specific O-linked glycoproteome in different types of sample. Detailed structural analysis of the sites identified revealed conserved motifs and topological orientations facing extracellular space, the cell surface, the lumen of the Golgi, and the endoplasmic reticulum (ER). EXoO was also able to reveal significant differences in the O-linked glycoproteome of tumor and normal kidney tissues pointing to its broader use in clinical diagnostics and therapeutics.

WEDNESDAY 9:50 – 11:10 am
LIFETIME ACHIEVEMENT IN PROTEOMICS AWARD SESSION,
Plaza Ballroom

Wed Talk 11:10 am - 12:00 pm: Adventures in the Garden of Proteomics

Catherine E. Costello

Boston Univ. School of Medicine, Boston, MA

The seeds for Proteomics were planted by insightful scientists who first noted that biopolymers were abundant in all living tissues, both within and outside the cell, and that they displayed changes during development, disease and ageing. The discoveries of the genetic code and its translation into proteins focused attention on the necessity for development of efficient methods for determining the sequences of these polymers, and relating their details to functions and interactions. The Human Genome project greatly accelerated the pace at which genes can be sequenced, and recent progress has starkly reduced the cost. Gene sequences provide the roots for formulating an understanding of the potential for life processes, but the actions depend on proteins that thus form the trunks and stems and post-translational modifications that regulate the flowering. I was lucky to have an early opportunity to rub my toes in the garden's soil and have been happy to be engaged with tending the garden over the course of my career. We've generated better hoes and pruning forks now, and have more opportunities to differentiate and share familiarity with our flowers. HUPO, at national and international levels, and the Human Proteome Project in particular, are the master gardeners, and mass spectrometry is our most useful tool, but it needs the support of other analytical and biophysical approaches, and the constant infusion of new bioniformatic fertilizers. This lecture will include some bits on past escapades but focus on recent adventures in the garden, with the intent to convey examples and insights that boost the enthusiasm of present and future farmers.

POSTER LIST

All posters will be displayed Monday and Tuesday.
Each day: odd-numbered posters present 1:30-2:15 pm; even-numbered posters present 2:15-3:00 pm.
ML = Mon. lightning talk; TL = Tues. lightning talk; WTT = Wed. tips & tricks talk.

Poster	*Topic*
001-008	Biomarkers and Targeted MS Assays
009-020	Cancer Proteomics
021-023	Cardiovascular Disease
024-025	Chemical Proteomics
026-027	Chromatin Dynamics
028-029	Clinical Proteomics
030-039	Computation, Informatics and Big Data
040	Cross-Linking / Molecular Painting
041-044	Data-Independent Acquisition Proteomics (DIA)
045-050	Glycoproteomics and Glycomics
29	High Resolution Mass Spectrometry
051	Imaging
052-056	Immunopeptidomics
057-058	Metabolomics
059	Microbiome Analysis
060-068	Multi-omics
069-077	Neuroproteomics / Neurological Diseases
078-088	New Technologies
089-096	Pathogen Proteomics
097-114	Post-Translational Modifications
115-116	Precision Medicine
117-120	Protein Complexes and Interactomics
121-125	Protein Quality Control
126-136	Proteomics in Ageing and Age-Related Diseases
137	Proteomics in Drug Development
138-139	Proteomics in Microbiology
140-149	Quantitative Proteomics
150-157	Signaling and Biochemical Pathways Proteomics
158	Single-Cell Proteomics
159	Top-Down Proteomics

Poster 001-ML **Multiplexed Quantification Strategy for Candidate Biomarker Discovery and Verification in Alzheimer's Disease;** Xiaofang Zhong; Qinying Yu; Fengfei Ma; Dustin Frost; Lingjun Li; *University of Wisconsin-Madison, Madison, WI.*

Poster 002 **A Multiple Reaction Monitoring Assay for Simultaneous Quantification of Mitochondrial Proteins in Mouse;** Alexsandr Stotland; Weston Spivia; Amanda Orosco; Allen Andres; Jennifer Van Eyk; Roberta Gottlieb; Sarah Parker; *Cedars Sinai Medical Center, Los Angeles, CA.*

Poster 003 **A Novel UHPLC-MRM-MS Methodology for Accurate, Reproducible, and fast quantification of histone PTMs;** Joseph Cesare; Zuofei Yuan; Steven Zhao; Peder Lund; Yekaterina Kori; Simone Sidoli; Josue Baeza; Hee Jong Kim; Kathryn E. Wellen; Benjamin A. Garcia; *University of Pennsylvania, Philadelphia, PA.*

Poster 004-TL **P4Ha1 Hydroxylation of Bradykinin Allows Blood-based Characterization of Tumor Hypoxia;** Yang Liu; Christopher Lyon; Jia Fan; Tony Hu; *Arizona State University, Tempe, AZ.*

Poster 005-ML **Utilizing Parallel Reaction Monitoring for a High Throughput Diagnostic Pipeline to Establish Cardiac Troponin I Phosphorylation as a Biomarker;** Daniel Soetkamp; Weston Spivia; Qin Fu; Jennifer E. Van Eyk; *Cedars-Sinai Medical Center, Beverly Hills, California.*

Poster 006-TL **Top-Down Proteogenomics Analysis of Serum Autoantibody Repertoire for the Discovery of Biomarker of Systemic Lupus Erythematosus;** Zhe Wang[1]; Xiaowen Liu[2]; Kenneth Smith[3]; Si Wu[1]; *[1]University of Oklahoma, Norman, OK; [2]School of Informatics and Computing, IUPUI, Indianapolis, IN; [3]Oklahoma Medical Research Foundation, Oklahoma City, OK.*

Poster 007 **Development of an MRM Assay to Distinguish Active and LAP TGF-β in Urine;** Chelsea Boo; Raghothama Chaerkady; Sonja Hess; *MedImmune, Gaithersburg, <Not Specified>.*

Poster 008-TL **Signatures of Ambient Exposure to Benzene and other Air Pollutants in the Human Serum Albumin Cys[34] Adductome;** Joshua Smith[1]; Robert O'Meally[1]; Derek Ng[1]; Thomas Kensler[1, 2]; Robert Cole[1]; John Groopman[1]; *[1]Johns Hopkins University, Baltimore, MD; [2]Fred Hutchinson Cancer Research Center, Seattle, WA.*

Poster 009 **Global Proteome and Phosphoproteome Alterations Reveal Novel Drug Targets to Circumvent 3rd-Generation EGFR TKI Resistance in Human Lung Adenocarcinoma;** Xu Zhang[1]; Tapan Maity[1]; Karen Ross[2]; Shaojian Gao[1]; Khoa Dang Nguyen[1]; Fatos Kirkali[1]; Cathy Wu[3]; Udayan Guha[1]; *[1]Thoracic and GI Malignancies Branch, CCR, NCI, NIH, Bethesda, MD; [2]Georgetown University Medical center, Washington DC, DC; [3]University of Delaware, Newark, DE.*

Poster 011 **Identifying Breast Cancer Vulnerabilities by Mapping Interactome Dysregulations in Primary Tumor Samples;** Robert Morris[1, 2]; Johannes Kreuzer[1, 2]; Ridwan Ahmad[1, 2]; Cyril H. Benes[1, 2]; Dennis C. Sgroi[1, 2]; Wilhelm Haas[1, 2]; *[1]Massachusetts General Hospital, Charlestown, MA; [2]Harvard Medical School, Boston, MA.*

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Poster 012 **A Novel Role α (1,6) Fucosyltransferase, a Glycosylated related Enzyme in the Biology of Castration Resistant Prostate Cancer;** Naser Uddin; *Johns Hopkins School of Medicine, Baltimore, MD*

Poster 014-WTT **Integrating** Kin**etic and** Quantitative P**roteomics to** Investigate Autophagy Substrates **in** Tum**ors;** Monique Speirs; John Price; *Brigham Young University, Provo, UT.*

Poster 015 **A Chemical Biology Screen Identifies a Metabolic Vulnerability of Neuroendocrine Cancer Cells to SQLE Inhibition;** Sebastian Hayes; *Agios, Cambridge, MA.*

Poster 016 **TRIM28 as a Candidate Mutant p53 Interacting Partner in Cancer Cells;** Mariel Mendoza; Katherine Alexander; Enrique Lin Shiao; Charly Ryan Good; Benjamin A. Garcia; Shelley L. Berger; *University of Pennsylvania, Philadelphia, PA.*

Poster 017-ML **Detection of Malignancy-Associated Proteome and Phosphoproteome Alterations in Human Colorectal Cancer Induced by Cell Surface Binding of Growth-Inhibitory Galectin-4;** Malwina Michalak[1]; Uwe Warnken[2]; Hans-Joachim Gabius[3]; Martina Schnölzer[2]; Jürgen Kopitz[1]; *[1]Heidelberg University Hospital, Heidelberg, Germany; [2]German Cancer Research Center (DKFZ), Heidelberg, Germany; [3]Ludwig-Maximilians-University Munich, Heidelberg, Germany.*

Poster 018 **Proteomic Analysis of Uveal Melanoma Derived Exosomes;** Blake Ebert; Alex J. Rai; *Columbia University, New York, NY.*

Poster 019-ML **Bioinformatics Approach for Understanding the Role of Intrinsic Disordered Regions in Cancer-related Proteins ;** Rita Hayford; Cathy Wu; Cecilia Arighi; *University of Delaware, Newark, DE.*

Poster 020 **Early Urine Proteome Changes in the Walker-256 Tail-Vein Injection Rat Model;** Jing Wei[1]; Na Ni[2]; Wenshu Meng[1]; Youhe Gao[1]; *[1]Beijing Normal University, Beijing, China; [2]Chongqing Medical University, Chongqing, China.*

Poster 021 **Identification of Smyd1's Chromatin Binding Partners via ChIP-MS;** Anna Bakhtina; Aman Makaju; Sarah Franklin; *University of Utah, Salt Lake City, UT.*

Poster 022 **Conservation and Divergence of Protein Pathways in the Vertebrate Heart;** Frank Conlon; *University of North Carolina, Chapel Hill, NC*

Poster 023-ML **Broad Time-Dependent Proteomic and Metabolomic Effects of Atorvastatin on Hepatocytes;** Akos Vertes[1]; Albert-Baskar Arul[1]; Andrew R. Korte[1]; Peter Avar[1]; Lida Parvin[1]; Ziad J. Sahab[1]; Deborah I. Bunin[2]; Merrill Knapp[2]; Andrew Poggio[2]; Carolyn L. Talcott[2]; Brian M. Davis[3]; Christine A. Morton[3]; Christopher J. Sevinsky[3]; Maria I. Zavodszky[3]; *[1]Dept. of Chemistry, The George Washington Univ., Washington, DC; [2]SRI International, Menlo Park, CA; [3]GE Global Research, Niskayuna, NY*

Poster 024 **Site-Specific Proteomic Profiling using a Novel Chemical Probe Identifies New Members in the Deubiquitinase Family;** Taylur Ma[1]; David Hewings[5]; Johanna Heideker[6]; Andrew Ah Young[1]; Farid El Oualid[2]; Alessia Amore[2]; Gregory Costakes[2]; Daniel Kirchhofer[1]; Bradley Brasher[3]; Thomas Pillow[1]; Nataliya Popovych[1]; Till Maurer[1]; Carsten Schwerdtfeger[3]; William Forrest[1]; John Flygare[3]; Matthew Bogyo[4]; Ingrid Wertz[1]; Kebing Yu[1]; *[1]Genentech, Inc, South San Francisco, ; [2]UbiQ Bio BV, Amsterdam, Netherlands; [3]Boston Biochem Inc, Cambridge, MA; [4]Stanford University, Stanford, CA; [5]Roche Inc, Basel, Switzerland; [6]Center for Aids Research, Thermo Fisher, San Diego, CA.*

Poster 025 **Use of Peptide Biosensor and PRM for Measuring Kinase Activity;** Tzu-Yi Yang[1]; Monica Johnson[2]; Laurie L. Parker[3]; *[1]University of Minnesota, Minneapolis, MN; [2]UMN, Minneapolis, MN; [3]University of Minnesota Twin Cities, Minneapolis, MN.*

Poster 026 **Changes to Human Chromatin Induced by Cytomegalovirus Immediate Early Proteins;** Abigail A. Lemmon[1]; Tyler T. Miller[2]; Jen Liddle[2]; Katarzyna Kulej[2]; Simone Sidoli[1]; Daphne C. Avgousti[2]; Benjamin A. Garcia[1]; Matthew D. Weitzman[1, 2]; *[1]University of Pennsylvania School of Medicine, Philadelphia, PA; [2]Children's Hospital of Philadelphia, Philadelphia, PA.*

Poster 028 **Adapting EasyPep™ MS Sample Preparation for 96-well Automated Liquid Handling Systems;** Sergei Snovida[1]; Ryan Bomgarden[1]; Amarjeet Flora[1]; Xinyu Zhang[2]; Emily I. Chen[2]; John C. Rogers[1]; *[1]Thermo Fisher Scientific, Rockford, IL; [2]Thermo Fisher Scientific, Cambridge, MA.*

Poster 029 **timsTOF Pro: Maximum Throughput, Robustness and Analytical Depth for Shotgun Proteomics;** Scarlet Koch[1]; Tharan Srikumar[2]; Christopher Swift[2]; Christopher Adams[3]; Heiner Koch[1]; Thomas Kosinski[1]; Gary Kruppa[2]; *[1]Bruker Daltonic, Bremen, Germany; [2]Bruker Daltonic, Billerica, MA; [3]Bruker Daltonic, San Jose, CA.*

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Poster 030 **Identification of Inconsistent Peptide Recovery and Aberrant Peptide Termini as Sources of Sample Variability in Patient-derived Tumor Samples;** Meghan Burke; Zheng Zhang; Yuri Mirokhin; Dmitrii Tchekhovskoi; Stephen Stein; *NIST, Gaithersburg, MD.*

Poster 031 **MaxQuant Software for Trapped Ion Mobility Enhanced Shotgun Proteomics;** Christoph Wichmann[1]; Nikita Prianichnikov[1]; Heiner Koch[2]; Marcus Lubeck[2]; Chris Adams[3]; Scarlet Koch[2]; Gary Kruppa[4]; Juergen Cox[1]; *[1]Max Planck Institute of Biochemistry, Martinsreid, Germany; [2]Bruker Daltonic, Bremen, Germany; [3]Bruker Daltonic, San Jose, CA; [4]Bruker Daltonic, Billerica , MA.*

Poster 032 **Reproducible Quantitative Mass Spectrometry-based Research: The MSstats Perspective;** Meena Choi[1]; Ting Huang[1]; Tsung-Heng Tsai[1]; Eralp Dogu[2]; Sara Mohammad Taheri[1]; Olga Vitek[1]; *[1]Northeastern University, Boston, MA; [2]Mugla Sitki Koçman University, Mugla, Turkey.*

Poster 033 **2018-2019 Metrics from the HUPO Human Proteome Project: Progress on Identifying and Characterizing the Human Proteome;** Gilbert Omenn[1]; Lydie Lane[2]; Christopher Overall[3]; Fernando Corrales[4]; Jochen Schwenk[5]; Young-Ki Paik[6]; Jennifer Van Eyk[7]; Liu Siqi[8]; Michael Snyder[9]; Mark Baker[10]; Eric Deutsch[11]; *[1]University of Michigan, Ann Arbor, MI; [2]CALIPHO Group, SIB Swiss Institute of Bioinformati, Geneva, Switzerland; [3]University of British Columbia, Vancouver, BC; [4]Centro Nacional de Biotecnologia, Madrid, Spain; [5]Science for Life Laboratory, Solna, N/A; [6]Yonsei Proteome Research Center, Seodaemoon-ku, Seoul, Korea; [7]Cedar Sinai Medical Center, Los Angeles, CA; [8]BGI Human Genome Center · Department of Bioinforma, Beijing, China; [9]Stanford University, Stanford, CA; [10]Macquarie University, Sydney, Australia; [11]Institute for Systems Biology, Seattle, WA.*

Poster 034 **Integrated Proteogenomic Data Analysis Pipeline and Its Applications to Post-translational Modification Investigation;** Yingwei Hu; Minghui Ao; Jianbo Pan; David J. Clark; Weiming Yang; Punit Shah; Michael Schnaubelt; Lijun Chen; Jiang Qian; Zhen Zhang; Daniel W. Chan; Hui Zhang; *Johns Hopkins University, Baltimore, Maryland.*

Poster 035 **Integrated Machine Learning Pipeline Reveals Fingerprints of the Oxidative Stress-Sensitive Post-translational Modification during Cardiac Remodeling;** Howard Choi[1]; Bilal Mirza[1]; Jie Wang[1]; Jessica M Lee[1]; Dominic CM Ng[1]; Neo Christopher Chung[1, 2]; Ding Wang[1]; David A. Liem[1]; J. Harry Caufield[1]; Henning Hermjakob[1, 3]; Wei Wang[1]; Yibin Wang[4]; John R. Yates III[1, 5]; Peipei Ping[1]; *[1]NIH BD2K Center of Excellence at UCLA, Los Angele, CA; [2]Institute of Informatics, University of Warsaw, Warsaw, Poland; [3]European Bioinformatics Institute (EMBL-EBI), Hinxton, United Kingdom; [4]Department of Anesthesiology, UCLA, Los Angeles, CA; [5]Department of Chemical Physiology, TSRI, La Jolla, CA.*

Poster 036-TL **Improved Protein Inference from Multiple Protease Bottom-Up Mass Spectrometry Data with MetaMorpheus;** Rachel Miller[1]; Rob Millikin[1]; Connor Hoffman[1]; Stefan Solntsev[1]; Gloria Sheynkman[2]; Michael Shortreed[1]; Lloyd Smith[1]; *[1]University Wisconsin-Madison, Madison, WI; [2]Dana-Farber Cancer Institute, Boston, MA.*

Poster 037-ML **EpiProfile 2.0: A Computational Platform for Processing Epi-Proteomics Mass Spectrometry Data;** Zuofei Yuan; Simone Sidoli; Dylan M. Marchione; Johayra Simithy; Kevin A. Janssen; Mary R. Szurgot; Benjamin A. Garcia; *University of Pennsylvania, Philadelphia, PA.*

Poster 038 **UniRef Clusters as a Resource for Protein Annotation Propagation and Prediction ;** Yuqi Wang[1, 3]; Hongzhan Huang[1, 3]; Peter McGarvey[2, 3]; Cecilia Arighi[1, 3]; Cathy Wu[1, 2]; UniProt Consortium[3]; *[1]CBCB, University of Delaware, Newark, DE; [2]BMCB, Georgetown University Medical Center, Washington, DC; [3]EBI-EMBL, UK; SIB, CH and PIR, USA, Washington, DC.*

Poster 039 **Proteomic Data Commons (PDC): A Node in the NCI Cancer Research Data Commons;** Ratna Thangudu[1]; Michael Holck[1]; Deepak Singhal[1]; Paul Rudnick[2]; Nathan Edwards[3]; Karen Ketchum[1]; Christopher Kinsinger[4]; Izumi Hinkson[5]; Anand Basu[1]; Michael Maccoss[6]; *[1]ESAC, Inc., Rockville, Maryland; [2]Spectragen Informatics LLC, Bainbridge Island, WA; [3]Georgetown University, Washington, DC; [4]Natl Cancer Institute, NIH, Bethesda, MD; [5]NCI CBIIT, Rockville, MD; [6]University of Washington, Seattle, WA.*

Poster 040-TL **Optimized Cross-linking Mass Spectrometry for *in situ* Interaction Proteomics;** Zheng Ser[1, 2]; Paolo Cifani[1]; Alex Kentsis[1, 2]; *[1]Sloan Kettering Institute, New York, NY; [2]Tri-Institutional PhD Program in Chemical Biology, New York, NY.*

Poster 041 **Accelerating DIA Studies to Extend Workflow Utility, Using Fast Microflow LC Gradients;** Christie Hunter[1]; Zuzana Demianova[3]; Nick Morrice[2]; *[1]SCIEX, Redwood City, CA; [2]SCIEX, Warrington, UK; [3]SCIEX, Darmstaedt, Germany.*

Poster 042 **Simplifying the Use of Ion Libraries During Data Processing of Data Independent Acquisition Proteomics Data;** Christie Hunter[1]; Nick Morrice[2]; *[1]SCIEX, Redwood City, CA; [2]SCIEX, Warrington, UK.*

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Poster 043-WTT **Rapid Qualitative and Absolute Quantification of Plasma based proteins using a Novel Scanning Quadrupole DIA Acquisition Method;** Roy Martin[1]; Lee Gethings[2]; *[1]Waters, Beverly, MA; [2]Waters, Wilmslow, UK.*

Poster 044 **Introducing the "DataCrusher SuperComputing Service": A DIA Pipeline that's ~10x Easier, ~1000x Faster, and ~10x More Robust;** Gautam Saxena; Japheth Odonya; Boutaskiouine Mustapha; *DeepDIA, Bethesda, MD.*

Poster 045-ML **The Nature of Phosphatidylinositol Mannosidases (PIMs) Interaction with the PPE68 Protein – Revealing Novel Insights in its Immunogenicity and Virulence;** Nagender Rameshwaram; Sangita Mukhopadhyay; *CDFD, India, Hyderabad, India.*

Poster 046-TL **Integrated Glycoproteomic Characterization of Human High-Grade Serous Ovarian Cancer;** Yingwei Hu; Jianbo Pan; Punit Shah; Minghui Ao; Stefani Thomas; Yang Liu; Lijun Chen; Michael Schnaubelt; David Clark; Qing Li; Jiang Qian; Zhen Zhang; Daniel Chan; Hui Zhang; *Johns Hopkins University, Baltimore,.*

Poster 048 **Organelle Glycoproteomics by using Lectin Chromatography and HCD/ETD Mass Spectrometry;** Junfeng Ma[1]; Jason Maynard[2]; Alma Burlingame[2]; Gerald Hart[3]; *[1]Georgetown University, Washington, DC; [2]UCSF, Sausalito, CA; [3]University of Georgia, Athens, GA.*

Poster 049-ML **Glycoproteomics-based Signatures for Tumor Subtyping and Clinical Outcome in Human High-Grade Serous Ovarian Cancer;** Jianbo Pan; Yingwei Hu; Shisheng Sun; Lijun Chen; Jian-Ying Zhou; Michael Schnaubelt; Minghui Ao; Jiang Qian; Zhen Zhang; Daniel Chan; Hui Zhang; *Johns Hopkins School of Medicine, Baltimore, MD.*

Poster 050 **Development of hybrid glycoproteomic workflows for site-specific characterization of Intact Glycopeptides;** Matthew Glover; Kristen Lekstrom; Raghothama Chaerkady; Sonja Hess; *MedImmune, Gaithersburg, MD.*

Poster 051-TL **Developing and Characterizing FLIM Probes to Detect Subcellular Tyrosine Kinase Activity;** Sampreeti Jena; Oscar Bastidas; Erica Pratt; Scout Allendorf; Blanche Cizubu; Laurie L. Parker; *University of Minnesota Twin Cities, Minneapolis, MN.*

Poster 052 **Enrichment and Identification of the Class I MHC Phosphopeptides from the Resected Head and Neck Cancer Tumors;** Mohammad Ovais Aziz-Zanjani[1]; Feng Shi[3]; Sean Sepulveda[3]; Jeffrey Shabanowitz[1]; Dina L. Bai[1]; Donald F. Hunt[1, 2]; Mark Cobbold[3]; *[1]Department of Chemistry, University of Virginia, Charlottesville, VA; [2]Department of Pathology, University of Virginia, Charlottesville, VA; [3]MGH Cancer Center, Harvard Medical School, Charlestown, MA.*

Poster 053 **Modulating Immunopeptidomes through *ex vivo* Manipulation;** Marlene Heberling; Niclas Olsson; Joshua Elias; *Stanford University, Stanford,.*

Poster 054 **Unbiased Solution for MS-based Immunopeptidomics with High Sensitivity and Accuracy;** Katherine Tran[1]; Hieu Tran[2]; Baozhen Shan[1]; *[1]Bioinformatics Solutions Inc, Waterloo, Canada; [2]University of Waterloo, Waterloo, Canada.*

Poster 055-ML **A New Strategy for the Global Identification and Validation of Post-Translationally Spliced Peptides with Neo-Fusion;** Zach Rolfs; Stefan Solntsev; Michael Shortreed; Brian Frey; Lloyd Smith; *University of Wisconsin - Madison, Madison, WI.*

Poster 056-TL **SILAC-based Quantitative Proteogenomics Unveil Altered MHC-associated Peptidome in Osimertinib Resistant Human Lung Adenocarcinoma;** Yue Qi; Tapan Maity; Meriam Bahta; Khoa Dang Nguyen; Constance Cultraro; Xu Zhang; Udanya Guha; *Thoracic and GI Malignancies Branch, CCR, NCI, NIH, Bethesda, MD.*

Poster 057-ML **Uncovering the Prognostic and Therapeutic Potential of N-Acetyl-Aspartyl-Glutamate Metabolism in Cancer;** Sunag Udupa[1]; Tu Nguyen[1]; Brian Kirsch[1, 2]; Ryoichi Asaka[1]; Karim Nabi[1]; Addison Quinones[1]; Jessica Tan[1]; Marjorie Antonio[1]; Felipe Camelo[1]; Ting Li[1]; Stephanie Nguyen[1]; Giang Hoang[1]; Kiet Nguyen[1]; Christos Sazeides[3]; Yao-An Shen[1]; Amira Elgogary[1]; Juvenal Reyes[1]; Liang Zhao[4]; Andre Kleensang[4]; Kaisorn Chaichana[1]; Thomas Hartung[4]; Michael Betenbaugh[2]; Suely Marie[5]; Jin Jung[1]; Tian-Li Wang[1]; Edward Gabrielson[1]; Anne Le[1]; *[1]Johns Hopkins Medicine, Baltimore, MD; [2]Johns Hopkins Whiting School of Engineering, Baltimore, MD; [3]University of Pennsylvania Perelman SOM, Philadelphia, PA; [4]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; [5]University of São Paulo, São Paulo, Brazil.*

Poster 058 **Uncovering the Role of N-Acetyl-Aspartyl-Glutamate as a Glutamate Reservoir in Cancer;** Giang Hoang[1]; Tu Nguyen[1]; Brian Kirsch[1, 2]; Ryoichi Asaka[1]; Karim Nabi[1]; Addison Quinones[1]; Jessica Tan[1]; Marjorie Antonio[1]; Felipe Camelo[1]; Ting Li[1]; Stephanie Nguyen[1]; Kiet Nguyen[1]; Sunag Udupa[1]; Christos Sazeides[3]; Yao-An Shen[1]; Amira Elgogary[1]; Juvenal Reyes[1]; Liang Zhao[4]; Andre Kleensang[4]; Kaisorn

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Chaichana[1]; Thomas Hartung[4]; Michael Betenbaugh[2]; Suely Marie[5]; Jin Jung[1]; Tian-Li Wang[1]; Edward Gabrielson[1]; Anne Le[1]; *[1]Johns Hopkins Medicine, Baltimore, MD; [2]Johns Hopkins Whiting School of Engineering, Baltimore, MD; [3]University of Pennsylvania Perelman SOM, Philadelphia, PA; [4]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; [5]University of São Paulo, São Paulo , Brazil.*

Poster 059 **The Intestinal Microbiome and its Metabolites Are Unaltered by Pathogen-Specific Monoclonal Antibodies ;** Omari Jones-Nelson[1]; Matthew Glover[1]; Andrey Tovchigrechko[1]; Taylor S. Cohen[1]; Fiona Fernandes[2]; Udaya Rangaswamy[2]; Liu Hui[2]; David E. Tabor[2]; Paul Warrener[1]; Jose Martinez[1]; Jamese Hilliard[1]; C. Ken Stover[1]; Wen Yu[1]; Gina Dangelo[1]; Sonja Hess[1]; Bret R. Sellman[1]; *[1]MedImmune, Gaithersburg, <Not Specified>; [2]Medimmune, South San Francisco, California.*

Poster 060 **Temporal Changes in Protein Abundance and Subcellular Location Reveal HL60 Cellular Response to Methotrexate Treatment;** Danielle B. Gutierrez[1]; Melissa A. Farrow[2]; Carrie E. Romer[1]; Jamie L. Allen[1]; Yuan-Wei Nei[3]; Zachary Jenkins[1]; Matthew Hensen[5]; KayCei Moton-Melancon[2]; Tina Tsui[1]; James C. Pino[1]; Michael Ripperger[1]; Nicole D. Muszynski[1]; Salisha Hill[1]; Kristie L. Rose[1]; Randi L. Gant-Branum[4]; Stacy D. Sherrod[1]; Carlos F. Lopez[1]; John A. McLean[1]; John P. Wikswo[1]; D. Borden Lacy[2]; Eric P. Skaar[2]; Jeremy L. Norris[1]; Richard M. Caprioli[1]; *[1]Vanderbilt University, Nashville, TN; [2]Vanderbilt University Medical Center, Nashville, TN; [3]Quest Diagnostics, Chantilly, VA; [4]Lackland Airforce Drug Testing Laboratory, Lackland AFB, TX; [5]Tempus, Chicago, IL.*

Poster 061 **Multi-omic Profiling of TKI Resistant K562 Cells Suggests Metabolic Reprogramming to Promote Cell Survival;** Laura Marholz; *University of Minnesota, Minneapolis, MN.*

Poster 062 **Integrative Proteomics for Non-Canonical Protein and Proteome Discovery using the Proteome Generator;** Paolo Cifani; Zining Chen; Avantika Dhabaria; Akihide Yoshimi; Abdel-Wahab Omar; John T. Poirier; Alex Kentsis; *Sloan-Kettering Institute, New York, NY.*

Poster 063-ML **Coordination between TGF-β Cellular Signaling and Epigenetic Regulation during Epithelial to Mesenchymal Transition;** Congcong Lu[1]; Simone Sidoli[1]; Katarzyna Kulej[1]; Karen Ross[2]; Cathy H Wu[2]; Benjamin A Garcia[1]; *[1]University of Pennsylvania, Philadelphia, PA; [2]University of Delaware, Newark, DE.*

Poster 064 **Hdac4 Interactions in Huntington's Disease Viewed through the Prism of Multiomics;** Joel Federspiel; Todd Greco; Ileana Cristea; *Princeton University, Princeton, NJ.*

Poster 065 **A Multi-omic Approach Identifies DLK1 as a Candidate Oncoprotein and Immunotherapeutic Target in High-Risk Neuroblastoma;** Amber K. Weiner[1, 2]; Alexander B. Radaoui[1]; Simone Sidoli[2]; Karina L. Conkrite[1]; Zalman Vaksman[1]; Komal S. Rothi[1]; Pichai Raman[1]; Jo Lynne Rokita[1]; Tina Glisovic-Aplenc[1]; Dan Martinez[1]; Tricia Bhatti[1]; Matthew Tsang[1]; Bruce Pawel[1, 2]; Benjamin A. Garcia[2]; John M. Maris[1, 2]; Sharon J. Diskin[1, 2]; *[1]Children's Hospital of Philadelphia, Philadelphia, PA; [2]University of Pennsylvania, Philadelphia, PA.*

Poster 066 **Network Integration of Omics Data for Fast-track Identification of the Mechanisms of Action for Drug Candidates;** Akos Vertes[1]; Albert Arul[1]; Peter Avar[1]; Andrew Korte[1]; Lida Parvin[1]; Ziad Sahab[1]; Deborah Bunin[2]; Merrill Knapp[2]; Andrew Poggio[2]; Carolyn Talcott[2]; Brian Davis[3]; Christine Morton[3]; Christopher Sevinsky[3]; Maria Zavodsky[3]; *[1]George Washington University, Washington, DC; [2]SRI International, Menlo Park, CA; [3]GE Global Research, Niskayuna, NY.*

Poster 067 **UniProt Genomic Mapping for Deciphering Functional Effects of Missense Variants;** Peter McGarvey[1]; Andrew Nightingale[2]; Hongzhan Huang[3]; Maria Martin[2]; Cathy Wu[3]; UniProt Consortium[2]; *[1]Georgetown University Med Center, Washington, DC; [2]European Bioinformatics Institute (EMBL-EBI), Hinxton, Cambridge, UK; [3]University of Delaware, Newark, DE.*

Poster 068 **Developing an Integrated Global, Glyco-, and Phosphoproteomic Workflow for Large Scale Analysis of Tissue Samples;** Yangying Zhou; Tung-Shing Mamie Lih; Ganglong Yang; Shao-Yung Chen; Lijun Chen; Hui Zhang; Qing Kay Li; *Johns Hopkins Medical Institutions, Baltimore, MD.*

Poster 069 **Mathematic, Machine Learning Methods on DESI Mass Spectrometry of Brain Tissue;** Austin Ahlstrom; *Brigham Young Univ., Provo, UT, Provo,.*

Poster 070-TL **An Integrated Multi-omic Analysis in iPSC-derived Motor Neurons from C9ORF72 ALS Patients;** Victoria Dardov[1]; Ryan Lim[2]; Vidya Venkatraman[1]; Jie Wu[2]; NeuroLINCS Consortium[3]; Leslie Thompson[2]; Clive Svendsen[1]; Jennifer Van Eyk[1]; *[1]Cedars Sinai Medical Center, Los Angeles, CA; [2]University of California, Irvine, Irvine, CA; [3]NIH, Bethesda, MD.*

Poster 071-ML **Host-centric Stool Proteomics Reveals Latent-phase-expressed Host Protease Inhibitors Modulate EAE Severity;** Carlos Gonzalez[1]; Stephanie Tankou[2]; Laura Cox[2]; Howard Weiner[2]; Josh Elias[1]; *[1]Stanford University, Stanford, CA; [2]BWH, Harvard School of Medicine, Boston, MA.*

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Poster 072 **Investigating Modified Lipid Metabolism in Brain Tissue Associated with Varying Isoforms of Apolipoprotein in Relation to Alzheimer's Disease;** John Holman; Peter Jones; Monique Speirs; Russell Denton; John Price; *Brigham Young University, Provo, NV.*

Poster 073-ML **Proteomic Analysis of the Developing Inner Ear in *Xenopus laevis*;** Aparna B. Baxi[1, 2]; Sally A. Moody[1]; Peter Nemes[1, 2]; *[1]George Washington University, Washington, DC; [2]University of Maryland, College Park, MD.*

Poster 074 **Identification and Validation Protein Phosphorylations Regulating Synapse Loss in Schizophrenia;** Matthew Macdonald[1]; Megan Garver[1]; Ying Ding[1]; David Lewis[1]; Nathan Yates[2]; Robert Sweet[1]; *[1]University of Pittsburgh, Pittsburgh, Pennsylvania; [2]University of Pittsburgh BioMS Center, Pittsburgh, Pennsylvania.*

Poster 075-ML **Understanding Epigenome and Proteome Remodeling Caused by Novel Germline Histone H3.3 Mutations during Neurodevelopment;** Khadija Wilson[1]; Geoffrey Dann[1]; Elizabeth J. Bhoj[2]; Hakon H. Hakonarson[2]; Benjamin A. Garcia[1]; *[1]University of Pennsylvania School of Medicine, Philadelphia, PA; [2]Children's Hospital of Philadelphia, Philadelphia , PA.*

Poster 076-TL **Measuring Parkison's Disease Mitochondrial Protein Turnover Rates in Human IPSC-Derived Organoids by Mass Spectrometry;** Anthony Duchesne; Nguyen-Vi Mohamed; Wei Yi; Jean Francois Trempe; *McGill University, Montréal, Canada.*

Poster 077-ML **Early Candidate Urine Biomarkers for Detecting Alzheimer's Disease before Amyloid-β Plaque Deposition in an APP (swe)/PSEN1dE9 Transgenic Mouse Model.;** Fanshuang Zhang[1]; Jing Wei[2]; Xundou Li[1]; Chao Ma[3]; Youhe Gao[2]; *[1]Basic Medicine Peking Union Medical College, Beijing, China; [2]Beijing Normal University, Beijing, China; [3]Chinese Academy of Medical Sciences, Beijing, China.*

Poster 078 **Single Step Protein Extraction from Trace-amount Human Hair for Genetically Variant Peptide Detection;** Zheng Zhang; Meghan Burke; William Wallace; Yuxue Liang; Sergey Sheetlin; Yuri Mirokhin; Dmitrii Tchekhovskoi; Stephen Stein; *NIST, Gaithersburg, MD.*

Poster 079-WTT **Spray-Capillary: An Electrohydrodynamic Spray Assisted Device for Quantitative Ultra-Low Volume Extraction;** Lushuang Huang; Zhe Wang; Si Wu; *University of Oklahoma, Norman, OK.*

Poster 080-WTT **Total Solubilization of FFPE Samples for High Throughput Clinical Proteomics;** John P. Wilson[1]; Ilyana Ilieva[2]; Darryl J. Pappin[1, 3]; John B. Wojcik[2]; *[1]ProtiFi, LLC, Farmingdale, NY; [2]University of Pennsylvania, Philadelphia, PA; [3]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.*

Poster 081 **Evaluation of a Novel LC System that Embeds Analytes in Pre-formed Gradients for Rapid, Ultra-Robust Proteomics;** Nicolai Bache[1]; Philipp Geyer[2]; Dorte Bekker-Jensen[3]; Ole Hoerning[1]; Lasse Falkenby[1]; Peter Treit[2]; Sophia Doll[2]; Igor Paron[2]; Florian Meier[2]; Jesper Olsen[3]; Ole Vorm[1]; Matthias Mann[2]; *[1]Evosep, Odense, Denmark; [2]Max Planck Institute of Biochemistry, Martinsried, Germany; [3]University of Copenhagen, Copenhagen, Denmark.*

Poster 082 **PASEF on a TIMS-QTOF Instrument Is Reproducible, Sensitive and High-Throughput for Shotgun Proteomic Workflows;** Heiner Koch[3]; Tharan Srikumar[4]; Marcus Lubeck[3]; Thomas Kosinski[3]; Romano Hebeler[3]; Florian Meier[1]; Christopher Adams[2]; Scarlet Koch[3]; Gary Kruppa[4]; Andreas Brunner[1]; Matthias Mann[1]; *[1]Max-Planck Inst. for Biochemistry, Martinsried, N/A; [2]Bruker Daltonic, San Jose , CA; [3]Bruker Daltonic, Bremen, Germany; [4]Bruker Daltonic, Billerica, MA.*

Poster 083-WTT **30 Second Analysis of Histone Post-Translational Modifications by Direct Infusion Mass Spectrometry;** Yekaterina Kori[1]; Simone Sidoli[1]; Mariana Lopes[2]; Zuo-Fei Yuan[1]; Hee Jong Kim[1]; Katarzyna Kulej[1]; Kevin Janssen[1]; Laura Agosto[1]; Julia Pinheiro Chagas da Cunha[2]; Benjamin A. Garcia[1]; *[1]University of Pennsylvania, Philadelphia, PA; [2]Instituto Butantan, Sao Paulo, Brazil.*

Poster 084-WTT **Kinetics of Acetone Precipitation: Optimizing Conditions to Efficiently Concentrate and Purify Protein Samples in Minutes with the ProTrap XG;** Jessica Nickerson; Alan A. Doucette; *Dalhousie University, Halifax, Canada.*

Poster 085 **Size Dependence for Protein Precipitation: Optimized Conditions for Efficient Recovery of Low-Mass Proteins in the ProTrap XG;** Venus Baghalabadi; Alan A. Doucette; *Dalhousie University, Halifax, Canada*

Poster 086 **Hands-off: Fully Automated & TMT-compatible Sample Preparation in less than 4 Hours on the PreOn Platform Employing the iST-NHS Technology;** Fabian Hosp; Doris Jansen; Nils Kulak; *PreOmics, Martinsried, Germany.*

Poster 087 **High-throughput and Robust Plasma Analysis with Capillary-flow LC and High-Resolution Accurate-Mass (HRAM) Mass-Spectrometry;** Oleksandr Boychenko[1]; Jenny Ho[2]; Christopher Pynn[1];

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Angelito Nepomuceno[3]; *[1]Thermo Fisher Scientific, Germering, Germany; [2]Thermo Fisher Scientific, Hemel Hempstead, UK; [3]Thermo Fisher Scientific, West Palm Beach, FL.*

Poster 088 **Quantifying Ubiquitination Signaling with a Chemical Proteomics Strategy;** Yunan Li; Ang Luo; Luke Erber; Yue Chen; *University of Minnesota, Minneapolis,.*

Poster 089-ML **Building an Antiviral Platform: Nuclear Protein Oligomerization as a Key Contributor to Innate Immune Response;** Tim Howard; Krystal Lum; Catherina Pan; Ileana Cristea; *Princeton University, Princeton, NJ.*

Poster 090-TL **Location, Location, Location: Using Spatial Proteomics to Uncover Functional Protein Translocations during Viral Infection;** Michelle Kennedy; Ileana Cristea; *Princeton University, Princeton, NEW JERSEY.*

Poster 091 **Identifying the Molecular Mechanisms of Sex-Specific Severity of the Middle East Respiratory Syndrome Coronavirus (MERS-CoV) using Proteomics;** Natarajan Bhanu[1]; Simone Sidoli[1]; Ranran Wu[1]; Neeltje van Doremalen[2]; Vincent Munster[2]; Angela Rasmussen[3]; Benjamin A Garcia[1]; *[1]University of Pennsylvania School of Medicine, Philadelphia, PA; [2]National Institutes of Health, Hamilton, MO; [3]Columbia University, New York, NY.*

Poster 092-TL **Antiviral Function of Mitochondrial Sirtuin 4 during Human Cytomegalovirus Infection;** Cora Betsinger; Elizabeth Rowland; Ileana Cristea; *Princeton University, Princeton, NJ.*

Poster 093 ***Developing an Analysis Pipeline for PfEMP1s in Parasites Isolated from Children Presenting with Malaria*;** Patricia Gonzales Hurtado[1]; Robert Morrison[1]; Jose M. C. Ribeiro[2]; Hussein Magale[1]; Oumar Attaher[3]; Bacary Diarra[3]; Almahamoudou Mahamar[3]; Amadou Barry[3]; Alassane Dicko[3]; Patrick Duffy[1]; Michal Fried[3]; *[1]NIAID/LMIV, Rockville, MD; [2]NIAID/LMVR, Rockville, MD; [3]MRTC-Univ of Sciences Techniques and Technologies, Bamako, Mali.*

Poster 094-TL **Dynamic Regulation of Mitochondria Morphology, Composition, Acetylation, and Function during Viral Infection;** Xinlei Sheng; Laura Murray; Ileana Cristea; *Princeton University, Princeton, NJ.*

Poster 095 **Quantitative Membrane Proteomics Analysis of Low Density and High Density Neutrophils from *Staphylococcus aureus* Infected Diabetic Mice;** Raghothama Chaerkady; Virginia Takahashi; Wen Yu; Taylor S. Cohen; Sonja Hess; *MedImmune, Gaithersburg, <Not Specified>.*

Poster 096-TL **Quantitative Proteomics Reveals Host Protein SLFN5 as a Target of HSV ICP0-mediated Ubiquitination and Degradation;** Joseph M. Dybas[1]; Eui Tae Kim[1]; Emigdio D. Reyes[1, 2]; Katarzyna Kulej[1]; Jennifer C. Liddle[1, 2]; Benjamin A. Garcia[2]; Matthew D. Weitzman[1, 2]; *[1]Children's Hospital of Philadelphia, Philadelphia, PA; [2]Perelman School of Med, University of Pennsylvania, Philadelphia, PA.*

Poster 097 **Quantitative Crotonylome Analysis Expands the Roles of p300 in the Regulation of the Lysine Crotonylation Pathway;** Mathew Perez-Neut; Huang He; Yejing Wang; Yingming Zhao; *University of Chicago, Chicago, IL.*

Poster 098 **Development of Mass Spectrometry-Compatible Peptide Biosensors to Detect Kinase Activity;** Nicole Wolter; Tzu-Yi Yang; Naomi Widstrom; Laurie Parker; *University of Minnesota-Twin Cities, Minneapolis, MN.*

Poster 099-ML **cGAS is Regulated by Phosphorylation and Acetylation during Infection with Herpes Simplex Virus 1;** Bokai Song; Krystal Lum; Ileana Cristea; *Princeton University, Princeton,.*

Poster 100 **Mapping Disulfide Linkages without Having to Detect Disulfide-linked Peptides;** Tommy K Cheung; Twyla Lombana; Marissa Matsumoto; David Arnott; *Genentech Inc, South San Francisco, CA.*

Poster 101-ML **Ethionine, Produced by Commensal *Lactobacillus reuteri*, Is Immunomodulatory, Proteogenic, and Leads to Ethylation of Human Proteins;** Daniel Röth[1]; Abby Chiang[1]; Gabriel Gugiu[1]; Christina Morra[2]; James Versalovic[2]; Markus Kalkum[1]; *[1]City of Hope, Duarte, CA; [2]Baylor College of Medicine, Houston, TX.*

Poster 102-TL **A Novel Method of Quantifying Protein Methylation Utilizing DIA-MS;** Aaron Robinson[1]; Ronald Holewinski[1]; Vidya Venkatraman[1]; Jose Mato[2]; Shelly Lu[1]; Jennifer Van Eyk[1]; *[1]Cedars Sinai Medical Center, Los Angeles, CA; [2]CIC bioGUNE, Bizkaia, Spain.*

Poster 103 **Method Development for Phosphorylation and Glycosylation Detection using Orbitrap Fusion Lumos;** Susanne B. Breitkopf; Jeffrey A. Culver; Michelle F. Clasquin; Bei Betty Zhang; Mara Monetti; *Pfizer, Inc., Cambridge,.*

Poster 104-TL **ELTA-MS: Labeling, enrichment and identification of ADP-ribosylated peptides by Mass Spectrometry;** Robert Lyle Mcpherson; Anthony Kar Lun Leung; *Johns Hopkins University, Baltimore, MD.*

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Poster 105 **Detection of BTK Activity using a Terbium-chelating Peptide Biosensor;** Naomi Widstrom[1]; Minervo Perez[1, 2]; John Blankenhorn[1]; Lindsay Breidenbach[1]; Hannah Peterson[1]; Laurie L. Parker[1]; *[1]University of Minnesota, Minneapolis, MN; [2]Purdue University, West Lafayette, IN.*

Poster 106 **Human Breast Cancer Cell Line Phosphoproteome Revealed by an Automated and Highly Selective Enrichment Workflow;** Shuai Wu; Linfeng Wu; *Agilent Technologies, Santa Clara, CA.*

Poster 107 **Ischemic Stress to Kidneys from SIRT5 Mice Is Mitigated by Succinylation Response;** Kevin Peasley[1]; Anja Holtz[2]; Nathan Basisty[2]; Takuto Chiba[1]; Birgit Schilling[2]; Sunder Sims-Lucas[1]; Eric Goetzman[1]; *[1]University of Pittsburgh, Pittsburgh, PA; [2]Buck Institute for Research on Aging, Novato, CA.*

Poster 108 **Adenoviral Proteins E1B55K and E4orf6 Use Non-Degradative Ubiquitination to Regulate Viral Late Protein Expression;** Christin Herrmann[1, 2]; Jen Liddle[1, 2]; Joseph Dybas[1, 2]; Benjamin A. Garcia[2]; Matthew Weitzman[1, 2]; *[1]Children's Hospital of Philadelphia, Philadelphia, PA; [2]University of Pennsylvania, Philadelphia, PA.*

Poster 109-ML **Lysine Benzoylation Is a Histone Mark Regulated by SIRT2;** Mathew Perez-Neut; He Huang; Di Zhang; Yingming Zhao; *University of Chicago, Chicago, IL.*

Poster 110 **Response to Variables for Mice on Caloric Restriction Experiments;** Elizabeth Wuerch; Lavender H Lin; Richard Carson; Nathan Zuniga; Eston Dunn; John Price; *Brigham Young University, Provo, UT*

Poster 112-TL **Acetylation of the Lamina Promotes the Integrity of the Nuclear Periphery and Inhibits Virus Production;** Laura Murray; Xinlei Sheng; Ileana Cristea; *Princeton University, Princeton, NJ.*

Poster 113 **Global PTM Analysis to Study Cross-Talk between Lysine Acetylome and Tyrosine Phosphoproteome in EGFR TKI Resistant Human Lung Adenocarcinoma;** Yue Qi; Tapan Maity; Xu Zhang; Udanya Guha; *Thoracic and GI Malignancies Branch, CCR, NCI, NIH, Bethesda, MD.*

Poster 113-ML **Tissue-Specific Protein Sulfhydrome Analysis in Mice as a Function of Age, Sex, and Diet;** Nazmin Bithi; Belinda Willard; Christopher Hine; *Cleveland Clinic Lerner Research Institute, Cleveland, OH.*

Poster 114-TL **Systems-Level Identification of PKA-Independent Vasopressin Signaling in Renal Epithelial Cells;** Arnab Datta; Chin-rang Yang; Raghuram Viswanathan; Mark A. Knepper; *NHLBI, NIH, Bethesda,.*

Poster 115 **Quantitative Protein Expression Biomarker Feasibility in Advanced Ovarian Cancer ;** Punit Kaur[1]; Alexzander Asea[1]; Luisa Manning[2]; Ernest Bognar[2]; Heidi Zupanc[2]; Gladice Wallraven[2]; Khalil Choucair[1]; Lance Dworkin[1]; John Nemunaitis[1]; *[1]University of Toledo, Toledo, OH; [2]Gradalis, Inc., Carrollton, TX.*

Poster 116-TL **Comparing Personalized Profiles of Host-expressed Proteins and Microbes in Human Stool Reveals Complementary Inter-Subject Distinction;** Ellen Casavant; Les Dethlefsen; Kris Sankaran; Daniel Sprockett; Susan Holmes; David Relman; Joshua Elias; *Stanford University, Stanford, CA*

Poster 117 **Development of mitoTurboID to Unveil Components of the Mitochondrial Signaling Hub Following Innate Immune Activation;** Clinton Bradfield; *SSS, LISB, NIAID,NIH, Bethesda, MD.*

Poster 118 **Protein ADP-Ribosylation and its Impact on Protein Complex Formation and Release in the LPS Activated Human Macrophage;** Casey Daniels[1]; Pauline Kaplan[1]; Clinton Bradfield[2]; Iain Fraser[2]; Aleksandra Nita-Lazar[1]; *[1]CNP, NIAID, NIH, Bethesda, N/A; [2]SSS, LISB, NIAID, NIH, Rockville, N/A.*

Poster 119-ML **Composition of the Myddosome during the Innate Immune Response;** Joseph Gillen[1]; Aleksandra Nita-Lazar[2]; *[1]NIH-NIAID, Bethesda, MD; [2]NIAID, NIH, Bethesda, MD.*

Poster 120 **Identification of Dynamic Nuclear Receptor Co-Regulatory Protein Complexes by Reverse Phase Protein Array and Mass Spectrometry;** Shixia Huang[1]; Kalpana D Acharya[2]; Sandy L. Grimm[1]; Sung Yun Jung[1]; Celetta G Callaway[1]; Kimal Pajapakshe[1]; Charles E Foulds[1]; Cristian Coarfa[1]; Marc J Tetel[2]; Dean P Edwards[1]; *[1]Baylor College of Medicine, Houston, TX; [2]Wellesley College, Wellesley, MA.*

Poster 121 **Determination of Host Cell Proteins In Antibody Preparations using PASEF on the timsTOF PRO;** Stuart Pengelley[1]; Scarlet Koch[1]; Christopher Swift[2]; Guillaume Tremintin[3]; Christopher Adams[3]; Detlev Suckau[1]; Jochen Boosfeld[1]; Gary Kruppa[2]; Michael Greig[3]; *[1]Bruker Daltonic, Bremen , Germany; [2]Bruker Daltonic, Billerica, MA; [3]Bruker Daltonic, San Jose, CA.*

Poster 122 **Multiplexed Interactomics Reveals Coordination of Proteostasis Network Remodeling and Mechanisms of Protein Quality Control;** Madison T. Wright; Lars Plate; *Vanderbilt University, Nashville, TN.*

Poster 123 **Influence of Post-Translational Modifications on Protein Stability;** Nathan Zuniga; Lavender Lin; John Price; *Brigham Young University, Provo, UT.*

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Poster 124-TL **Quantitative Interactomics to Determine Protein Quality Control Mechanisms Dictating Thyroglobulin Secretion;** Madison Wright; Lars Plate; *Vanderbilt University, Nashville, TN.*

Poster 125 **Reference Materials for Proteomic Investigations;** Ashley Beasley-Green; Lisa Kilpatrick; Mark Lowenthal; Karen Phinney; David Bunk; *NIST, Gaithersburg, MD.*

Poster 126 **SASP Atlas: A Comprehensive and Unbiased Proteomic Database of the Senescence Associated Secretory Phenotype;** Nathan Basisty[1]; Abhijit Kale[1]; Okhee Jeon[1]; Chisaka Kuehnemann[1]; Therese Payne[1]; Chirag Rao[1]; Anja Holtz[1]; Samah Shah[1]; Luigi Ferrucci[3]; Judith Campisi[1, 2]; Birgit Schilling[1]; *[1]The Buck Institute, Novato, CA; [2]Lawrence Berkeley Laboratory, Berkeley, CA; [3]National Institute on Aging, Baltimore, MD.*

Poster 127 **Physical Activity Associated Proteomics of Skeletal Muscle: Being Physically Active in Daily Life Protect Skeletal Muscle from Aging;** Ceereena Ubaida-Mohien; Marta Gonzalez-Freire; Alexey Lyashkov; Ruin Moaddel; Chee Chia; Eleanor Simonsick; Ranjan Sen; Luigi Ferrucci; *NIA/NIH, Baltimore, Maryland.*

Poster 128-TL **Multifaceted Proteomic Evaluation of Lysosome Dysfunction in Neurodegeneration via Human iPSC-Derived Neurons;** Ling Hao[1]; Ryan Prestil[1, 2]; Michael Fernandopulle[1, 2]; Stewart Humble[1]; Daniel Lee[3]; Saadia Hasan[1]; Maia Parsadanian[1]; Richard Youle[1]; Michael Ward[1]; *[1]National Inst of Neurological Disorders and Stroke, Bethesda, MD; [2]University of Cambridge, Cambridge, UK; [3]Cornell University, Ithaca, NY.*

Poster 129 **Effects of Oxidized Lipoprotein (oxLDL) on the Proteome in Retinal Pigment Epithelial Cells;** Sarka Beranova-Giorgianni; Francesco Giorgianni; *University of Tennessee Health Science Center, Memphis, TN.*

Poster 130 **Calorie Restriction Conditions May Modulate Aging Rates by Altering Ribosomal Maintenance and Quality;** Richard Carson[1]; Bradley Naylor[2]; John Price[1]; *[1]Brigham Young University, Provo, UT; [2]University of Utah, Salt Lake City, UT.*

Poster 132 **Characterization of Changes in the Insolublome with Aging and Age-related Diseases;** Xueshu Xie; Dipa Bhaumik; Kathleen Dumas; Manish Chamoli; Anja Holtz; Suzanne Angeli; Renuka Sivapatham; Julie Andersen; Gordon Lithgow; Birgit Schilling; *Buck Institute for Research on Aging, Novato, CA.*

Poster 133 **Changes in Skeletal Muscle Proteins: A Story of Splicing, Mitochondria, and Immune Function;** Ceereena Ubaida-Mohien[1]; Alexey Lyashkov[1]; Marta Gonzalez-Freire[1]; Ravi Tharakan[1]; , Michelle Shardell[1]; Ruin Moaddel[1]; Richard Semba[2]; Chee Chia[1]; Ranjan Sen[1]; Luigi Ferrucci[1]; *[1]National Institute on Aging, Baltimore, MD; [2]Johns Hopkins Medical Institute, Baltimore, MD.*

Poster 134-TL **Using Protein Stability as a Matric of Protein Quality in Protein Homeostasis;** Lavender Hsien-Jung Lin; Nathan Zuniga; Joseph Creery; Marcus Hadfield; John Price; *Brigham Young University, Provo, UT.*

Poster 135 **Identifying ApoE Isoform-dependent Changes for Protein Turnover in the Brain;** Joseph Creery; Russell Denton; John Price; *Brigham Young University, Provo, UT.*

Poster 136-ML **Proteomic Approaches to Study Cell Death Mechanisms in Human Stem Cell-Derived Retinal Ganglion Cells;** Joseph Mertz; Xitiz Chamling; Ah Young Lee; Xiaoli Chang; Byoung-Kyu Cho; David Clark; Cynthia Berlinicke; Hui Zhang; Donald Zack; *Johns Hopkins Medical School, Baltimore, MD.*

Poster 137 **Body-wide Dynamics of Organ Proteomes for Health and Diseases;** Bingyun Sun; *Simon Fraser University, Burnaby, Canada.*

Poster 138-WTT ***Burkholderia* Rewires its Proteome to Lower Antibiotics Sensitivities and to Support Biofilm Formation;** Mohd M. Khan[1, 5]; Supaksorn Chattagul[2]; Bao Q. Tran[3, 6]; Jeffrey A. Freiberg[4]; Aleksandra Nita-Lazar[5]; Mark E. Shirtliff[4]; Rasana W. Sermswan[2]; Robert K. Ernst[4]; David R. Goodlett[3]; *[1]University of Maryland School of Medicine, Baltimore, MD; [2]Melioidosis Research Center, Khon Kaen University, Khon Kaen, Thailand; [3]University of Maryland School of Pharmacy, Baltimore, MD; [4]University of Maryland School of Dentistry, Baltimore, MD; [5]LISB, NIAID, National Institutes of Health (NIH), Bethesda, MD; [6]U.S. Army Edgewood Chemical Biological Center, Gunpowder, MD.*

Poster 139 **Rapid Diagnosis of New and Relapse Tuberculosis by Quantification of a Circulating Antigen in HIV-Infected Adults;** Jia Fan[1]; Christopher J Lyon[1]; Zhen Zhao[2]; Edward A Graviss[3]; Ye Hu[1]; *[1]Arizona State University, Tempe, AZ; [2]Weill Cornell Medicine, New York, NY; [3]Houston Methodist Research Institute, Houston, TX.*

Poster 140 **Development of a Quality Control Standard for Tandem Mass Tags (TMT) Workflows;** Jae Choi[1]; Aaron Robitaille[2]; Tabiwang Arrey[3]; Rosa Viner[2]; Julian Saba[4]; Andreas Huhmer[2]; John Rogers[1]; Ken

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Miller[2]; *[1]Thermo Fisher Scientific, Rockford, IL; [2]Thermo Fisher Scientific, San Jose, CA; [3]Thermo Fisher Scientific, Bremen, Germany; [4]Thermo Fisher Scientific, Mississauga, Canada.*

Poster 141-ML **Statistical Testing using Multiple Levels of Quantitative Information in DIA Experiments;** Ting Huang[1]; Roland Bruderer[2]; Jan Muntel[2]; Olga Vitek[1]; Lukas Reiter[2]; *[1]Northeastern University, Boston, MA; [2]Biognosys AG, Schlieren, Switzerland.*

Poster 142 **Quantitative Analysis of the Fetal Tissue Translatome by Mass Spectrometry Reveals Temporal and Tissue-Specific Regulatory Networks *in utero*;** Josue Baeza[1]; Barbara Coons[2]; William Peranteau[2]; Benjamin A. Garcia[1]; *[1]University of Pennsylvania School of Medicine, Philadelphia, PA; [2]Children's Hospital of Philadelphia, Philadelphia, PA.*

Poster 143 **Model-Free SILAC Data Analysis Is Possible, Reproducible, and Essential;** David Chiang; Patrick Chu; *Sage-N Research, Milpitas, CA.*

Poster 144-TL **Bringing KINATEST-ID to Everyone: A Pipeline for Studying Tyrosine Kinases;** John Blankenhorn; James Johnson; Laurie Parker; *UMN, Minneapolis, MN.*

Poster 145 **Achieving Robust Deep Proteome Coverage on 2D Multiplexed Samples with the Evosep One LC System while Reducing Analysis Time;** Jonathan Krieger[1]; Leanne Wybenga-Groot[1]; Jiefei Tong[1]; Nicolai Bache[2]; Ming Tsao[3, 4]; Michael F. Moran[1, 4]; *[1]The Hospital for Sick Children, Toronto, Canada; [2]Evosep, Odense, Denmark; [3]Princess Margaret Cancer Centre, Toronto, Canada; [4]University of Toronto, Toronto, Canada.*

Poster 146-TL **Global Quantification of Proteome and Phosphoproteome Revealed Novel Cellular Signaling Mechanisms Responsive to Hypoxia and Iron Deficiency;** Luke Erber; Yao Gong; Maolin Tu; Phu Tran; Yue Chen; *University of Minnesota, Minneapolis,.*

Poster 147 **Quantitative Proteomic Profiling of a Model MCF10A-KRas^{G12V} Cell Line Reveals Dysregulated Pathways;** Jian-Jiang Hao[1, 2]; Mason Tao[1, 2]; *[1]Poochon Scientific, Frederick, MD; [2]Poochon Scientific, Frederick, Maryland.*

Poster 148-TL **Quantitative Top-Down Proteomics in Complex Samples using Protein-Level Tandem Mass Tag (TMT) Labeling;** Dahang Yu[1]; Zhe Wang[1]; Qiang Kou[2]; Kenneth Smith[3]; Xiaowen Liu[2]; Si Wu[1]; *[1]University of Oklahoma, Norman, OK; [2]Indiana University-Purdue University Indianapolis, Indianapolis, IN; [3]Oklahoma Medical Research Foundation, Oklahoma City, OK.*

Poster 149-ML **Proteomic Characterization of the Spemann Organizer in *Xenopus laevis* (frog) Embryos;** Vi Quach; Aparna Baxi; Peter Nemes; *University of Maryland, College Park, MD.*

Poster 150 **Processed by Angiotensin-Converting Enzyme: A Plethora of Non-Angiotensin-I Peptide Substrates and Products;** Margarita Semis[1]; Gabriel Gugiu[1]; Ellen A Bernstein[2]; Kenneth E Bernstein[2]; Markus Kalkum[1]; *[1]City of Hope, Duarte, CA; [2]Cedars-Sinai Medical Center, Los Angeles, CA.*

Poster 151 **Targeted Proteomics-Driven Computational Modeling of the Mouse Macrophage Toll-like Receptor Signaling Pathway;** Nathan Manes; Jessica Calzola; Pauline Kaplan; Martin Meier-Schellersheim; Iain Fraser; Aleksandra Nita-Lazar; *National Institutes of Health, Bethesda, MD.*

Poster 152-TL **Mechanical Stimulation Induces Rapid Phosphorylation-dependent Signaling in *Xenopus* Embryos;** Yutaka Hashimoto[1, 2]; Noriyuki Kinoshita[2]; Todd Greco[1]; Joel Federspiel[1]; Pierre Jean Beltran[1]; Naoto Ueno[2]; Ileana Cristea[1]; *[1]Princeton University, Princeton, NJ; [2]National Institute for Basic Biology, Okazaki, Japan.*

Poster 153-ML **Systems-Wide Hijacking of Host Cells during Herpes Simplex Virus (HSV-1) Infection;** Katarzyna Kulej[1]; Ashley N. Della Fera[1]; Eui Tae Kim[1]; Matthew J. Charman[1]; Simone Sidoli[2]; Benjamin A. Garcia[2]; Matthew D. Weitzman[1]; *[1]Children's Hospital of Philadelphia, Philadelphia, PA; [2]University of Pennsylvania School of Medicine, Philadelphia, PA.*

Poster 154-TL **Towards Elucidation of Muscle-Specific Receptor Tyrosine Kinase (MuSK) Signaling Pathway by Differential Agonists;** Hanna Budayeva; Arundhati Sengupta Ghosh; Lilian Phu; Donald Kirkpatrick; *Genentech Inc., South San Francisco, CA.*

Poster 155 **Applications of Mass Spectrometry Targeted Assays for Quantitative Analysis of Cancer Signaling Proteins;** Penny Jensen[1]; Bhavin Patel[1]; Leigh Foster[1]; Aaron Gajadhar[2]; Sebastien Gallien[2]; Jonathan R. Krieger[3]; Jiefei Tong[4]; Ming S. Tsao[5]; Michael F. Moran[3]; Rosa Viner[2]; Andreas Huhmer[2]; Kay Opperman[1]; John Rogers[1]; *[1]Thermo Fisher Scientific, Rockford, IL; [2]Thermo Fisher Scientific, San Jose, CA; [3]SPARC Biocentre, The Hospital for Sick Children, Toronto, Canada; [4]Program in Cell Biology, The Hospital for Sick Chi, Toronto, Canada; [5]Departments of Laboratory Medicine and Pathobiolog, Toronto, Canada.*

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Poster 156-TL **Global Immunoproteomic Profiling of Endotoxin-stimulated Macrophages Uncovers Specifics of TLR4- and Caspase11- mediated Recognition;** Orna Rabinovich Ernst[1]; Mohd M. Khan[2]; Benjamin Oyler[2]; Jing Sun[1]; Nathan Manes[1]; Iain Fraser[1]; Aleksandra Nita-Lazar[1]; David Goodlett[2]; *[1]National Institutes of Health, BETHESDA, MD; [2]University of Maryland, Baltimore, MD.*

Poster 157 **Epidermal Growth Factor-Induced Phosphorylation Responses in Rat Inner Medullary Collecting Duct;** Chung-Lin Chou; Mark Knepper; *National Institutes of Health, Bethesda,.*

Poster 158 **Single-cell mass spectrometry for proteomic analysis of patch-clamp electrophysiology identified dopaminergic neurons;** Sam Choi[1]; Abigail Polter[2]; Peter Nemes[1]; *[1]University of Maryland, College Park, MD; [2]George Washington University, Washington Dc, DC.*

Poster 159-ML **Proteoform Family Identification and Quantification using Proteoform Suite;** Leah V. Schaffer[1]; Michael R. Shortreed[1]; Anthony J. Cesnik[1]; Jarred W. Rensvold[2]; Adam Jochem[2]; Trisha Tucholski[1]; Mark Scalf[1]; Brian L. Frey[1]; Ying Ge[1]; David J. Pagliarini[1, 2]; Lloyd M. Smith[1]; *[1]University of Wisconsin-Madison, Madison, WI; [2]Morgridge Institute for Research, Madison, WI.*

POSTER ABSTRACTS

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Poster 001-ML: Multiplexed Quantification Strategy for Candidate Biomarker Discovery and Verification in Alzheimer's Disease

Xiaofang Zhong; Qinying Yu; Fengfei Ma; Dustin Frost; Lingjun Li
University of Wisconsin-Madison, Madison, WI

Absolute quantification in targeted proteomics is challenging due to a variety of factors, including low specificity in complex backgrounds, limited analytical throughput, and wide dynamic range. To address these problems, we developed a hybrid offset-triggered multiplex absolute quantification (HOTMAQ) strategy that combines cost-effective mass difference and isobaric DiLeu (N,N-dimethyl leucine) tags to enable simultaneous construction of an internal standard curve in the MS1 precursor scan, real-time identification of peptides at the MS2 level, and mass offset-triggered accurate quantification of target proteins in synchronous precursor selection (SPS)-MS3 spectra. This approach increases the analytical throughput of targeted quantitative proteomics by up to 12-fold. The HOTMAQ strategy was employed to verify candidate protein biomarkers in preclinical Alzheimer's disease (AD) with high accuracy. The greatly enhanced throughput and quantitative performance, paired with sample flexibility, makes HOTMAQ broadly applicable to targeted peptidomics, proteomics, and post-translational modifications.

Besides of protein biomarkers, the glycosylation-based biomarker is another promising direction, as glycosylation is crucial for many brain functions, including learning and memory. In our quantitative N-glycoproteome study, cerebrospinal fluid (CSF) samples from 48 healthy controls, patients at mild cognitive impairment (MCI) and AD dementia were compared by 12-plex isobaric DiLeu labeling strategy. After enrichment, above 2000 N-glycopeptides corresponding to 112 N-glycoproteins were quantified in Fusion Lumos. The majority of proteins in CSF are either fucosylated or sialylated. Through the integrated proteomic and glycoproteomic study, we demonstrated N-glycoproteins could be candidate biomarkers in Alzheimer's disease.

Poster 002: A Multiple Reaction Monitoring Assay for Simultaneous Quantification of Mitochondrial Proteins in Mouse

Alexsandr Stotland; Weston Spivia; Amanda Orosco; Allen Andres; Jennifer Van Eyk; Roberta Gottlieb; Sarah Parker
Cedars Sinai Medical Center, Los Angeles, CA

Mitochondria are the major source of cellular energy (ATP), as well as critical mediators of widespread functions such as cellular redox balance, apoptosis, and metabolic flux. Mitochondrial dysregulation occurs in a variety of cardiovascular pathologies, including atherosclerosis, heart failure, and vascular aneurysms. Mitochondrial protein abundance is a key indicator of mitochondrial quantity as well as composition. Methods to quantify mitochondrial proteins are limited to low throughput immunoassays or untargeted, relative quantification by shotgun data-dependent or data-independent mass spectrometry. We developed a targeted, scheduled multiple reaction monitoring assay, termed 'Mitoplex', to enable the precise and focused quantification of a set of 32 mitochondrial proteins critical to central carbon chain metabolism and overall mitochondrial function. Mitoplex quantification of proteins was reproducible and sensitive in both mitochondrial enrichments and total lysates from cultured cell lines (mouse C2C12 skeletal muscle myoblasts) and tissue (mouse soleus muscle). Further, the Mitoplex replicated and extended previous observations of a large increase in mitochondrial protein expression during the C2C12 myoblast to myotube differentiation process. We then quantified a novel reduction in mitochondrial protein expression following exposure of C2C12 cells to the HMG-CoA reductase inhibitor simvastatin, which was further associated with a failure of myoblasts to fully differentiate and a reduction in overall mitochondrial ATP production. Finally, the Mitoplex quantified widespread reduction of skeletal muscle protein abundance in vivo following treatment of mice with simvastatin. We conclude that Mitoplex is a focused, sensitive, and high-throughput quantitative tool to monitor mitochondrial protein content. We've demonstrated its the biological relevance by characterization of mitochondrial protein depletion as a potential mechanism driving the skeletal muscle myopathy following statin treatment, which may ultimately aid in identification of ways to prevent this debilitating drug side effect in humans.

Poster 003: A Novel UHPLC-MRM-MS Methodology for Accurate, Reproducible, and Fast Quantification of Histone PTMs

Joseph Cesare; Zuofei Yuan; Steven Zhao; Peder Lund; Yekaterina Kori; Simone Sidoli; Josue Baeza; Hee Jong Kim; Kathryn E. Wellen; Benjamin A. Garcia
University of Pennsylvania, Philadelphia, PA

Histones are structural proteins that modulate chromatin structure and gene expression. Their post translational modifications (PTMs) recruit transcription factors and directly affect chromatin state through chemical interactions. Aberrant levels of these modifications are found in many diseases such as over methylation of Histone 3 Lysine 4 (H3K4) and Histone 3 Lysine 27 (H3K27) in acute myeloid leukemia (AML) and multiple lineage leukemia (MLL) (Chi et al, Nature Reviews Cancer 2010). The ability to quantify these changes reliably, at low costs, and in high throughput experiments will allow for large scale studies and has the potential to revolutionize clinical diagnostics. Mass spectrometry (MS) is the method of choice for this analysis; however, it requires expensive instrumentation, each sample requires at least one hour of run time, and the use of nano-liquid chromatography is susceptible to batch effects. To address these limitations, we coupled analytical flow ultra-high-pressure liquid chromatography (UHPLC) to a triple quadrupole mass spectrometer using multiple reaction monitoring (MRM) acquisition mode to quantify 93 peptides and 80 known PTMs. Our method obtained highly reproducible results with a median coefficient of variation 3.9% in 20 minutes using a heavy labeled peptide library. This method was applied to human derived cell lines and mouse liver tissue and demonstrated more accurate quantification than nano-liquid chromatography coupled to data independent acquisition (DIA). For quality control and correction for batch effects, we included a peptide to indicate efficient digestion and chemical derivatization in each analysis. Furthermore, we corrected for ionization biases. The data analysis was integrated into our EpiProfile 2.0 software and found to be comparable to data analysis completed through manual inspection and Skyline software. Our new UHPLC-MRM-MS method has reduced the analysis time and increased reproducibility and accuracy in quantification opening new opportunities for translational medicine and clinical diagnostics.

Poster 004-TL: P4Ha1 Hydroxylation of Bradykinin Allows Blood-based Characterization of Tumor Hypoxia

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Tumor hypoxia is linked to poor prognosis due to its role in promoting cancer progression and metastasis. Evaluating tumor hypoxia to predict patient outcomes is challenging, however, due to the lack of rapid, accurate and non-invasive methods. Hypoxia positively regulates the α1 subunit of prolyl-4-hydroxylase (P4H) in several tumors, and P4Hα1 expression is involved in hypoxia-inducible modification of the plasma protein bradykinin (BK) to hydroxyprolyl BK (Hyp-BK), leading us to hypothesize tumor hypoxia could be detected

by plasma Hyp-BK analysis. Here we show that P4Hα1 expression is selectively increased in malignant vs. non-malignant pancreatic cancer tissue, corresponding to tumor expression of Carbonic Anhydrase 9, a reported hypoxia marker. Hyp-BK/BK ratios in pre-treatment plasma samples of pancreatic cancer patients are also inversely correlated with their response to treatment, suggesting that plasma Hyp-BK/BK may have value as a non-invasive, surrogate marker for tumor hypoxia and disease prognosis in response to anticancer therapy.

Poster 005-ML: Utilizing Parallel Reaction Monitoring for a High Throughput Diagnostic Pipeline to Establish Cardiac Troponin I Phosphorylation as a Biomarker

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Introduction and Objectives

Cardiac Troponin I (cTnI) is the preferred diagnostic biomarker for various myocardial diseases. Phosphorylation of specific Ser/Thr residues on cTnI are relevant for several pathological conditions. In heart failure (HF) phosphorylation of residues S22 and S23 are reduced, whereas residues T143 and S199 are increased and are associated with decreased force generation and hypertrophic cardiomyopathy, respectively. This study focuses on introducing a highly sensitive, high throughput quantitative screening for cTnI, and its disease-associated posttranslational modifications in blood samples by mass spectrometric analysis.

Methods

An automated robotic high throughput intact protein immunoprecipitation assay was developed and optimized for identification and quantification of cTnI phosphorylation in patient plasma. Targeted approaches on different MS instruments (Qtrap 6500, Obitrap Elite and Orbitrap Fusion Lumos) using alternate flow velocities (high, low and nano) were tested for optimal sensitivity.

Results and Discussion

Multiple factors in cTnI immunoprecipitation workflow were tested and optimized manually using recombinant cTn-complex in a cTnI depleted plasma matrix, e.g., optimal antibody concentrations, pull down duration, bead washing and the elution process. An established parallel reaction monitoring method on the Orbitrap Fusion Lumos was proven to provide the desired sensitivity to measure cTnI phosphorylation in plasma from patients with myocardial infarcts. Further optimization of automated antibody based cTnI enrichment from actual undiluted patient plasma led to alterations in the workflow of the automated IP assay, e.g., increased antibody-antigen incubation period, reduced washing and increased elution periods.

Conclusion

An automated high-throughput assay was successfully established that can quantify total cTnI and phosphorylation on residues S22, S23, T143 and S199 using a targeted MS approach in patient plasma samples with approximately 90% accuracy for total cTnI when compared to an ELISA based method.

Poster 006-TL: Top-Down Proteogenomics Analysis of Serum Autoantibody Repertoire for the Discovery of Biomarker of Systemic Lupus Erythematosus

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Systemic Lupus Erythematosus (SLE) is a multi-organ, systemic autoimmune disorder, affecting over 1.5 million Americans. The hallmark of SLE is the production of serum autoantibodies that are directly pathogenic, eventually causing organ damage and early mortality. Autoantibodies accrue years before clinical symptoms arise which may allow early diagnosis and treatment. While current diagnostic platforms can screen total autoantibodies, finding specific autoantibody biomarkers is impossible. Top-down proteomics analyzes intact proteoforms with sequence variations and post-translational modifications, holding great potential to analyze serum autoantibodies of high similarity and diversity. However, there are two major challenges: (1) The serum autoantibody repertoire is extremely complexed (*e.g.,* a wide range of highly homologous autoantibodies), requiring advanced separation approaches; (2) Personalized autoantibody database must be generated due to high diversity of autoantibody repertoire.

Herein, we developed a top-down proteogenomics platform integrating (1) a long-column ultra-high-pressure RPLC to efficiently separate highly homologous autoantibodies based on the hydrophobicity differences from sequence variations; (2) a personalized protein database from the B-cell next-generation sequencing. 86 Fab-related mass features were identified in longitudinal SLE serum samples from a patient, representing first top-down demonstration of serum autoantibody repertoire analysis. Two monoclonal autoantibodies with same CDR3 sequence were identified, one of which was functional characterized to have high affinity to a known antigen SmD through the fully human recombinant antibody. These two autoantibodies may result from the somatic gene conversion. However, interestingly, they have different expression patterns over the patient's disease progress, which may relate to the affinity maturation of individual antibodies. Only CDR3 sequences (*i.e.*, possibly from bottom-up) cannot pinpoint mature autoantibodies which are potential disease biomarkers.

Our results demonstrate the potential application of top-down proteogenomics analysis of serum autoantibody repertoire. It holds great promise for discovering novel serum autoantibody biomarkers, as well as promoting our understanding of pathogenic autoimmune processes.

Poster 007: Development of an MRM Assay to Distinguish Active and LAP TGF-β in Urine

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Transforming growth factors β (TGF-β) are important cytokines that regulate cell proliferation, differentiation, and other biological processes. After cleavage of the signal peptide, they form latent TGF-β complexes in which TGF-β is noncovalently bound to latency-associated peptide (LAP). TGF-β mediates immune and anti-inflammatory responses. A method to distinguish between active TGF-β and LAP would provide critical insight into biological/disease processes; however, to the best of our knowledge, there are currently no mass spectrometry (MS)-based assays that differentiate these forms. Herein we have developed a multiple reaction monitoring (MRM) assay to distinguish active TGF-β and LAP.

A critical evaluation of sample solubility, reduction, and alkylation was performed in TGF-β-spiked urine and sample preparation conditions were selected to prevent protein precipitation and encourage complete carbamidomethylation of nine cysteines. Urine was obtained from normal and chronic kidney disease (CKD) donors and following optimized reduction and alkylation, the proteins were fractionated using a C3 column (acidic HPLC conditions). Fractions containing total active TGF-β and total LAP were spiked with iRT peptides and digested with Lys-C followed by trypsin. Relative quantitation was performed using isotopically-labeled ($^{13}C^{15}N$) synthetic standards to peptide targets from both active TGF-β and LAP.

All posters will be displayed Monday and Tuesday.
Each day: odd-numbered posters present 1:30-2:15 pm; even-numbered posters present 2:15-3:00 pm.
ML = Mon. lightning talk; TL = Tues. lightning talk; WTT = Wed. tips & tricks talk.

The LC-MS/MS method is a robust method for the optimized detection of total active TGF-β and total LAP in normal and CKD donor urine. Active TGF-β and LAP levels were markedly elevated in the urine of CKD donors versus normal donors. The detection and quantitation of LAP was particularly critical to this project since previously, an ELISA assay was used to detect and quantitate active TGF-β and the presence of LAP in urine was unconfirmed. Current investigations now involve measuring fibrosis levels in urine donor samples and correlating to active TGF-β levels.

Poster 008-TL: Signatures of Ambient Exposure to Benzene and other Air Pollutants in the Human Serum Albumin Cys^{34} Adductome

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Benzene and other components of outdoor air pollution are human carcinogens. However, airborne concentrations of pollutants may not accurately predict exposure or internal dose. Covalent adducts to the Cys^{34} thiol in human serum albumin (HSA) can serve as internal dosimeters, while the $t_{1/2}$ of HSA facilitates biomonitoring of the previous ~3 months. Analyzing the HSA adductome can simultaneously reveal unique biomarkers for many pollutants in a complex mixture. We have used Cys^{34} adductomics to characterize longitudinal exposures in Qidong, China, which experiences substantial airborne pollution and a rising incidence of lung cancer. Non-smoking women (n=8) from the placebo arm of a published clinical chemoprevention trial were selected for this pilot study. HSA was precipitated from serum collected at baseline (day 0) and day 56, digested with lysyl endopeptidase (LysC), and Cys^{34}-adducted peptides were separated and quantified by online nanoflow LC interfaced with a Thermo Scientific Orbitrap Fusion Tribrid mass spectrometer. We detected four Cys^{34} adducts arising from three benzene metabolites: benzoquinone (BQ; BQ1, BQ2), benzene oxide (BO), and benzene diolepoxide (BDE). Incubation of control serum with 1,4-BQ *in vitro* demonstrated dose-dependence of BQ adducts and that they may reflect keto-enol tautomerism of a 1,4-BQ-derived adduct, but do not arise from 1,2-BQ. Along with benzene-derived adducts, we identified other putative adducts (ethylene oxide, nitriles, aldehydes, oxidation, disulfides) and revealed shifts in the Cys^{34} adductome during the 8-week follow-up: decreased BQ2 (p=0.08), BDE (p=0.06), acrylonitrile (p=0.08), homocysteine (p=0.05), and cysteinylglycine (p=0.08), but increased unassigned adducts (p=0.02). To our knowledge, this is the first report of benzene-derived adducts in humans with ambient exposures, detected with an adductomics approach. Work is underway to expand sample number, exposure timeline, and adduct identification. Cys^{34} adductomics represents a promising approach for the discovery of air pollutant biomarkers and quantification of an individual's exposure to air pollutants.

Poster 009: Global Proteome and Phosphoproteome Alterations Reveal Novel Drug Targets to Circumvent 3rd-Generation EGFR TKI Resistance in Human Lung Adenocarcinoma

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Osimertinib, a 3rd generation epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) is approved for the treatment of lung adenocarcinoma patients harboring TKI-sensitizing or resistant (EGFRT790M) EGFR mutants. Here, we sought to identify novel resistance mechanisms to two 3rd-generation EGFR TKIs, osimertinib and rociletinib using SILAC-based quantitative mass spectrometry. The parental lung adenocarcinoma cell line, H1975 (EGFRL858R/T790M), and the isogenic osimertinib (AZR3,4) and rociletinib (COR1,10) resistant cells were employed in 3-5 biological replicates of global proteome and phosphoproteome estimation with/without TKIs. TiO2-enriched phosphopeptides underwent tandem mass spectrometry in QE-HF. Overall, we identified 6752 proteins and 17,646 phosphosites. The abundance of hundreds of proteins and phosphopeptides was significantly altered between the TKI-sensitive and resistant cells. Surprisingly, there was a greater degree of inhibition of phosphorylation in resistant cells, including of EGFR and the downstream MAPK pathway. Several phosphatases, including PTPN11, PTPN12, and PTPN1 were predicted to be activated in the resistant cells. Proteins involved in epithelial mesenchymal transition (EMT) were expressed more in TKI-resistant cells, suggesting EMT as a mechanism of resistance. IPA analysis of proteins with significantly altered phosphorylation identified several signaling pathways activated (such as p53, PTEN, RhoGDI, RhoA and PPAR) or inhibited (including ERBB, AMPK, ERK/MAPK, RAC) in the resistant cells. We used iPTMnet, a bioinformatic resource that integrates data from text mining of scientific literature and other PTM databases to identify upstream kinases of phosphorylation targets. AKT and PKA kinase substrates were hyper-phosphorylated in TKI-resistant cells. We also used kinase-inhibitor target data (KinomeScan) and phosphoproteomic data (P100) generated by the NIH LINCS Program to identify drugs that might overcome resistance. The TKI resistant cells were indeed sensitive (EC50:18-130nM) to one of the predicted drugs, dactolisib, a PI3K/MTOR inhibitor. In vivo studies are underway in mouse models to demonstrate the efficacy of dactolisib in circumventing TKI resistance.

Poster 011: Identifying Breast Cancer Vulnerabilities by Mapping Interactome Dysregulations in Primary Tumor Samples

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The promise of cancer precision medicine is to treat patients based on the specific molecular properties of their tumors. However, identifying patient-specific tumor vulnerabilities still remains an enormous challenge. We have developed a novel technology that allows mapping dysregulations of protein-protein interactions (PPI) in an interactome-wide manner. The method is based on (i) high-throughput quantitative proteome mapping of tumor samples using TMT-11 reagents, and MS2/SPS-MS3 method on Orbitrap Fusion and Lumos instruments, (ii) identification of PPIs through protein co-regulation analysis, and (iii) mapping of PPI dysregulations through monitoring sample specific deviations of protein-protein co-regulations to identify tumor vulnerabilities. Our previous work with cancer cell lines has shown the high potential of this approach, and we have now applied the method in 320 samples of primary breast cancer tumors (254) and normal breast tissue (66) received from the MGH breast cancer tumor bank. The samples represent all subtypes according to the Perou classification – luminal A, luminal B, HER2+, and triple-negative (TNBC). To our knowledge this is currently the largest proteomics dataset of primary tumor samples. Across all 300 samples, we have quantified more than 11,000 proteins with an average of 7,995 proteins quantified for each sample. Protein concentration co-regulation analysis resulted in identifying more than 20,000 protein-protein association. PPI dysregulation analysis revealed an average

of more than 200 dysregulated interactions tumor. We will present tumor and subtype-specific vulnerabilities and therefore potential targets in subtype-specific treatment strategies. We found that the identified interactome dysregulation include pathways known to be perturbed in the specific pathways based on the known subtype-specific driver mutations. We will also present a strategy to identify novel treatment target candidates by overlaying interactome dysregulation data from tumors and cancer cell lines, which vulnerabilities have been mapped by genome-wide genetic drop-out screens.

Poster 012: A Novel Role α (1,6) Fucosyltransferase, a Glycosylated Related Enzyme in the Biology of Castration Resistant Prostate Cancer

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Glycosylation is recognized as one of the most common modifications on proteins. Recent studies have shown that aberrant expression of α (1,6) fucosyltransferase (FUT8), which catalyzes the transfer of fucose from GDP-fucose to core-GlcNAc of the N-linked glycoproteins, modulates cellular behavior that could lead to the development of aggressive prostate cancer. While the relationship between the abnormal expression of FUT8 and glycoprotein fucosylation in different prostate cancer cells has been demonstrated, there is no evidence that shows dysregulated fucosylation might be involved in prostate cancer progression from androgen-dependent to castration-resistant disease. In this study, using a proteomics approach, we analyzed androgen-dependent and androgen-resistant LAPC4 cells and identified FUT8 to be significantly overexpressed in the androgen-resistant LAPC4 cells. These findings were independently confirmed in LAPC4 cells that were treated with non-steroidal anti-androgen (bicalutamide) and in the *in vivo* castrated tumor xenograft models. We further evaluated whether FUT8 expression alone might be responsible for the androgen resistant phenotype, by exogenously overexpressing FUT8 in prostate cancer cells, we found a substantial decreased in cellular PSA expression that was consistent with FUT8 expression and PSA production in prostate cancer specimens. To our knowledge, this is the first study reporting the functional role of fucosylated enzyme in the development of castration-resistant prostate cancer.

Poster 014-WTT: Integrating Kinetic and Quantitative Proteomics to Investigate Autophagy Substrates in Tumors

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The activation of the catabolic pathway autophagy is strongly connected to cancer stress tolerance and chemoresistance. Pharmacological inhibition of autophagy is a potential strategy to sensitize tumors to traditional chemotherapeutics. Current clinical trials are attempting to control autophagy flux using broad-spectrum inhibitors with limited knowledge of the side effects of these drugs on protein homeostasis in healthy surrounding tissues. We used D_2O-based metabolic labeling and mass spectrometry combined with standard quantitative proteomics techniques to measure changes in protein degradation in a xenograft mouse model upon autophagy inhibition. We found that autophagy substrates differ in the tumor versus liver tissue. Our results suggest that autophagy is used to selectively degrade mitochondria (mitophagy) in tumor cells, perhaps to regulate mitochondrial function and biogenesis. In the liver, autophagy is used broadly as a bulk degradation system to maintain global proteostasis. These experiments support a model of modified mitochondrial metabolism, enforced in part by rapid autophagic degradation, as a pro-survival mechanism in cancer. We also observed off-target effects in the liver consistent with a toxicity response to treatment. These methods are directly applicable to humans and could test for subject-specific differences in autophagy targeting and guide the development and translation of substrate-specific autophagy inhibitors.

Poster 015: A Chemical Biology Screen Identifies a Metabolic Vulnerability of Neuroendocrine Cancer Cells to SQLE Inhibition

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Aberrant metabolism of cancer cells is well appreciated, but the identification of cancer subsets with specific metabolic vulnerabilities remains challenging. We conducted a chemical biology screen and identified a subset of neuroendocrine tumours displaying a striking pattern of sensitivity to inhibition of the cholesterol biosynthetic pathway enzyme squalene epoxidase (SQLE). Using parallel approaches, including deep proteomic profiling of 42 small cell lung cancer lines with varying degrees of sensitivity to SQLE inhibition, we were able to identify a subset of cell lines with distinct proteomic and transcriptomic signatures as being the most susceptible to SQLE inhibition. We employed mass spectrometry-based metabolomics analyses to demonstrate that sensitivity to SQLE inhibition results not from cholesterol biosynthesis pathway inhibition but from the specific and toxic accumulation of the SQLE substrate, squalene. These findings highlight SQLE as a potential therapeutic target in a subset of neuroendocrine tumors, particularly small cell lung cancers.

Poster 016: TRIM28 as a Candidate Mutant p53 Interacting Partner in Cancer Cells

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p53 is a transcription factor that is mutated in over 50% of cancers. Missense mutations in the DNA binding domain of p53 can result in a gain-of-function (GOF) phenotype, leading to increased cell proliferation and tumor formation. Our lab previously showed that prevalent mutant p53 (mtp53) forms modify chromatin through their interaction with ETS2 and activation of non-canonical transcriptional targets (MOZ, MLL1, and MLL2). Aside from ETS2, other mtp53 partners that have been identified, including Sp1, NF-Y, and PML. However, whether specific proteins are critical for the stability and the GOF effect of mtp53 remains to be seen. To this end, we developed a quantitative mass spectrometry-based strategy, combined with molecular and genomic approaches, to identify and validate novel mtp53 binding partners from cancer cell lines with varying GOF p53 mutations. Our preliminary data identified the transcriptional corepressor TRIM28 as a candidate mtp53 interacting partner, as it was identified in all 4 GOF cell lines tested (VU1365, HUPT3, MDA468, and PANC1). Knockdown of TRIM28 in MDA468 cells (contains R273H mutation in p53) caused a decrease in cell viability. TRIM28 has been shown to interact with MDM2 to promote wild type p53 ubiquitylation and degradation; however, its role in regulating mtp53 has not been determined. Ultimately, our studies will identify and validate further novel proteins critical for the GOF activity of mtp53. Characterizing these novel interacting partners of mtp53 will shed light into the molecular mechanisms underlying cancer and thus will provide new therapeutic targets to destabilize mutant p53 interactions in cancer cells.

Poster 017-ML: Detection of Malignancy-associated Proteome and Phosphoproteome Alterations in Human Colorectal Cancer Induced by Cell Surface Binding of Growth-Inhibitory Galectin-4

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Carbohydrate-binding proteins, called lectins, translate glycan-encoded information on the cell surface into effects on fundamental biological processes, including cell growth, differentiation and adhesion. One of them, galectin-4 (gal-4), exhibits tumor suppressing actions and has been shown to influence colorectal cancer tumorigenesis and progression. Accordingly, we have found that gal-4 cell surface binding induced cell differentiation and reduced proliferation in five human colorectal cancer cell lines (LS 180, Vaco 432, Colo 205, CX 1, HCT 116). In order to explore changes underlying observed shifts in growth behavior and morphology on a molecular level, SILAC-based proteomics and phosphoproteomics were applied in LS 180 cells treated with gal-4. Proteome investigation disclosed down-regulation of DNA replication-associated processes, while protein presence for secretory and transport functions appeared increased. Profound down-regulation was observed for malignancy-associated proteins, such as EPCAM (epithelial cell adhesion molecule; ~6-fold), LMNB1 (lamin-B1; ~5-fold) and common cell proliferation marker Ki-67 (~4-fold), accompanied by strongly up-regulated CALB2 (calretinin; ~24-fold) and transglutaminase TGM2 (~11-fold), among others. As a part of interactive galectin network, gal-4 caused an intracellular increase of adhesion/growth regulatory galectin-1 (~4-fold). Further phosphoproteomic analysis at two time points revealed early and sustained effects induced by gal-4. Early reaction indicated modulation of membrane organization, vesicle-mediated transport as well as microtubule-kinetochore attachment by BET1 hypophosphorylation (~3-fold) and CENPF hyperphosphorylation (centromere protein F; ~2-fold), among others. Sustained phosphorylation changes suggested the role of cytoskeleton organization and glutamine metabolism by altered phosphorylation of CFL1 (cofilin S3; ~5-fold) and glutamine transporter SLC1A5 (S503; ~3-fold), proven by significant effect of gal-4 treatment on glutamine uptake in the analyzed cell lines. Altogether, detected proteome and phosphoproteome changes are potentially relevant for the observed phenotypic shift, giving new insights into gal-4 functions and revealing the capacity of galectins to affect human colon cancer cells at multiple sites.

Poster 018: Proteomic Analysis of Uveal Melanoma Derived Exosomes

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Uveal melanoma (UM) is a rare form of non-cutaneous melanoma that develops from melanocytes in the uveal tract, forming a malignant tumor of the eye. Metastatic disease frequently involves the liver and is associated with poor outcomes – resulting almost always in death within 15 months. Recent evidence suggests that organotropic metastasis can be mediated through exosomes, which are membrane bound nanovesicles released from all cells and contain proteins, nucleic acid, and other small molecules. We have characterized the exosomal proteome in an effort to gain insight into the molecular mechanisms of metastasis. We first demonstrated successful exosomal isolation through western blotting of core exosomal proteins and visualization of these vesicles using transmission electron microscopy. We treated exosomes with crizotinib, a chemotherapeutic agent currently in clinical trials for UM, and performed comprehensive, label-free, proteomics on exosomes from the treated and untreated cells. We identified >600 proteins in total and ~150 proteins unique to each group. Based on gene ontology and literature review, we identified 16 high-value biomarker candidates and assessed their levels in exosomes derived from 92.1 (UM) cell culture media and clinical samples from UM patients. Finally, to determine the effect of exosomes and drug treatment on metastasis, we treated liver cells with exosomes isolated from cell culture media. Exosomes from untreated cells induced multiple changes in target hepatocytes, including alterations in signaling pathways affecting angiogenesis, extracellular matrix invasion, and cell-cell adhesion. These changes were abrogated when hepatocytes were treated with UM exosomes isolated after incubation with crizotinib. We demonstrate the effect of crizotinib on the selective packaging and distribution of proteins within exosomes, and develop a list of protein biomarkers potentially useful for clinical management of UM patients. Further, our studies suggest that exosomes can induce changes in hepatocytes and that crizotinib treatment dampens this response.

Poster 019-ML: Bioinformatics Approach for Understanding the Role of Intrinsic Disordered Regions in Cancer-related Proteins

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Intrinsically disordered regions (IDRs) are protein regions that do not fold into stable secondary or tertiary structure under physiological conditions. They are usually biologically active and associated with important cellular activities such as regulation, signaling, and control. IDRs have been implicated in many diseases, such as cancer, cardiovascular and neurodegenerative diseases. Given that IDRs provide structural plasticity and functional diversity, we hypothesize that proteins associated with cancer contain disordered regions which play a critical role in regulating their function. Here we collected a list of human proteins involved in cancer from the UniProt Knowledgebase and the curated cancer gene list from the Catalogue of Somatic Mutations in Cancer (COSMIC). The cancer-related proteins were screened for the presence of disordered regions using MobiDB, a database for disordered proteins. Then, we mapped potential regulatory features, such as post-translational modifications (PTMs), motifs, regions, and repeats from the individual proteins to the IDRs, and we also looked for variants or mutagenesis information on these regions. Our data suggest that a large percentage of the cancer-related proteins contain IDRs spanning at least nine amino acid residues long (~70% of 278 total proteins collected). We found that IDRs of proteins associated with cancer were rich in PTMs (mainly phosphorylated sites); their IDRs encompassed regions involved in protein interactions, and approximately 11% of the non-synonymous SNPs mapped to IDR regions. Additionally, the functional classification of the IDR protein set under study was analyzed and the terms enriched in this set included transcriptional regulation and transcription. The pathways enriched in the IDRs set include the Wnt signaling pathway. Our data integrates the current knowledge on IDRs and functional information in cancer proteins and may provide new light into cancer disease mechanism and development of therapeutic approaches.

Poster 020: Early Urine Proteome Changes in the Walker-256 Tail-Vein Injection Rat Model

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Detection of cancer at its early stage is important for treatment. Urine, which is not regulated by homeostatic mechanisms, reflects early systemic changes throughout the whole body and can be used for the early detection of cancer. In this study, the Walker-256 tail-vein injection rat model was established to find whether the urine proteome could reflect early changes if tumor grown in lung. Urine samples from

the control group (n=7) and Walker-256 tail-vein injection group (n=7) on days 2, 4, 6 and 9 were analyzed by label-free proteomic quantitative methods. On day 2, when lung tumor nodules did not appear, 62 differential proteins were identified. They were associated with epithelial cell differentiation, regulation of immune system processes and the classical complement activation pathway. On day 4, when lung tumor nodules appeared, 72 differential proteins were identified. They were associated with the innate immune response and positive regulation of phagocytosis. On day 6, when body weight began to decrease, 117 differential proteins were identified. On day 9, the identified 125 differential proteins were associated with the B cell receptor signaling pathway and the positive regulation of B cell activation. Our results indicate that (1) the urine proteome changed even on the second day after tail-vein injection of Walker-256 cells and that (2) compared to previous studies, the urine proteomes were different when the same cancer cells were grown in different organs.

Keywords: cancer biomarkers, urine proteome, lung tumor, early detection

Poster 021: Identification of Smyd1's Chromatin Binding Partners via ChIP-MS

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As the heart experiences stress, it compensates by undergoing hypertrophic growth which, left unchecked, eventually progresses to heart failure. Pathological cardiac hypertrophy is preceded by the re-expression of genes normally only activate during development which can only be accomplished by significant remodeling of the chromatin in order for key regions of DNA to be silenced or reactivated. Although recent studies have demonstrated that altering key epigenetic mechanisms can inhibit these gene-expression changes and prevent disease-induced growth, our knowledge regarding the chromatin modifiers driving cardiac pathology is quite limited. We have previously shown that loss of the histone methyltransferase, Smyd1, can be deleterious for the heart, however, the mechanisms by which the two Smyd1 variants (Smyd1a and Smyd1b) regulate gene expression changes and how this maintains basal cardiac function and influences heart failure onset is not known. In this study we used chromatin-immunoprecipitation coupled with tandem mass spectrometry (ChIP-MS) to identify the Smyd1 binding partners on chromatin and the post-translational modifications found on nucleosomes they bind. Specifically, we utilized adenoviral-mediated expression of Flag-tagged Smyd1a or Smyd1b in C2C12 cells and primary rat ventricular myocytes (under normal and hypertrophic conditions). Our analyses identified a number of novel interactors of Smyd1a and Smyd1b on chromatin (31 and 12, respectively) which included both shared and variant-specific proteins. Interestingly, we identified BTF3 and Ybx1 as novel transcription factors which were found to exclusively bind Smyd1a and Smyd1b, respectively. Additionally, we showed that nucleosomes bound by Smyd1 are enriched in histone H3 di- or tri-methylated on K37, mono-methylated on R40/K80, and void of methylation or acetylation marks on K9/K14. In addition, these nucleosomes are enriched in histone H2A type-1. Overall, these results begin to unravel the mechanistic basis by which these histone methyltransferases regulate gene expression changes in the genome to influence myocyte morphology and physiology.

Poster 022: Conservation and Divergence of Protein Pathways in the Vertebrate Heart

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Congenital malformations, or structural birth defects, are now the leading cause of infant mortality in the United States and Europe. Of the congenital malformations, congenital heart disease (CHD) is the most common. Studies of human heart disease and treatments have relied on vertebrate model systems with a dual circulatory system, including *Xenopus*, mouse, and pig. However, little is known about which proteins and protein pathways are conserved and which have diverged between these model systems. To address these questions we examined heart tissue proteomes of *Xenopus tropicalis*, *Xenopus laevis, Mus musculus*, and *Sus scrofa* and assessed protein abundance changes in the context of pathways, protein complexes, and enrichment of corresponding genes in human heart diseases. This was achieved by dissecting heart tissue and subjecting the tissue to differential solubilization followed by gel fractionation, digestion, and analysis on a Q Exactive HF (ThermoScientific). Differential heart proteome assessment was performed by label-free quantitation using Proteome Discoverer 2.1 and Scaffold. Over 9,000 proteins were identified across all four species and were mapped to orthologous human proteins to assist with bioinformatic analysis. Quantitative differences revealed species-specific enrichments in selected pathways. One example is a pronounced enrichment of cell cycle associated proteins in *Xenopus laevis* compared to the other species, which we confirmed via targeted mass spectrometry. Thus, the dataset provided here will facilitate selection of appropriate models for studies of specific genes. To demonstrate the utility of the dataset, we tested the role of the Kcp protein, which is present in the hearts of humans and frogs, but not present in mice or pigs. Strikingly, germ-line null mutations in Kcp in *Xenopus* lead to cardiac failure and death. Thus, our data provide a new and better road-map for establishing animal models for the genetic basis human cardiac development and disease.

Poster 023-ML: Broad Time-dependent Proteomic and Metabolomic Effects of Atorvastatin on Hepatocytes

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Cholesterol plaques built up in arteries can slowly reduce blood flow leading to ruptures and heart disease. Atorvastatin, and other drugs from the statin class, provide cardiovascular protection by blocking hydroxymethylglutaryl coenzyme A reductase (HMGCR), an essential enzyme in the cholesterol biosynthesis pathway. Over and above its primary action, atorvastatin has a range of well-established adverse effects and cholesterol-independent beneficial or "pleiotropic" effects, including reduction of inflammatory mediators, improvement of endothelial function and antioxidant mechanism.

While hypothesis driven investigations on the cellular level have unveiled many of these effects, systematic mass spectrometry-based experiments have the potential to provide further insight into related molecular mechanisms. Here we show that cell culture based untargeted profiling of time-dependent proteomic and metabolomic effects of atorvastatin can capture changes related to the known mechanism of action, and also provides a comprehensive view of adverse and pleiotropic effects. Our metabolome analysis captured the changes in many lipid features, for example the decreases of lanosterol and 4-methylzymosterol levels, in terpenoid biosynthesis and related pathways, whereas comprehensive analysis of the protein abundances showed significant changes for 86 proteins, including

increased abundances of HMGCR, squalene synthase and apolipoprotein-A. As most features affected by atorvastatin are already well identified in the literature, we were able to pinpoint changes that either support existing hypotheses or represent undiscovered details of the diverse effects of atorvastatin. Among others we observed decreased abundances for DNA topoisomerase 2-alpha and increased abundances for Alpha-1-anti-trypsin. Our time-dependent analysis (based on 10 timepoints) revealed that approximately one fifth of the significant changes were missed by traditional single timepoint analyses, which demonstrates the importance of temporal profiling and systemic approaches.

Poster 024: Site-specific Proteomic Profiling using a Novel Chemical Probe Identifies New Members in the Deubiquitinase Family

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Activity-based protein profiling (ABPP) may be a powerful tool to explore in situdeubiquitinase (DUB) activity to aid the discovery of ubiquitin-based therapeutics. A common workflow consists of DUB enrichment by an activity-based probe and subsequently identified with mass spectrometry. In principle, inferring DUB activity from probe reactivity requires that the probe reacts with the enzyme at its active site; however, this is rarely verified since the labeling sites are usually not determined. In some cases, there is no indication that a protein enriched by the probe is truly an active DUB, or merely an ubiquitin binder. Here we present an enhanced chemoproteomic approach to discover deubiquitinating enzymes and study their activity through reaction sites.

Poster 025: Use of Peptide Biosensor and PRM for Measuring Kinase Activity

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Our group uses peptide biosensors for measuring kinase activity in cells. Peptide sequences that can be selectively phosphorylated by the Abl kinase are synthesized. Stable isotope labeled Alanine is incorporated to create four isocratic biosensors (mass differences +0, +4, +7, +10 Da). Each isocratic biosensor was used for measuring Abl kinase activity in K562 cells under different treatment conditions. Equal amounts of lysates from different treatments were mix and the biosensors' Tyrosine phosphorylation was quantified using PRM. PRM results show about 2% and 20% biosensor phosphorylation under vehicle and pervanadate treatments, respectively. Phosphorylation was not detected when cells were treated with Gleevec, an Abl kinase inhibitor. Our next goal is to quantify both biosensor and endogenous kinase phosphorylation using SWATH-MS.

Poster 026: Changes to Human Chromatin Induced by Cytomegalovirus Immediate Early Proteins

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The herpesvirus Human Cytomegalovirus (HCMV) latently infects over half the adult population and can cause serious disease in immunocompromised patients. The double-stranded DNA herpesvirus expresses immediate early proteins, most abundantly immediate early 1 (IE1) and immediate early 2 (IE2), to promote viral infection through largely unknown mechanisms. IE1 interacts with host chromatin by binding to host histones through its chromatin-tethering domain (CTD), which binds the acidic patch between histones H2A and H2B. We investigate the effects of IE1 binding on chromatin. Using mass spectrometry, we assessed the differences in histone post-translational modifications (PTMs) in cells expressing IE1, IE1Δ (deleted CTD), or IE2. Our findings reveal significant changes in several histone post-translational modifications with over-expression of IE1. Modifications to the core histones correspond with cell cycle progression and changes in gene transcription, and the functional significance of a double modified linker histone H1 peptide is still under investigation.

Poster 028: Adapting EasyPep™ MS Sample Preparation for 96-Well Automated Liquid Handling Systems

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Introduction
Advances in mass spectrometry (MS) instrumentation has enabled routine analysis of complex protein samples. However, sample preparation methods are not standardized with many protocols taking 8-24hrs in addition to suffering from low peptide yields, poor digestion efficiency and low reproducibility. Recently, we developed a new, simplified sample prep kit containing pre-formulated reagents and a standardized protocol for processing 10µg to 100µg protein samples in less than 2 hours. In this study, we adapted our chemistry to use a 96-well filter plate for peptide clean up and assessed this format using an automated liquid handling system.
Methods
Cellular protein extracts and human plasma samples were diluted in lysis buffer. A universal nuclease was added to cellular extracts to reduce sample viscosity. Protein samples were heated at 50°C for 10 minutes in the presence of a combined reduction/alkylation solution before digestion using a trypsin/LysC protease mixture. A mixed mode peptide clean-up procedure using a 96-well filter plate format was evaluated using a vacuum manifold or automated positive-pressure system. Peptides were quantified and normalized using the Pierce™ Quantitative Colorimetric Peptide Assay prior to LC-MS analysis using a Thermo Scientific® Q Exactive® HF hybrid quadrupole-Orbitrap® mass spectrometer.
Preliminary Data
Although our optimized protocol using in our EasyPep kit significantly reduces both hands-on and total sample processing time, peptide clean up using microcentrifuge spin columns is still time consuming with larger sample numbers. We developed a new 96-well filter plate format to support higher sample processing throughput. This format showed nearly identical performance in terms of peptide yield, identification rates, alkylation efficiency and digestion efficiency compared to the manual spin column protocol with better reproducibility among replicates.
Conclusion
Overall, we demonstrate that our chemistry is readily adaptable to automated liquid handling system which provides excellent reproducibility and greatly simplifies proteomic sample preparation.

Poster 029: timsTOF Pro: Maximum Throughput, Robustness and Analytical Depth for Shotgun Proteomics

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Sample throughput (>40 runs in 24 hr) in proteomics with reasonable proteome depth is highly desirable. The timsTOF Pro with trapped ion mobility spectrometry (TIMS) offers additional separation power and increased peak capacity. TIMS enables the powerful Parallel Accumulation Serial Fragmentation (PASEF) method [1,2] for very high sequencing speed. These features are perfectly suited for proteome analysis on short gradients. Here, we demonstrate the performance of the timsTOF Pro mass spectrometer using short gradients, small sample loads and hundreds of LC MSMS runs in a 24 hr time period.

Hela digest (< 250 ng) was LC separated either using the nanoElute HPLC (Bruker Daltonics) or Evosep One system (Evosep) on-line coupled to a high-resolution TIMS-QTOF instrument (timsTOF Pro, Bruker Daltonics). For all analyses we applied the PASEF method. Data analysis was performed using PEAKS (Bioinformatics solutions Inc.) and MaxQuant (Jürgen Cox, Max Planck Institute of Biochemistry).

When analyzing 50 ng of HeLa digest using the Evosep One system in combination with a TIMS-QTOF mass spectrometer, 1400 proteins could be reproducibly (R2 > 0.97) identified using short gradients of 5.6 min. This very short gradient gives the possibility to analyze up to 200 samples/day. Increasing the measurement time per sample (measurement of up to 50 samples/day) and using a nanoElute HPLC system allows the identification of more than 4000 protein groups from 250 ng of HeLa digest, enabling the analysis of large sample cohorts with a reasonable proteome depth of coverage and short measurement time. Moreover, measurements of 24 concatenated fractions of HeLa digest allowed the identification of more than 100,000 unique peptides and 9,052 protein groups in less than 12 h measurement time. Our results show that an ultimate analytical depth can be achieved using short gradients.

Poster 030: Identification of Inconsistent Peptide Recovery and Aberrant Peptide Termini as Sources of Sample Variability in Patient-derived Tumor Samples

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NIST performance metrics (Rudnick et al, MCP, 2009) have been extended to individual samples in multiplexed experiments, such as iTRAQ- or TMT-labeled peptides, as well as results obtained from the recently developed hybrid mass spectral library search. In addition, we have compared normalized reporter ion abundance, relative to a reference channel, across retention time between digests which has identified tumor samples with inconsistent peptide recovery thereby resolving the previously unexplained bimodal reporter ion intensity distribution observed in data obtained from Clinical Proteomic Tumor Analysis Consortium (CPTAC).

Variation in reporter ion relative abundance across over 300 distinct DeltaMass values, obtained from hybrid search results, was also analyzed. The hybrid search can identify peptides that contain unanticipated modifications by matching both ion m/z and mass losses (Burke et al, JPR, 2016). The DeltaMass, or mass difference between the query and reference spectral library peptide, is expected to correspond to a modification. Outlier modifications identified include amino acid losses and additions. Further analysis revealed amino acid additions were found to be localized to the C-terminus and losses localized to the N-terminus. Moreover, select amino acids are preferred, meaning that not all amino acids are equally likely to be truncated or added to a peptide sequence, and this preference also varies across laboratory and experiment. The presence and variability observed in peptides containing aberrant termini, which may be due to non-tryptic proteolytic activity or sample degradation, is found to contribute to sample variation.

Poster 031: MaxQuant Software for Trapped Ion Mobility Enhanced Shotgun Proteomics

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Trapped ion mobility spectrometry (TIMS) provides an additional dimension of separation to LC-MS which boosts proteome coverage, quantification accuracy and dynamic range in shotgun proteomics experiments. Required for this is suitable software that extracts the information contained in the 4D data space spanned by m/z, retention time, trapped ion mobility and signal intensity. Here, we describe the trapped ion mobility enhanced MaxQuant software, which utilizes the added data dimension.

We performed benchmark measurements on a Bruker timsTOF Pro instrument with 'Parallel Accumulation Serial Fragmentation' (PASEF) functionality for the acquisition of MS/MS spectra. We generated benchmark datasets in which HeLa proteins were spiked with E. coli and yeast proteomes at 1:2 and 1:5 ratios and compared the replicates with the MaxLFQ algorithm for label-free quantification.

We adapted the complete MaxQuant shotgun proteomics workflow to process data with the added trapped ion mobility dimension. Most adaptations were done in the feature detection workflow which now produces 4D peaks. De-isotoping and assembling of MS1 labeling multiplets utilize intensity profile correlations also over the ion mobility direction. Matching MS1 features between runs, the transfer of identifications without MS/MS identifications, is making use of the ion mobility coordinates to become more specific. Peptide identification, FDR, protein assembly, label-free quantification and isobaric labeling quantification algorithms are included. Application to benchmark dataset showed unprecedented identification depth in single shot experiments and precise ratio quantification. MaxQuant offers an end to end computational workflow for the identification and quantification in LC-TIMS-MS/MS shotgun proteomics data including CCS value determination to reduce proteomics FDR and enhance the LFQ performance and the match between runs functionality using the 3D (m/z, RT and CCS).

Poster 032: Reproducible Quantitative Mass Spectrometry-based Research: The MSstats Perspective

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We present a statistical perspective on reproducible quantitative mass spectrometry-based proteomics. Statistical components of reproducibility include experimental design, from both biological perspective (which proteins and samples, and how many, do we need to quantify?) and technological perspective (are the assays appropriate for the task? Do the experimental steps run properly?). Statistical components of reproducibility also include data processing (which features should we use to quantify a protein?) and downstream statistical analysis (how to detect changes in protein abundance? Are our conclusions consistent with prior results?). Answering these

questions requires the availability of statistical methods, and but also of publicly available data that help understand the advantages and the limitations of the methods. We developed a family of R/Bioconductor packages, called MSstats, for protein significance analysis in mass spectrometry-based proteomic experiments. MSstats supports DDA, SRM, and DIA acquisition, label-free experiments or experiments with tandem mass tag (TMT) labeling. MSstats supports assay characterization and longitudinal profiling and statistical quality control. MSstats includes new functionalities for selecting informative features, and works seamlessly with numerous data processing tools. We will highlight the new aspects and extensions of these packages and the contributions of our lab to these components of reproducible research.

Poster 033: 2018-2019 Metrics from the HUPO Human Proteome Project: Progress on Identifying and Characterizing the Human Proteome

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The Human Proteome Project (HPP) annually reports on progress throughout the field in credibly identifying and characterizing the human protein parts list and making proteomics an integral part of multiomics studies in medicine and the life sciences. NeXtProt release 2018-01-17 contains 17 470 PE1 proteins, 89% of all neXtProt predicted PE1-4 proteins, up from 17 008 in release 2017-01. Conversely, the number of neXtProt PE2,3,4 missing proteins (MPs) was reduced from 2579 to 2186. Of the PE1 proteins, 16 092 are based on mass spectrometry results, and 1378 on other protein studies, notably protein-protein interaction. PeptideAtlas 2018-01 has 15 798 canonical proteins, up 625 over the previous year, including 269 from SUMOylation studies. The largest reason for MPs is low abundance. The 6th annual HPP special issue of JPR presents evidence for 104 MPs that meet the HPP guidelines plus 107 identified in MassIVE (JPR 2018; 17:4023). The 2019 releases from PeptideAtlas and neXtProt will be available for updates in time for the US HUPO meeting.

Meanwhile, the Human Protein Atlas has released its Cell Atlas, Pathology Atlas, updated Tissue Atlas, and recommendations on antibody validation. Finally, multiplex organ-specific popular proteins targeted proteomics has been applied to various diseases.

Poster 034: Integrated Proteogenomic Data Analysis Pipeline and Its Applications to Post-translational Modification Investigation

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Proteogenomic analysis provides comprehensive and integrated characterization of specimens using multiple genomic and proteomic data generation pipelines, which is critical to understand the regulations in the alterations of genes, proteins, and post-translational modifications (PTMs). There are needs for a data analysis pipeline to integrate genomic and proteomic data as well as PTM information. Here, we describe an proteogenomic data analysis pipeline with the purpose of investigation of PTMs (e.g. Phosphorylation and Glycosylation).

With the standardized analysis templates implemented in the pipeline, it provides a series of routine data analysis outputs for further investigation by integration of existing tools and in-house scripts, which include quality control assessment, differential expression analysis, pathway analysis, phenotype associations analysis, PTMs identification and investigation, and etc. The pipeline has been applied in the latest studies of Clinical Proteomic Tumor Analysis Consortium (CPTAC) and the HIV latency study from an amfAR project. In practice, the pipeline not only significantly shortened the period from data quality assessment to preliminary conclusion generation, but also showed the potential of the utility of using integrated data analysis on multi-omics data. For example, the integrated analysis of the expression of glycosylated peptides and glycosylation enzymes revealed the differential glycosylation mechanisms in ovarian tumor and non-tumor tissues in the ovarian cancer study.

To conclude, our results showed that the pipeline provided standard quality control assessment as well as efficient integrated data analysis methods to accurate knowledge discovery from multi-omics studies.

Poster 035: Integrated Machine Learning Pipeline Reveals Fingerprints of the Oxidative Stress-Sensitive Post-translational Modification during Cardiac Remodeling

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Oxidative stress is a common pathological stimulus contributing to cardiac disease, yet our understanding of oxidative stress-induced protein targets and their dynamics remain elusive. This is largely due to the complex nature of oxidative stress-induced post-translational modifications (O-PTMs) of proteins. Accordingly, machine learning (ML) approaches can unveil complex patterns in large biological datasets. However, the successful application of ML to elucidate O-PTM signatures in cardiac pathology present challenges in sparsity, temporal nature, and high-dimensionality of data that require further innovations.

Longitudinal datasets over a 14-day duration were generated from 6 genetically distinct murine strains with variable disease susceptibility. Mice were infused with isoproterenol, a model of cardiac disease and elevated oxidative stress. Functional phenotyping ensued via quantifying 7 chemically-distinct O-PTMs and developing a novel ML-based platform to link O-PTM fingerprints with cardiac pathological progression. Molecular signatures of cardiac phenotypes and O-PTM dynamic patterns were identified using feature selection algorithms following random forest-based missing data imputation and cubic spline-based clustering, respectively. Unique molecular signatures were validated using three independent mechanisms: computational, biological, and translational.

Cardiac remodeling potentiated O-PTMs in 6,478 of 8,227 murine cardiac proteins, including 25,148 modified sites in 549 cellular

pathways. ML analysis produced 8 O-PTM sites representing a cardiac pathological phenotype; known hypertrophic pathways such as muscle contraction, TCA, beta-oxidation, branched chain amino acid catabolism (BCAA), and Ca2+ regulation showed enrichment. Importantly, O-PTM combinations in these pathways exhibited distinct temporal profiles, discovering novel molecular signatures that define different phases of cardiac disease progression.

Our findings provide the first O-PTM molecular map that serves as an avenue for molecular signature discovery and design of therapeutics. Our novel ML-based integrative analytics approaches discerned the relationships of O-PTM alterations and functional remodeling. This pipeline has been demonstrated to be effective for conducting deep molecular phenotyping in disease progression of cardiovascular medicine.

Poster 036-TL: Improved Protein Inference from Multiple Protease Bottom-Up Mass Spectrometry Data with MetaMorpheus

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Protein inference is a process in bottom-up proteomics to infer the presence of a set of proteins from the identified peptides. Peptides with only one protein of origin ("unique peptides") provide enough evidence for confident identification of that protein. Identified peptides with multiple possible proteins of origin yield ambiguous protein identifications ("protein groups"). One strategy employed to increase the number of identified unique peptides is to use multiple proteases. Each protease produces a different set of peptides from the same set of proteins, which increases the chances of identifying a unique peptide and subsequently identifying each parent protein. Protein inference, when performed separately for each protease's identified peptides, necessitates reconciling the protein inference results, which is time consuming and can lead to errors. We recently updated the search software MetaMorpheus with the ability to perform coincident protein parsimony using bottom-up data from multiple different proteases. Combined protein inference disambiguates many protein groups into single protein identifications and reduces the number of erroneous protein identifications. In addition, PTM identification and localization are markedly improved.

Poster 037-ML: EpiProfile 2.0: A Computational Platform for Processing Epi-Proteomics Mass Spectrometry Data

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Histone post-translational modifications (PTMs) contribute to chromatin function through their chemical properties which influence chromatin structure, and their ability to recruit chromatin interacting proteins. Nanoflow liquid chromatography coupled with high resolution tandem mass spectrometry (nanoLC-MS/MS) has emerged as the most suitable technology for global histone modification analysis due to the high sensitivity and the high mass accuracy that provide confident identification. However, the histone nanoLC-MS/MS data analysis is even more challenging due to the large number and variety of isobaric histone peptides, and the high dynamic range of histone peptide abundances. Therefore, quantification of histone PTMs has become a high priority to investigate cell regulation and epigenetics.

Here, we present EpiProfile 2.0, an extended version of our 2015 software (v1.0) for accurate quantification of histone peptides based on liquid chromatography – tandem mass spectrometry analysis. EpiProfile 2.0 is now optimized for data-independent acquisition through the use of precursor and fragment extracted ion chromatography to accurately determine the chromatographic profile and to discriminate isobaric forms of peptides. The software uses fragment ions and an intelligent retention time prediction trained on the analyzed samples to enable accurate peak detection. EpiProfile 2.0 supports label-free and isotopic labeling, different organisms, known sequence mutations in diseases, different derivatization strategies, and unusual PTMs (such as acyl-derived modifications). In summary, EpiProfile 2.0 is a universal and accurate platform for the quantification of histone marks via LC-MS/MS. Being the first software of its kind we anticipate that EpiProfile 2.0 will play a fundamental role in epigenetic studies relevant to biology and translational medicine. EpiProfile is freely available at https://github.com/zfyuan/EpiProfile2.0_Family.

Poster 038: UniRef Clusters as a Resource for Protein Annotation Propagation and Prediction

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UniRef databases provide full-scale clustering of UniProt protein sequences to hide the redundant sequences and obtain complete coverage of the sequence space at three resolutions: 100, 90 and 50 % identity, respectively. They have been utilized in a broad range of applications. UniRef100 has become a standard sequence database for Mass Spectrometry search while UniRef90/50 are used in similarity-based functional annotation. We have assessed the intra-cluster molecular function consistency with Gene Ontology (GO), and further found that such results were highly coherent in all three GO domains.

Recently, we have implemented GO annotations to UniRef90/50 clusters when all members with GO annotation contained common GO terms, or had a meaningful common ancestor. Such GO terms should be safely propagated to other UniProtKB members in the cluster lacking any GO annotation. In other words, we could predict the GO terms using the UniRef clusters.

To evaluate this, we took all the UniRef90/50 clusters that provided any GO annotation in a previous release and examine if an un-annotated member from such cluster has received GO annotations from UniProtKB in a later release. A new GO term would be counted as false positive if it does not match previous prediction, but has a non-root common ancestor with it. We compared the GO annotations between Releases through 2017 and 2018 for UniRef90/50 and the results indicate that more than 97% of the new GO annotations match the previous predictions.

In conclusion, the members of a UniRef90/50 cluster are highly coherent and provide a good potential method for annotation prediction/propagation. In fact UniRef clusters have been widely used for the purpose, such as GO enrichment, gene prediction, metagenomics functional profiling and transcriptome assembly. Here, we provided a formal evaluation of the UniRef-based annotation method.

Web access at www.uniprot.org/uniref and ftp://ftp.uniprot.org/pub/databases/uniprot/uniref

Poster 039: Proteomic Data Commons (PDC): A Node in the NCI Cancer Research Data Commons

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The creation of a National Cancer Data Ecosystem to establish data science infrastructure necessary to connect repositories, analytical tools, and knowledge bases was a key recommendation of the 2016 Beau Biden Cancer Moonshot[SM] initiative. These resources serve to support data aggregation, query, analysis, and visualization in unique and powerful ways within and across data types.

To this end, the National Cancer Institute (NCI) has begun the development of the NCI Cancer Research Data Commons (CRDC) that included a Genomic Data Commons, Proteomic Data Commons and Cloud Resources. In the near future, the CRDC will launch an Imaging Data Commons and a Cancer Data Aggregator.

As a node in the CRDC, the overarching goal of the NCI Proteomic Data Commons (PDC) project is to democratize access to cancer-related proteomic datasets as well as to provide sustainable computational support to the cancer research community. The PDC seeks to enable and empower the cancer-research community, including scientists working in both intramural and extramural laboratories, with the necessary informatics capabilities to carry out large-scale, multi-omic data analysis.

We recently launched a minimal viable product (MVP) for the PDC with the goal of getting a prototype product quickly to the user community in order to collect feedback to drive the future development of features and capabilities of the final product. The prototype consists of a data portal and workspace components, deployed in Amazon Web Services. In this presentation we will describe the current status of the PDC, including: available datasets, application programming interface, analysis pipelines, data model, underlying architecture, integration with the CRDC, and the overall philosophy of the PDC. The prototype can be accessed from https://pdc.esacinc.com/pdc.

Poster 040-TL: Optimized Cross-linking Mass Spectrometry for *in situ* Interaction Proteomics

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Recent advancements in mass spectrometer cleavable protein cross-linkers and algorithms for cross-linked peptide identification now enable proteome-scale cross-linking mass spectrometry (XL-MS). Here, we optimized the use of mass spectrometer cleavable cross-linker, disuccinimidyl sulfoxide (DSSO), for labeling protein complexes in live human cells. To control the sensitivity and specificity of identified cross-linked peptides, we applied a generalized linear mixture model to calibrate cross-link peptide-spectra matching (CSM) scores. Using specific CSM score thresholds to control the false discovery rate, we found that higher-energy collisional dissociation (HCD) and electron transfer dissociation (ETD) can both be effective for proteome-scale XL-MS protein interaction mapping. We demonstrate that the density and coverage of protein-protein interaction maps can be significantly improved through the use of multiple proteases. In addition, the use of sample-specific search databases can be used to improve the specificity of cross-linked peptide spectral matching. Application of this approach to cross-linked human chromatin in live cells recapitulated known and revealed new protein interactions of nucleosomes and other chromatin-associated complexes in situ. In particular, we found unanticipated interactions of HMGN proteins with the H1 linker histone, providing the sought-after mechanism to explain its structural effects on chromatin directly. Live cell cross-linking was performed on human cancer cells to define the architecture of the mutated chromatin remodeling BAF complex. These studies identified aberrant protein-protein interactions retained by the mutant BAF complex in rhabdoid tumor cells, thereby identifying neomorphic BAF complexes induced by oncogenic mutations. The optimized approach for mapping native protein interactions can be applied to study a wide range of unanswered biological problems.

Poster 041: Accelerating DIA Studies to Extend Workflow Utility, Using Fast Microflow LC Gradients

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Microflow LC has been used increasing in quantitative proteomics in combination with SWATH® Acquisition, to provide better robustness and higher throughput when measuring larger sample cohorts. With higher flow rates, sample loading, trap/column washing, equilibration and gradient formation are all accelerated, allowing much faster run times to be achieved. Here, the impact of shortening gradient length on protein quantitation results with DIA was explored. Using microflow LC on the TripleTOF® 6600 System, a range of gradient lengths from 5 – 45 mins was explored, and key acquisition parameters for SWATH® Acquisition were varied to optimize for the much faster run times.

Using complex digested cell lysates, SWATH protein quantitation results were assessed as a function of gradient length. Fast MS/MS acquisition rates were found to be critical because this enabled more smaller variable Q1 windows to improve S/N for quantitation. Even with the fastest gradients, methods with 60-100 windows with very fast accumulation times of 15 msec improved results. As expected, total # of protein quantified decreased when shortening the gradient from 45 to 5mins. However, with the 10min gradient, the peptide and protein quantified was ~60 and 70% respectively of results from the 45min gradient for the three matrices tested (on 2 instruments). With the Pan Human Library on a 1ug load of HEK lysate, ~2100 and ~3400 proteins were quantified with the 5 and 10 min gradients respectively. The full optimization results will be presented.

Poster 042: Simplifying the Use of Ion Libraries During Data Processing of Data Independent Acquisition Proteomics Data

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As the use of data independent acquisition (DIA) grows in proteomics research, the need for improved data processing workflows increases. The most common data processing workflow is to use spectral ion libraries to drive targeted extraction of peptide / fragment areas from the data, using the m/z and retention time information contained in the library. Increasing the size and quality of the ion library has been shown to increase the proteins reliably quantified from a dataset. Retention time (RT) correlation between ion library and the dataset is another key factor that determines quality of data extraction. Two algorithms were explored to simplify the data extraction workflow for SWATH® Acquisition data within the OneOmics® Project cloud processing pipeline, the automatic merging of related ion libraries followed by auto RT calibration.

The quality of library merging on SWATH results was explored with a series of small datasets. Non-linear retention time alignment was shown to be effective for merging of libraries with identical or differing gradient lengths. By merging libraries from replicates of a 1D dataset, a 20% gain in peptides was observed. The gain in peptides identified and quantified by SWATH is primarily attributed to an increase in peptide coverage rather than improved ion selection. Full pipeline was then used on some biological datasets (PBMC and Mouse cell lines) to measure performance, where a series of libraries were available to merge. Results will be discussed.

Poster 043-WTT: Rapid Qualitative and Absolute Quantification of Plasma based proteins using a Novel Scanning Quadrupole DIA Acquisition Method

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Quantitative proteomics often incorporates the use of stable isotope labels (SILs) to provide absolute quantification. Recent advancements have seen the introduction of peptide panels allowing the quantification of over 500 proteins in plasma sample sets. However, this is technically challenging when attempting to acquire the data using more traditional MS acquisition modes such as multiple reaction monitoring (MRM), since the duty cycle of the instrument is compromised and therefore results in under sampling. Data independent analysis (DIA) methodology provides high throughput while also ensuring high rates of data acquisition and specificity. Here we describe the use of a kit consisting of SIL's capable of quantifying >500 plasma-based proteins in conjunction with a novel scanning quadrupole DIA acquisition schema in order to quantify proteins of interest for patient cohorts diagnosed with respiratory disorders.

Undepleted human plasma originating from controls and patients diagnosed with chronic obstructive pulmonary disorder (COPD) and asthma were reduced, alkylated and tryptically digested overnight. Prior to LC-MS analysis, samples for their individual groups were pooled and spiked with PQ500 SIL peptides (Biognosys). In all cases, samples were separated using various LC gradients (15 and 45 minutes) whilst employing 1mm scale chromatography for high throughput analyses. MS data were acquired using SONAR, whereby the quadrupole (MS1) was continuously scanned between m/z 400-900 using a quadrupole transmission width of approximately 20 Da, whilst the TOF scanned between m/z 50-2000. In all cases, precursor and product ion information were collected. The LC-MS data were processed with Spectronaut Pulsar X (Biognosys AG and Progenesis QI for Proteomics (Non-linear Dynamics). Multivariate statistical analysis showed distinct differences between all three cohorts and proteins corresponding to 86% of the spectral library were quantified. CV's for each group were all found to be <5% in all cases and was maintained across all gradient lengths.

Poster 044: Introducing the "DataCrusher SuperComputing Service": A DIA pipeline that's ~10x Easier, ~1000x Faster, and ~10x More Robust

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Data Independent Acquisition (DIA) has gained tremendous popularity in the last five years, but its tremendous power is rarely fully harnessed due to three main factors:

1) the complexity of the experimental setup (eg the tremendous informatics and MS time needed to create DDA-based spectral libraries)
2) the computing resource requirements, which results in days/weeks of computation time for even small projects
3) the lack of a reasonably simple, single informatics workflow that can be executed with minimal training

Herein, we present for the first time the "DataCrusher SuperComputing Service," a beginning-to-end informatics pipeline which runs on a supercomputer, can process both MS files created from a traditional DIA protocol as well as those created using a novel DIA protocol called "Global Staggered DIA" (gsDIA), and which can be accessed by the scientific MS community using a modern web browser.

The DataCrusher SuperComputing Service
1) Does not require a spectral library to be generated
2) Runs in approximately 15 minutes, irrespective of the number of samples submitted
3) Does not require retention time standards to be injected
4) Demonstrates substantively superior number of low-abundant analytes quantified reproducibly (CV < 5%) than traditional DIA protocols
5) Is dead-easy to use (most of the relevant information can be derived from the MS data itself without querying user)
6) Includes an automated, near-real-time, and extraordinarily fast MS data importer (~30s/file)
7) Is accessed through a user's browser, and so does not involve any installation by the average user
8) Exists simultaneously in multiple parts of the world (US, Europe, Asia) and therefore provides low latency, high fault tolerance, and adheres to various legal requirements for data storage
9) Includes an interactive graphical system for all processed data, from raw MS data to MS2 features to pseudo mgfs to lists of peptides and proteins.

Poster 045-ML: The Nature of Phosphatidylinositol Mannosidases (PIMs) Interaction with the PPE68 Protein – Revealing Novel Insights in its Immunogenicity and Virulence

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Background: Mycobacterial protein glycosylation is understudied and for only very few glycoproteins glycosylation sites have been described. Mycobacteria have been suggested to possess O-linked glycosylation pathways that display many commonalities with their eukaryotic and archaeal counterparts as well as some unexpected variations. Studies have demonstrated that PPE68 is a glycoprotein and plays an important role in *M. tuberculosis* pathogenicity and represent as potential vaccine candidate and diagnostic tool. Our preliminary results and shreds of evidence have shown that *M. tuberculosis* PPE68 protein stained positive for glycosylation *in vitro* (PAS staining) and glycosylated PPE68 is highly immunogenic in contrast to the deglycosylated PPE68. However, it is still unclear why does PPE68 wears an attire of such a modification? If PPE68 has the potential of being a therapeutic target it seems interesting to understand the protein glycosylation pattern to identify attributes connected to its immunogenicity using advanced strategies of glycomics and glycoproteomics.
Methods: Dot-blotting/electroblotting, Release of *N*-linked glycans, Release/reduction of *O*-linked glycans, Desalting of reduced *N*- and *O*-linked glycans, PGC-LC-ESI MS/MS of glycans
Results: Using glycomics and glycoproteomics, we demonstrated that the glycosylation of the PPE68 protein is due to its attachment to PIMs. The preliminary finding suggests that this is a covalent bond, rather than just non-covalent interaction. The bond is labile against acidic and alkaline hydrolysis and the glycosylation can partly be removed from the protein core by nucleophiles. Results also suggested the participation of aspartic and glutamic acid carboxylic side chain in the binding of the PIMs to PPE68.
Conclusions: Our study hints to a novel role of PPE68 as a PIM anchored protein that implicates its role in immune signaling in *M. tuberculosis* survival inside the host. This would also improve our understanding of the intricacies of mycobacterial protein glycosylation systems.

Keywords: PGC-LC-ESI MS/MS of glycans, glycoproteomics, glycomics

Poster 046-TL: Integrated Glycoproteomic Characterization of Human High-Grade Serous Ovarian Cancer

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Many gene products exhibit great structural heterogeneity due to an array of modifications. These modifications are not directly encoded in the genomic template but often affect the functionality of proteins. Protein glycosylation plays a vital role in proper protein functions. However, the analysis of glycoproteins has been limited compared to other protein modifications, such as phosphorylation. Here, we performed an integrated proteomic and glycoproteomic analysis of 83 prospectively collected high-grade serous ovarian carcinoma (HGSC) and 23 non-tumor tissues. Integration of the expression data from global proteomics and glycoproteomics revealed tumor-specific glycosylation, uncovered different glycosylation associated with three subtypes of HGSC, and identified glycosylation enzymes that were correlated with glycosylation. In addition to providing a valuable resource, these results provide new insights into the potential roles of glycosylation in the pathogenesis of HGSC with the possibility of distinguishing pathological outcomes of ovarian tumors from non-tumors, as well as tumor subtypes.

Poster 048: Organelle Glycoproteomics by using Lectin Chromatography and HCD/ETD Mass Spectrometry

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Glycosylation, a large and structurally diverse category of post-translational modifications, regulates a wide range of protein functions and cellular processes. However, it has been a major challenge to study glycosylation due to technical difficulties for site mapping and structural characterization. To enrich sub-stoichiometric glycosylation, a number of methods have been developed recently. Meanwhile multiple mass spectrometric approaches have been adopted to facilitate site assignment and structural elucidation. In this study, we compiled a map for glycoproteins (including N-glyco-, O-GalNAc, and O-GlcNAc proteins) in different organelles by analyzing glycan-containing peptides. Specifically, we prepared organelle-enriched samples from human cancer cells. Glycopeptides were enriched by multiple rounds of wheat germ agglutinin (WGA) affinity chromatography. Enriched samples were then dried and fractioned by high pH RPLC. To improve HCD/ETD detection, each fraction was injected twice, once allowing for fragmentation of precursor ions 3+ and above, and one injection for only 2+ precursor ions. Indeed, we found a wide distribution of glycoproteins identified: O-GlcNAcylation occurs mainly on proteins in nuclear and plasma membrane while N-glycosylation and O-GalNAcylation occur on proteins localized in ER, Golgi, lysosome, and plasma membrane. Specifically, we identified 106 unique O-GlcNAc peptides with unambiguous site localization, 293 O-GlcNAc peptides with ambiguous site localization, 918 unique peptides with other glycosylation forms (unambiguous sites assigned), and 287 unique peptides with ambiguous localization of N or O-GalNAc modifications. More importantly, besides the identity of glycosylation sites, our results reveal the nature of the oligosaccharide structures on these proteins. We also report an iterative approach that allows us to explore the diverse nature and distribution of oligosaccharides present in glycopeptide mixtures.

Poster 049-ML: Glycoproteomics-based Signatures for Tumor Subtyping and Clinical Outcome in Human High-Grade Serous Ovarian Cancer

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In different individuals, tumor heterogeneity could exhibit significant differences at the molecular level due to an array of co-occurring genomic, transcriptional, translational, and post-translational regulations. As one of the most abundant and complex protein-translational modifications, protein glycosylation is known to be associated with tumor progression, metastasis and survival. To investigate the role of protein glycosylation in tumor heterogeneity of high-grade serous ovarian carcinoma (HGSC), we performed mass-spectrometry (MS)-based glycoproteomic quantification on 119 TCGA HGSC tissues using 4-plex iTRAQ reagents. Bi-cluster analysis of intact glycoproteomics identified 3 major tumor subtypes and 5 groups of intact glycopeptides, and showed a strong relationship between N-glycan structures and molecular subtypes, for example, fucosylation and mesenchymal subtype. Further survival analysis displayed that intact glycopeptide signature of mesenchymal subtype indicated a poor clinical outcome in HGSC. In addition, glycoprotein synthesis involving a series of reactions and enzymes is regulated by several factors that are related to gene expression, protein expression, and protein functions. Through integration of genomics, transcriptomics and proteomics in previous studies with glycoproteomics, we studied the expression correlation between different types of glycoprotein product (i.e. mRNA, protein, deglycosite peptide and intact glycopeptide). Although glycoprotein expression was major regulated by substrates in this study, we investigated and found some glycosylation enzymes could relate to the production of different types of glycan peptide, and further coordinate the tumor heterogeneity. Deep understanding of the glycosylation process and production in different types of HGSC may provide clues for precision medicine and targeted therapy on glycosylation genes.

Poster 050: Development of Hybrid Glycoproteomic Workflows for Site-Specific Characterization of Intact Glycopeptides

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Protein glycosylation is an abundant post-translational modification that regulates a variety of important molecular and cellular processes. The analysis of intact glycopeptides remains an enormous analytical challenge. Because of this, the majority of glycoproteomic studies have relied on enzymatic removal of the glycan side chain. Although this is an effective strategy for identifying glycosylation sites, valuable information about glycan microheterogeneity and the biological function of specific glycans is lost. Here, we describe the development of a hybrid analytical workflow that combines hydrophilic interaction liquid chromatography (HILIC) and strong anion exchange cartridges operated in electrostatic repulsion-hydrophilic interaction chromatography mode (SAX-ERLIC) for glycopeptide enrichment with electron-transfer/higher-energy collision dissociation (EThcD) fragmentation for site-specific characterization of intact glycopeptides.

Preliminary analysis of CHO digest (1 mg starting material) enriched with HILIC and analyzed in a 90-minute LC-MS/MS method with EThcD fragmentation yielded > 1000 N-linked glycopeptide identifications. In agreement with previous results, preliminary results suggest that SAX-ERLIC is superior for enrichment of O-linked glycopeptides compared to HILIC. Therefore, ongoing experiments

are being performed to analyze the utility of a serial enrichment strategy in which HILIC enrichment is performed prior to SAX-ERLIC. The aim of this method is to use HILIC to remove the majority of N-linked glycopeptides prior to SAX-ERLIC to increase the O-glycoproteome coverage. This serial enrichment strategy combined with EThcD-enabled glycopeptide characterization has the potential to provide valuable site-specific information about the role of N- and O-linked glycans in a variety of biological systems.

Poster 051-TL: Developing and Characterizing FLIM Probes to Detect Subcellular Tyrosine Kinase Activity

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Tyrosine kinases are regulators of the signal transduction process and play a key role in cellular processes such as proliferation, migration, metabolism, differentiation and survival. The goal of this project is to develop biosensors (probes) to measure and spatially map kinase activity in live, intact cells with subcellular resolution. The fluorophore labeled peptide probes comprise a motif that facilitates cell penetration and a motif recognized by a specific kinase family. Fluorescence lifetime imaging microscopy (FLIM) measures the decay rate of fluorescence excitation at a picosecond scale resolution and enables single cell mapping of fluorophore lifetime. In this study, fluorophore labeled probes were incubated with cultured MDA-MB-231 cells. Fluorescence lifetime distributions of the probes were mapped within cells, before and after kinase stimulation to detect changes induced due to phosphorylation. The effect of the respective inhibitor drugs on kinase activity were also gauged from shifts in fluorophore lifetime. Identical probes with a phosphorylated tyrosine and a Y to F mutant served as the positive and negative controls for these experiments. FLIM is capable of superior multiplexed analysis and intensity based read out techniques since it enables fluorophores to be distinguished by their lifetime behaviors in addition to their excitation and emission properties.

The shifts in fluorescence lifetime upon phosphorylation arise due to Src homology 2 (SH2) domains within the kinase serving as 'reader' proteins that recognize and interact with phosphorylated probes (protein-protein interaction). In vitro, high-throughput-compatible fluorescence polarization assays and Isothermal Titration Calorimetry (ITC) were employed to characterize the binding affinity between recombinantly expressed SH2 domains and their corresponding peptide probes (phospho-, unphospho- and Y to F mutants). This work should lead to a suite of novel, complementary probes for quantification and localization of PTM enzymatic function in cells as well as serve a high throughput pipeline for drug screening.

Poster 052: Enrichment and Identification of the Class I MHC Phosphopeptides from the Resected Head and Neck Cancer Tumors

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Patients with recurrent or metastatic head and neck cancers (HNCs) have a median overall survival of less than 1 year. [1] The recurrence rate is ~50% in patients with locally advanced HNCs within 3 years. [1] The effectiveness of cancer immunotherapies for the treatment of HNCs patients[1] are based on the fact that they have T lymphocyte capable of recognizing tumor antigens. [2] Tumor antigens are presented to the immune system through the major histocompatibility complex (MHC)-I pathway. [2] Previous studies revealed that deregulated protein phosphorylation in cancer results in the presentation of class I MHC phosphopeptides on the cell surface[3] that are potential targets for T cell recognition. [3,4] In this study, class I MHC phosphopeptides were identified from five resected HNC tumors. After the cell lysis and the immunopurification of class I MHC molecules, MHC-bound peptides were eluted by the acid. Then, samples were desalted via the STop and Go Extraction (STAGE) tip technique. [5] Finally, the immobilized metal affinity chromatography (IMAC) protocol was used for the enrichment of the class I MHC phosphopeptides before the analysis with high-performance liquid chromatography coupled to electrospray ionization–tandem mass spectrometry (HPLC-ESI-MS/MS). [6] Complementary data obtained from the collisional activated dissociation (CAD) and electron transfer dissociation (ETD) fragmentation techniques were used for the manual sequencing and determination of the phosphosite. This technology enabled the identification and sequencing class I MHC phosphopeptides at the attomole level from resected tumor samples as low as 0.1g. About two-thirds of 122 identified class I MHC phosphopeptides from HNCs tumor samples were already identified in other cancer types like melanoma, colorectal, liver, and ovarian cancers. [5] The overlapping of the identified class I MHC phosphopeptides among various cancer types and between different cancer patients shows their immunotherapeutic merits.

Poster 053: Modulating Immunopeptidomes through *ex vivo* Manipulation

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Tumor neoantigens hold immense potential for cancer immunotherapies but are limited by challenges with *in vivo* detection and validation. This is due to a dearth of neoantigens presented on cell surfaces via Human Leukocyte Antigen–Class I and –Class II, otherwise known as the immunopeptidome. I hypothesize that HLA–Class I and –Class II immunopeptidomes can be modulated to increase neoantigen presentation, expanding the therapeutic opportunities for adoptive T-cell therapy or personalized cancer vaccines. Initial experiments demonstrate that rapamycin treatment causes protein abundance changes that are apparent in the proteome. Normally less abundant HLA molecules such as HLA-C and HLA-DQ are consistently unpregulated. NetMHCpan-4.0 binding affinity estimates of eluted HLA–I peptides show that mean binding affinity is significantly lower for alleles HLA–B*15:10 and HLA–C*04:01. There is also a reproducible increase in high-affinity pHLA–I for all alleles following 48 hours of rapamycin treatment. For both HLA–C*03:04 and HLA–C*04:01, the increase in unique pHLA–I is 2-fold. Thus, rapamycin-induced changes in HLA expression consistently alter the immunopeptidomic landscape.These data indicate a potential role for rapamycin in increasing neoantigen presentation and expanding immunotherapy opportunities.

Poster 054: Unbiased Solution for MS-based Immunopeptidomics with High Sensitivity and Accuracy

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Identification of tumor-specific antigens (neoantigens) is needed for development of effective cancer immunotherapy and a good source for such antigens are the pools of HLA-bound peptides presented exclusively by the tumor cells. Mass spectrometry (MS) has evolved as the method of choice for the exploration of the human immunopeptidome (HLA class-I and class-II peptides). Workflows for

immunopeptidomics are different from ones for more established shotgun proteomics which is biased to tryptic peptides, yet inherent differences between these two fields create significant drawbacks of current data analysis algorithms for the former. We provide de novo sequencing for peptide identification to address the barriers for data analysis, e.g., diverse C-termini of HLA-peptides, lack of sequence library for spliced peptides. With deep learning technology, we first time proposed peptide de novo sequencing algorithms for data independent acquisition (DIA) method. Further, de novo sequencing was integrated with motif-constrained database search for HLA-peptide identification to increase peptide sequencing coverage, depth, and confidence. Our approach was tested with several public data sets for the identification of HLA-peptides or neopeptides, including DDA (PXD007596, PXD006939, PXD004964, and PXD002431) and DIA (PXD001094). On average, 60% more HLA-peptides were identified by validation with the public immunopeptidome databases, IEDB and NeuroPep. Preliminary results showed that the de novo sequencing-based data analysis would provide a novel solution for immunopeptidomics with high sensitivity and accuracy.

Poster 055-ML: A New Strategy for the Global Identification and Validation of Post-Translationally Spliced Peptides with Neo-Fusion

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A controversy has emerged regarding the existence and prevalence of post-translationally spliced peptides in biology. Such peptides have been reported as both autoantigens in pancreatic islets and as HLA-I associated antigens in tumors, and thus have major potential implications for autoimmunity and cancer immunotherapy. The investigation of spliced peptides by mass spectrometry is currently limited by the nontrivial nature of spliced peptide identification and is typically accompanied by a high false-positive rate. We developed Neo-Fusion, a software program to assist in the identification and validation of post-translationally spliced peptides in complex samples. Neo-Fusion uses separate b- and y-ion databases to identify the N-terminal and C-terminal halves of each spliced peptide and determine the best matching sequence. We present a new and comprehensive strategy to increase the sensitivity and confidence of spliced peptide identification. This strategy uses Neo-Fusion and the auto *de novo* program PEAKS to first identify putative spliced peptide sequences. These candidate sequences are then manually validated through comparisons of theoretical and experimental retention times and peptide fragmentation patterns. Through this work, we have identified common pitfalls in the field that can lead to misidentification of post-translationally spliced peptides and massively underestimated false discovery rates. Thorough reanalysis of published data from manuscripts reporting large scale presentation of post-translationally spliced peptides indicates that most of the reported spliced peptides were assigned incorrect sequences. Freely available tools exist for the rapid evaluation of reported spliced peptide identifications, and these tools should be employed whenever spliced peptide discovery is attempted. Using our strategy, we estimate that, at most, 1-4% of all HLA-I associated peptides can be explained through post-translational splicing.

Poster 056-TL: SILAC-based Quantitative Proteogenomics Unveil Altered MHC-associated Peptidome in Osimertinib Resistant Human Lung Adenocarcinoma

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The 3rd generation epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI), osimertinib is very effective against EGFR mutant lung adenocarcinoma. Unfortunately, all patients develop resistance. EGFR mutant patients respond poorly to immune checkpoint inhibitors. Adoptive T-cell therapy (ACT) holds great potential for treating metastatic cancers. Since cytotoxic T cells largely recognize the major histocompatibility complex (MHC) class I-associated peptides, tumor-specific neo-peptides are attractive targets for ACT. Here, we employed a proteogenomic approach using SILAC-based quantitative proteomics to quantify the class I-associated neo-peptides from parental PC9 ($EGFR^{Del}$) lung adenocarcinoma cells, and isogenic osimertinib resistant PC9 (PC9-OsiR-NCI-1) following pan-MHC class I pull-down from cellular lysates and detection of class I-associated peptides by tandem mass spectrometry in a QE-HF mass spectrometer. Cell line-specific databases were built using whole exome sequencing (WES) data. PEAKS and Maxquant were used for identification and quantification of peptides. We identified 10711 class I-associated peptides which were 6-15 amino acid residues in length and 11 variant peptides, many of which were not predicted as neoantigens using epitope prediction algorithms. Interestingly, 90% of the peptides contained either a lysine, arginine or both; therefore, SILAC could be used for quantitation. Among the peptides identified in both cell lines, 17 and 440 had increased and decreased abundance, respectively in the resistant cells. 178 peptides were identified only in PC9 parental cells and 10 peptides only in resistant cells. The SILAC data of class I-associated peptidome demonstrate that there may be significant reduction of class I presentation by osimertinib resistant cells. In summary, we have established an integrated proteogenomic platform to provide direct evidence of neo-peptide presentation and to estimate the abundance of the MHC peptidome. Further experiments are underway to identify such variant neo-peptides in tumor tissue from osimertinib resistant patients enrolled in our clinical protocol at the NIH Clinical Center.

Poster 057-ML: Uncovering the Prognostic and Therapeutic Potential of N-Acetyl-Aspartyl-Glutamate Metabolism in Cancer

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N-acetyl-aspartyl-glutamate (NAAG) is a peptide-based neurotransmitter that has been extensively studied in many neurologic diseases. In this study, we unveiled NAAG's role in the promotion of cancer growth and its elevated presence in more aggressive cancers. In cancers expressing glutamate carboxypeptidase II (GCPII), the enzyme that catalyzes the hydrolysis of NAAG to glutamate and N-acetyl-aspartate (NAA), we demonstrated that NAAG acted as a glutamate provider, restoring cell proliferation upon glutamate deprivation. GCPII was thus identified as an alternative target enzyme for cancer therapy, especially in cancers showing resistance to glutaminase inhibition therapy in current clinical trials. In GCPII deficient-cancers, NAAG also enhanced cancer cell growth but through a metabotropic glutamate receptor 3 (mGlu3)-dependent

pathway. Moreover, NAAG was found to be a potential non-invasive metabolite monitor for cancer progression. This newly discovered role of NAAG has greatly reshaped our understanding of its multi-faceted prognostic and therapeutic significance.

Poster 058: Uncovering the Role of N-Acetyl-Aspartyl-Glutamate as a Glutamate Reservoir in Cancer

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N-acetyl-aspartyl-glutamate (NAAG) is a peptide-based neurotransmitter that has been extensively studied in many neurological diseases. In this study, a specific role of NAAG is identified for the first time in cancer. We found that NAAG is more abundant in higher-grade cancers and is a source of glutamate in cancers expressing glutamate carboxypeptidase II (GCPII), the enzyme that hydrolyzes NAAG to glutamate and N-acetyl-aspartate. Knocking down GCPII expression through genetic alteration or pharmacological inhibition of GCPII results in decreased glutamate concentrations and reduced cancer cell proliferation in vitro, as well as tumor weights in vivo. Moreover, targeting GCPII in combination with glutaminase inhibition accentuates the reduction of glutamate concentrations, cell proliferation and tumor weights. These findings suggest that NAAG serves as an important reservoir to provide glutamate to cancer cells through GCPII when glutamate production from other sources is limited. Thus, GCPII is a viable target for cancer therapy, either alone or in combination with glutaminase inhibition.

Poster 059: The Intestinal Microbiome And Its Metabolites Are Unaltered by Pathogen-Specific Monoclonal Antibodies

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The microbiome plays a key role in human health and its perturbation is increasingly recognized as contributing to many human diseases. Although antibiotic therapy for bacterial infections has revolutionized medicine, it is now clear that broad-spectrum antibiotics alter the composition and function of the host microbiome. This adverse effect on the microbiome coupled with the emergence of multi-drug resistant pathogens has spurred development of pathogen-specific strategies, such as monoclonal antibodies (mAbs), to combat bacterial infection. We hypothesized that pathogen-specific mAbs against prevalent antibiotic resistant pathogens would not significantly disrupt the intestinal microbiota as compared to conventional antibiotics. We treated 7-week-old, female, C57BL/6 mice with either a single systemic mAb dose or (saline as a control) or human equivalent doses of the antibiotics vancomycin, linezolid, or levofloxacin for 5 consecutive days. Independent experiments were conducted for each indication. We performed microbiome and metabolomic studies using fecal pellets collected from individual animals prior to treatment and for up to 14 days. The taxonomic abundance and diversity of the bacterial genera and key metabolites (short-chain fatty acids, bile acids and tryptophan metabolites) in the fecal pellets from mice treated with pathogen-specific mAbs were minimally affected and were similar to that in the control groups. In contrast, as expected, metabolites were drastically changed in antibiotic-treated mice (12 hr, and day 7). This correlated with dramatic changes in the relative abundance, as well as alpha- and beta-diversity in the feces of all antibiotic treated groups. While the metabolites showed some restoration at day 14, changes in the microbiome persisted until the end of the study. Taken together, these results indicate that pathogen-specific mAbs do not alter the fecal microbiome like broad-spectrum antibiotic therapies do and may therefore represent an approach to antibacterial therapy that leaves a microbiome intact.

Poster 060: Temporal Changes in Protein Abundance and Subcellular Location Reveal HL60 Cellular Response to Methotrexate Treatment

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Elucidation of protein responses to biological stimuli (*e.g.*, therapeutics) is primarily based on the measurement of abundance changes. Recently, large-scale measurement of protein translocation events across subcellular compartments in an untargeted fashion has been demonstrated in a comparison of control and treated cells. However, the comprehensive understanding of cellular response to perturbation requires knowledge of both abundance and localization changes over time. The spatial proteome is dynamic, complicating the validation of a protein's assignment to a particular subcellular compartment and its translocation between two compartments. We present a strategy to investigate both protein abundance and localization changes in a temporally resolved fashion with specific application to elucidating, in HL60 cells, a comprehensive mechanism of action for methotrexate. HL60 cells were exposed to methotrexate for durations ranging from 20 seconds to 48 hours. Vehicle-treated control and methotrexate-treated samples were processed for whole cell lysate (4 replicates, 12 time points), phosphoenrichment (SILAC labeled, 2 replicates, 10 time points) or fractioned via centrifugation to obtain nuclear, membrane, and cytosolic compartments (3 replicates, 5 time points). Samples were processed by automated digestion (abundance changes) or filter-aided sample preparation (subcellular fractions), desalted, and analyzed by mass spectrometry. In total, 2,102 proteins and 3,253 phosphoproteins changed significantly in abundance and 4,265 proteins from subcellular fractions changed significantly. Of these, 3,228 proteins changed significantly in abundance within a compartment and 2,795 proteins translocated from one compartment to another, with 1,758 proteins overlapping. Temporal abundance and location changes were incorporated into a custom data analysis pipeline for efficient integration and extraction of biological relevance. DNA damage, cell cycle arrest, and apoptosis were explored. This approach provides efficient workflows to gain comprehensive insight into the dynamics of temporal and spatial proteomics during cellular response to perturbation.

POSTER ABSTRACTS

All posters will be displayed Monday and Tuesday.
Each day: odd-numbered posters present 1:30-2:15 pm; even-numbered posters present 2:15-3:00 pm.
ML = Mon. lightning talk; TL = Tues. lightning talk; WTT = Wed. tips & tricks talk.

Poster 061: Multi-omic Profiling of TKI resistant K562 Cells Suggests Metabolic Reprogramming to Promote Cell Survival

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Resistance to chemotherapy can occur through a wide variety of mechanisms. Typically, resistance to tyrosine kinase inhibitors (TKIs) is thought to arise from kinase mutations or signaling pathway reprogramming—however, "off-target" adaptations enabling survival in the presence of TKIs without resistant mutations are poorly understood. Previously, we established cell line resistance models for the three most commonly used TKIs in chronic myeloid leukemia treatment, and found that their resistance to cell death was not attributed entirely to failure of kinase inhibition. In the present study, we performed global, integrated proteomic and transcriptomic profiling of these cell lines to describe the mechanisms of resistance at the protein and gene expression level. The proteomic and transcriptional data were correlated to generate an integrated understanding of the gene expression and protein alterations associated with TKI resistance. We identified mechanisms of resistance that were unique to each TKI. Additionally, we defined mechanisms of resistance that were common to all TKIs tested. Resistance to all of the TKIs was associated with the oxidative stress responses, hypoxia signatures, and apparent metabolic reprogramming of the cells. Metabolite profiling and glucose dependence experiments showed that the resistant cells relied on glycolysis (particularly through the pentose phosphate pathway) more heavily than the sensitive cells, which supported the idea that metabolism alterations were associated with resistant cell survival. These experiments are the first to report a global, integrated proteomic and transcriptomic analysis of TKI resistance. These data suggest that targeting metabolic pathways along with TKI treatment may overcome pan-TKI resistance.

Poster 062: Integrative Proteomics for Non-Canonical Protein and Proteome Discovery using the ProteomeGenerator

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Modern mass spectrometry now permits genome-scale measurements of biological proteomes. However, analysis of specific specimens is hindered by the incomplete representation of protein sequence variability, and by the technical demands for the construction a sample-specific sequence databases. Here, we describe ProteomeGenerator, a framework for de novo and reference-assisted proteogenomic analysis based on sample-specific genome and transcriptome sequencing, and high-accuracy mass spectrometry proteomics. This enables the assembly of sample-specific proteomes encoded by mutant and non-canonical genes, including protein isoforms resulting from alternative mRNA transcription, splicing, or editing. To improve the accuracy of protein identification in non-canonical proteomes, this method relies on statistical target-decoy database matching calibrated using sample-specific controls. The current version of ProteomeGenerator integrates automated peptide-spectral matching using MaxQuant, and is implemented as a Snakemake workflow within a Singularity container for one-step installation in diverse computing environments. We applied ProteomeGenerator for the proteogenomic analysis of splicing factor SRSF2 mutant leukemia cells, demonstrating high-confidence identification of non-canonical protein isoforms arising from alternative transcriptional start sites, intron retention, and cryptic exon splicing as well as improved accuracy of genome-scale proteome discovery. Additionally, we report proteogenomic performance metrics for SEQUEST HT, MaxQuant, Byonic, and PEAKS mass spectral analysis algorithms, obtained using a generalized target-decoy strategy based on inclusion of both decoy spectra and decoy sequences. Thus, ProteomeGenerator should facilitate the discovery of non-canonical and neomorphic biological proteomes, as required for metaproteomic, immune, and cancer proteomic studies.

Poster 063-ML: Coordination between TGF-β Cellular Signaling and Epigenetic Regulation during Epithelial to Mesenchymal Transition

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Epithelial to mesenchymal transition (EMT) is a reversible and plastic process in which epithelial cells lose their junctions and polarity to gain a motile mesenchymal phenotype. It plays a crucial role in cancer propagation. EMT can be orchestrated by the activation of multiple signaling pathways, where have been found highly coordinated with many epigenetic regulators. Although the mechanism of EMT has been studied over decades, crosstalk between signaling and epigenetic regulation is not fully understood.

Here we present a time-resolved multi-omics strategy, which featured the identification of the correlation between protein changes (proteome), signaling pathways (phosphoproteome) and chromatin modulation (histone modifications) dynamics during TGF-β-induced EMT. By taking the advantages of SILAC (stable isotope labeling by amino acids in cell culture) based quantification, proteins concentrated at areas of cell-cell adhesion and cell migration were grouped after 1-day TGF-β stimulation, overall suggesting that structural proteins rearrangements evoked in the later transition state. Significant regulations of phosphorylations were detected after only 5 min treatment, leading to the prediction that Erk signaling was activated upon immediate stimulation. The comprehensive profiling of histone post-translational modifications identified H3K27me3 (histone H3 lysine 27 trimethylation) as the most significantly up-regulated mark. This PTM is catalyzed by Ezh2, which is itself regulated by phosphorylations. We thus speculated and confirmed that a combined inhibition of Erk signaling and Ezh2 was more effective in blocking EMT progress than individual inhibitions.

In summary, our data provided a more detailed map of cross-talk between signaling pathway and chromatin regulation comparing to previous EMT studies. Our findings point to a promising therapeutic strategy for EMT-related diseases by combining Erk inhibitor (singling pathway) and Ezh2 inhibitor (epigenetic regulation).

Poster 064: Hdac4 Interactions in Huntington's Disease Viewed through the Prism of Multiomics

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Huntington's disease (HD) is a monogenic disorder, driven by the expansion of a trinucleotide (CAG) repeat within the huntingtin (Htt) gene and culminating in extensive neuronal degeneration in the brain, predominantly in the striatum and cortex. The histone deacetylase 4 (Hdac4) was found to contribute to the disease progression, providing a potential therapeutic target. Hdac4 knockdown reduced the accumulation of misfolded Htt protein and improved HD phenotypes. However, the underlying mechanism remains unclear, given its independence on deacetylase activity and the predominant cytoplasmic Hdac4 localization in the brain. Here, we undertook a multiomics approach to study the Hdac4 contribution to HD pathogenesis. We characterized the interactome of endogenous Hdac4 in the brains of HD mouse models. Alterations in interactions

were investigated in response to Htt polyQ length, comparing mice with normal (Q20) and disease (Q140) Htt, at both pre- and post-symptomatic ages (2 and 10 months, respectively). Parallel interaction analyses for Hdac5, a related class IIa Hdac enzyme, highlighted the unique interaction network established by Hdac4. To validate interactions and distinguish those specifically enhanced in an HD-vulnerable brain region, we next characterized endogenous Hdac4 interactions in dissected striata from this HD mouse series. We discovered that Hdac4 associations were largely polyQ-dependent in the striatum, but not in the whole brain, particularly in symptomatic mice. In contrast, Hdac5 interactions did not exhibit polyQ dependence. To identify which functions and proteins within Hdac4 interactions could participate in HD pathogenesis, we integrated our interactome with proteome and transcriptome datasets generated from striata of the same HD mouse models. We found an overlap in enriched functional classes with the Hdac4 interactome, particularly in vesicular trafficking and synaptic functions. This study expands the knowledge of Hdac4 regulation and functions in the context of HD, adding to the understanding of the molecular underpinning of HD phenotypes.

Poster 065: A Multi-omic Approach Identifies DLK1 as a Candidate Oncoprotein and Immunotherapeutic Target in High-Risk Neuroblastoma

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Immunotherapeutic strategies have produced remarkable results in some malignancies; however, optimal cell surface targets in many cancers remain elusive. We have developed a multi-omic approach to identify high-confidence cell surface oncoproteins for immunotherapy development and applied it to neuroblastoma (NB), an often fatal childhood cancer. We first utilized an optimized sucrose density gradient methodology followed by nano-liquid chromatography coupled to mass spectrometry (nLC-MS/MS) to identify proteins on the surface of NB cells, including 12 human-derived NB cell lines and 10 patient-derived xenograft (PDX) models. This yielded on average 66% (range:60-68%) membrane protein enrichment with high reproducibility between biological replicates (80%; range:78-84%) and identified 1,010 unique proteins containing an extracellular domain. Proteomic data was integrated with extensive NB and normal tissue expression data to discover NB-specific (or enriched) proteins for validation and assessment of biological relevance. This approach confirmed known cell surface proteins under development as immunotherapeutic targets in NB (ALK, GPC2, DLL3, NCAM1, CD276), and prioritized Delta Like Non-Canonical Notch Ligand 1 (DLK1) for further study. High DLK1 expression in patient tumors obtained at diagnosis predicted poor outcome. Integration of H3K27ac chromatin immunoprecipitation (ChIP) sequencing and RNA-sequencing from NB cells revealed that *DLK1* expression was uniquely driven by the presence of a super-enhancer at the locus. DLK1 protein expression was validated by Western blotting, immunofluorescence, flow cytometry, and immunohistochemistry on both NB and normal tissue microarrays (TMAs). Silencing of DLK1 in NB cell models using short hairpin RNAs (shRNAs) resulted in significant growth inhibition and induction of neuronal differentiation. Taken together, we have defined the first MS-based surfaceome of NB and identified DLK1 as a candidate oncoprotein and immunotherapeutic target. Ongoing *in vivo* studies in PDX models of NB will assess the efficacy of DLK1-based immunotherapy and efforts are underway to extend this multi-omic approach to additional high-risk childhood cancers.

Poster 066: Network Integration of Omics Data for Fast-track Identification of the Mechanisms of Action for Drug Candidates

Akos Vertes[1]; Albert Arul[1]; Peter Avar[1]; Andrew Korte[1]; Lida Parvin[1]; Ziad Sahab[1]; Deborah Bunin[2]; Merrill Knapp[2]; Andrew Poggio[2]; Carolyn Talcott[2]; Brian Davis[3]; Christine Morton[3]; Christopher Sevinsky[3]; Maria Zavodsky[3]
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Identification of the mechanism of action (MoA) for drug candidates through targeted assays typically takes years and contributes to the lengthy drug approval process. This challenge is best addressed through high-throughput omics technologies to monitor the cellular response to drug exposure, followed by network mapping of the resulting data for the reconstruction of their MoAs. The response of hepatocytes (HepG2/C3A) exposed to Nexturastat A—an HDAC-6 inhibitor and leukemia drug candidate—was followed at 10 different time-points ranging from 10 s to 48 h. Transcripts and proteins were then analyzed by microarray-based transcriptomics and multiplex shotgun proteomics, respectively. Non-targeted metabolomics experiments were also conducted using UPLC-MS and laser desorption ionization MS from silicon nano-post arrays (NAPA). A network was constructed based on correlation analysis of the molecular time profiles, and the MoA was extracted based on Markov clustering and modularity class analysis. Conventional assays were used to validate the MoA. Omics analyses resulted in the identification and quantitation of >67,000 transcripts, >3,000 proteins, and >400 metabolites over 10 time-points. The statistically-significant regulation of 2,089 transcripts (|FC| >2.0), 235 protein groups (|FC| >1.5), and 203 metabolites (|FC| >1.3) was observed. Spearman correlation-based network mapping of these biomolecules (rs > 0.96) resulted in the formation of 20,286 edges and 19 modularity classes. Gene set enrichment analysis (GSEA) was then performed to identify the biological processes overrepresented in each class. This linked the MoA to cell cycle arrest, DNA damage/apoptosis, and chromatin modification, and to lipid accumulation in the cells. Flow cytometry analysis confirmed G1/S checkpoint inhibition. BODIPY stain for lipid droplets and immunofluorescence against pan-acetyl-lysine confirmed the accumulation of lipid droplets and the upregulation in lysine acetylation, respectively. Our omics approach followed by network mapping rapidly identified the MoA of a drug candidate.

Poster 067: UniProt Genomic Mapping for Deciphering Functional Effects of Missense Variants

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Understanding the association of genetic variation with its functional consequences in proteins is essential for the interpretation of genomic data and identifying causal variants in diseases. Integration of protein functional knowledge with genome annotation can assist in comprehending genetic variation within complex biological processes. Here, we describe mapping UniProtKB human sequences and positional annotations such as active sites, binding sites, and variants to the human genome (GRCh38) and the release of a public genome track hub for genome browsers.

To demonstrate the power of combining protein and genome annotations, we present specific biological examples in disease-

related genes and illustrate how missense variants in key functional features of a protein can alter a protein's structure and function and if severe enough are classified as harmful. Comparisons of UniProtKB annotations and protein variants with ClinVar clinically annotated SNP data show that 32% of UniProtKB variants co-locate with 8% of ClinVar SNPs. The majority of co-located UniProtKB disease-associated variants (86%) map to 'pathogenic' ClinVar SNPs. To get an overview of variants in different functional features we examined SNPs from ClinVar that overlap selected protein features and plot the percentage of ClinVar SNPs, in each ACMG annotation category, that overlap each protein feature. Six features have more pathogenic variants than either benign or uncertain (Disulfide Bonds, Initiator Methionine, Intramembrane Region, Natural Variant, DNA Binding Domain, Active Site).

In summary, linking annotated data with assertions, publications and other evidence from UniProtKB, ClinVar or other datasets via co-location on the genome, can help integrate protein and genomic analyses and improve interoperability between the genomic and proteomic communities to determine the functional effects of genome variation on proteins. The location of a variant within functional features may correlate with pathogenicity and would be a useful attribute for use in variant prediction algorithms, including machine-learning approaches.

Poster 068: Developing an Integrated Global, glyco-, and Phosphoproteomic Workflow for Large Scale Analysis of Tissue Samples

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The rapid technology development and applications of mass spectrometry-based technologies have enabled the comprehensive proteomic analysis of global proteins and protein post-translational modifications (PTMs). The conventional workflows for characterization of global proteome and proteins with PTMs are often carried out independently and separately. Thus, there is a crucial need for developing an effective and integrated proteomic workflow for global proteins and their PTMs. In this study, we first investigated the impact of protein glycosylation and phosphorylation to global proteomics and found that the use of glycosidase (PNGase F) and phosphatase (CIP) to remove the modifications of global proteins could improve the number of peptide and protein identification of global proteome of breast cancer xenograft tissue samples. We then developed a new integrated workflow for global, glyco-, and phospho-proteomics analysis where samples (after trypsin digestion) went through IMAC enrichment for phosphopeptides followed by MAX enrichment for glycopeptides. The peptides after depletion of phophopeptides and glycopeptides were analyzed for global proteomics. Similar to what we observed by enzymatic de-glycosyaltion and de-phosphorylation, the proteomic analysis of peptides with depleted glycopeptides and phosphopeptides showed 1.18-, 1.19- and 1.30-fold increase in the number of identified proteins, peptides, and PSMs comparing to those without depletion (i.e., global proteome obtained before going through IMAC and MAX enrichment). There was high reproducibility of our integrated workflow for global proteome and suggesting that using PTM-depleted peptides improved the identification of global proteome adequately. Moreover, for the enriched phosphopeptides, we identified an average 5,798 and 9,317 unique phosphopeptides and phospho-PSMs from three replicates with specificity values over 98%. For glycopeptides, we also confirmed the phenomenon of co-capture and elution of glycopeptides from phosphopeptide enrichment and observed different glycan distribution profiles of glycopeptides enriched by MAX. In summary, our integrated workflow provided a new dimension of multi-proteomics analysis of tissue samples.

Poster 069: Mathematic, Machine Learning Methods on DESI Mass Spectrometry of Brain Tissue

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Imaging mass spectrometry generates information rich patterns of chemical spectra. Application of imaging mass spectrometry to spatial regulation of metabolism is an exciting new opportunity. Independent of this research, methods in mathematics, programming, and machine learning have continued to progress, providing new tools to solve address complex problems. Effective application of such methods could allow significant headway to be made into analysis of mass spectrometric data, uncovering more about the molecular processes that accompany neurodegeneration. We utilized mass spectrometry to collect images of mouse brain tissue. Here, I demonstrate methods for automatic brain region detection and analysis by applying mathematical and machine learning algorithms to mass spectrometry based images of brain tissue.

Poster 070-TL: An Integrated Multi-omic Analysis in iPSC-Derived Motor Neurons from C9ORF72 ALS Patients

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The NeuroLINCS consortium produced a detailed molecular characterization of motor neurons from induced pluripotent stem cells (iPSCs) derived from patients with amyotrophic lateral sclerosis who carried hexanucleotide expansions in C9orf72 - the most common known cause of ALS. Searching for early molecular differences, we characterized cellular states through ATAC-seq, RNA-seq, and data-independent acquisition mass-spectrometry (DIA-MS) proteomics. Several pathways, including biological adhesion and extracellular matrix organization were altered across epigenomic, transcriptomic, and proteomic data. In addition, protein isoforms predicted from the transcriptomics data were analyzed in the matching proteomics data set to determine if isoforms were translated and if there were quantity changes between C9ORF72 ALS and control. A similar workflow was employed to investigate the presence of novel splice variants identified in the transcriptomic data within the DIA-MS data and if there were quantity changes between C9ORF72 ALS and control. We were able to detect 11 potentially novel splice variants within the DIA data, with five of these being differential between ALS and Control.

Poster 071-ML: Host-centric Stool Proteomics Reveals Latent-Phase-Expressed Host Protease Inhibitors Modulate EAE Severity

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Diseases are often diagnosed once classically recognized symptoms arise, ignoring the latent period prior to disease when biological pathways contributing to disease are altered but disease state is not outwardly detectable. For example, experimental autoimmune encephalomyelitis (EAE), a model for multiple sclerosis, exhibits a latent period of approximately 10 days before overt symptom onset, but changes prior to these symptoms are not well characterized. Recent evidence suggests microbial community structures and specific microbial constituents influence disease outcome of EAE, yet

its impact on the latent phase remains unknown. Here, we show the phenotypically latent period between immunization and EAE's overt symptom onset is characterized by clear host responses, as measured by host-centric proteomics. In particular, we found a sharp, transient increase in protease inhibitor abundance inversely correlated with EAE severity. Antibiotic administration largely attenuated the increase in protease inhibitors. These results strengthen previously discovered ties between proteases and their inhibitors play critical roles in the severity of EAE. More broadly, our findings highlight the utility host-derived stool-omics can have for revealing complex, dynamic biology.

Poster 072: Investigating Modified Lipid Metabolism in Brain Tissue Associated with Varying Isoforms of Apolipoprotein in Relation to Alzheimer's Disease

John Holman; Peter Jones; Monique Speirs; Russell Denton; John Price
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Lipid transport and metabolism are connected to multiple predictors of Alzheimer's Disease (AD) risk. Apolipoprotein (APoE), a protein involved in lipid transport is the strongest genetic risk factor. APoE2 demonstrates a low risk for AD development, APoE3 contains neutral risk for AD development, and APoE4 is associated as a risk factor for AD development. Bioactive lipid signaling, has been associated across a variety of cell-signaling pathways and plays a key role in determining cell fate. While it has been shown that possessing the APoE4 isoform increases likeliness of AD development, the connection between APoE4 and modified lipid metabolism has not been fully explored. Using mouse brain tissue collected from the 3 APoE isoforms, I will present our recent work investigating changes in lipid transport and metabolism. This will help determine how lipid transport and metabolism impacts biochemical pathways within the brain to modify AD risk.

Poster 073-ML: Proteomic Analysis of the Developing Inner Ear in *Xenopus laevis*

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Congenital hearing loss is one of the most prevalent birth defects in which 1.4 in 1000 infants screened in the US have hearing loss. To understand congenital hearing loss, we need to expand our current knowledge of the proteins that play a role in forming the inner ear during development.

The South African clawed frog (*Xenopus laevis*) is a well-characterized animal model in developmental biology whose inner ears are evolved for land-based hearing. Recent studies in *Xenopus* have explored the transcriptomic profiles of developed inner ears to reveal human hearing loss gene orthologs in this animal model. However, the information on the numerous proteins and their dynamics during the formation of the inner ear is still lacking. To address this knowledge gap, we here developed a methodology using liquid chromatography and high-resolution mass spectrometry to quantify protein production in the developing inner ear (otic tissue) of *Xenopus laevis*. Briefly, otic tissues were manually dissected from five larval stages that represent key transitions in otic patterning starting from the otic vesicle up to the formation of all primary inner ear components. Proteins were extracted from the dissected tissues and digested with trypsin. The resulting peptides from each larval stage were barcoded with a unique tandem mass tag (TMT) and analyzed together to quantify relative proteomic changes between stages of inner ear formation. Our preliminary analysis led to the identification of over 3,000 protein groups, of which ~74 proteins are associated with hearing loss in humans. We are now exploring this information to identify unique proteomic trends during inner ear patterning. Our approach to study the temporal dynamics of proteins during otic development holds a potential to identify novel proteins guiding the formation of the vertebrate ear.

Poster 074: Identification and Validation Protein Phosphorylations Regulating Synapse Loss in Schizophrenia

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Reductions in dendritic spines, the postsynaptic component of excitatory synapses, have been reproducibly observed in multiple brain areas in schizophrenia (Sz) and are believed to underlie cortical processing deficits. Synaptic plasticity is regulated by synaptic protein network features such as protein trafficking and activity, both of which are mediated by posttranslational modifications (e.g. phosphorylation). Additionally, a significant number of Sz risk genes code for synaptic proteins. Here, in brain tissue from 50 Sz and 50 matched control subjects, we utilized parallel microscopy, proteomic, and phosphoproteomic approaches to identify protein phosphorylations highly correlated with both dendritic spine loss and synaptic protein level alterations in Sz. The effects of one candidate phosphorylation (MAP2 S426) were evaluated in HEK293 cells and mice.

We observed robust changes to synaptic and phosphorylation levels of canonical postsynaptic proteins in Sz ($q < 0.05$) that were not explained by postmortem interval or protein expression. WGCNA and cross-network analyses observed significant correlations ($q < 0.05$) between synaptosome, phosphorylation, and dendritic spine alterations in Sz. Nine phosphorylations on eight proteins were highly correlated with both synaptic protein alterations and spine loss.

Our findings suggest that Sz genetic risk and synaptic protein network pathology manifests in processes beyond gene and protein expression, such as protein trafficking and activity. Of the eight proteins with spine and synaptic protein level correlated phospho-alterations, all but one have well-documented roles in vesicular trafficking of postsynaptic glutamate receptors and/or regulating dendritic spines, indicating that they could be upstream of Sz spine loss. Preliminary analysis of one candidate phosphorylation (MAP2 S426E) found that this single modification was capable of impairing MAP2-microtuble binding and resulted in decreased cortical volume, a well replicated finding in Sz, further supporting a role for these phosphorylations in Sz pathology.

Poster 075-ML: Understanding Epigenome and Proteome Remodeling Caused by Novel Germline Histone H3.3 Mutations during Neurodevelopment

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Histone H3.3 (H3.3) is a histone variant often found at actively transcribed loci. H3.3 also plays a role in cellular inheritance as ablation of H3.3 expression leads to loss of active gene states and dysfunction of heterochromatin telomeric structures.H3F3Aand H3F3B, the two genes known to encode H3.3, are ubiquitously expressed in all human cells with higher expression in the ovaries, testes, uterus and brain. Somatic mutations in the H3F3Agenes have

been reported as oncogenic drivers of pediatric glioblastomas. More recently, here at the Center for Applied Genomics at the Children's Hospital of Philadelphia novel germline mutations in both H3F3genes have been discovered in a small cohort of patients who suffer from a common pattern of neurodevelopmental disorders, seizures and craniofacial abnormalities. Here we hypothesize that mutant H3.3 (mH3.3) change the regulatory capacity of mH3.3 containing chromatin and therefore modulate gene expression and ultimately the cellular proteome. To this end, we developed stable murine astrocyte cell lines expressing either wild type H3.3 or mH3.3 (G90R, T45I) to study by quantitative mass spectrometry the histone post translational modification changes to the mH3.3 containing nucleosomes and subsequent proteome alterations. Our preliminary data has identified downregulation of chromatin remodeler proteins, as well as upregulation of mitochondrial proteins following reprogramming of the reprogrammed mH3.3 encrusted epigenome. Ultimately, our studies aim to identify and validate potential epigenetic and proteome-wide factors involved in these novel neurodevelopmental disorders. Understanding the basic mechanisms of how these new histone mutations function in neurodevelopment may allow re-classification of epigenome reprogramming in neurological contexts.

Poster 076-TL: Measuring Parkison's Disease Mitochondrial Protein Turnover Rates in Human IPSC-Derived Organoids by Mass Spectrometry

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Parkinson's Disease (PD) is a currently incurable neurodegenerative disorder that manifests in the elderly through motor symptoms of bradykinesia, rigidity and tremor. PD causes a dopamine deficit, which leads to ineffective neural motor function. For unknown reasons, certain neuronal populations involved in the disease will die whilst others nearby that are very similar the same will survive. One of the prevalent theories explaining this selective death is the mitochondria stress hypothesis, where the neuron populations associated with PD are under more stress than others, resulting in their death from mitochondrial quality control mechanisms. Therefore, understanding the mechanisms of mitochondrial quality control in these PD-associated neural populations is critical. There are two PD-associated proteins: PINK1, a mitochondrial-targeted kinase, and Parkin, a ubiquitin ligase. Previous studies have found that the regulation of mitochondrial proteins in fruit flies is impaired by mutations in Parkin and PINK1, characterized by a deficit in mitochondrial protein turnover. Whether mitochondrial proteins are similarly regulated in mammals has yet to be confirmed. In the current study, our aim was to validate the human induced pluripotent stem cell organoids model in measuring protein turnover. We used mass spectrometry proteomics to examine the effect of a Parkin mutation in a human induced pluripotent stem cell (IPSC) organoid model. We used stable isotope labelling (SILAC) in the amino acid leucine to measure protein half-life and turnover in time-course experiments. Our preliminary results have shown the incorporation of heavy isotope labels into the organoids is associated with a decrease in protein turnover in Parkin mutated organoids. Additional experiments are underway to optimize mass spectrometry methods and proteomic software to maximize the detection and identification of proteins affected by the mutation. In the future, we will expand to other PD-related organoid models to test drugs that stimulate mitochondrial quality control.

Poster 077-ML: Early Candidate Urine Biomarkers for Detecting Alzheimer's Disease before Amyloid-β Plaque Deposition in an APP (swe)/PSEN1dE9 Transgenic Mouse Model

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Alzheimer's disease (AD) is an incurable age-associated neurodegenerative disorder that is characterized by irreversible progressive cognitive deficits and extensive brain damage. The identification of candidate biomarkers before amyloid-β plaque deposition occurs is therefore of great importance for the early intervention of AD. Urine, which is not regulated by homeostatic mechanisms, theoretically accumulates changes associated with AD earlier than cerebrospinal fluid and blood. In this study, an APP (swe)/PSEN1dE9 transgenic mouse model was used to identify candidate biomarkers for early AD. Urine samples were collected from 4-, 6-, and 8-month-old transgenic mouse models, and the urinary proteomes were profiled using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). The levels of 29 proteins differed significantly between wild type and 4-month-old mice, which had not started to deposit amyloid-β plaque. Among these proteins, 13 have been associated with the mechanisms of AD, while 9 have been suggested as AD biomarkers. Our results indicated that urine proteins enable detecting AD before amyloid-β plaque deposition, which may present an opportunity for intervention.

Key words: Alzheimer's disease (AD), urine proteome, early diagnosis, APP (swe)/PSEN1dE9

Poster 078: Single Step Protein Extraction from Trace-amount Human Hair for Genetically Variant Peptide Detection

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Recent reports have demonstrated that genetically variant peptides (GVPs) derived from human hair shaft proteins can be used to differentiate individuals of different biogeographic origin. Human hair has the potential to be a suitable alternative to DNA for human identification if GVPs existing in hair cuticular keratins and keratin related proteins can be reliably and accurately identified. To detect them, we first need a sensitive method to extract proteins out of trace-amount human hair shaft. However, hair protein extraction remains challenging due to the extensive cross-linking and poor solubility. We developed a new method called single-step extraction that can extract hair proteins, mainly hair cuticular keratins, from as little as 1 cm-long human hair shaft. We also compared this newly developed method to two previously published methods: 1) ProteaseMax based method; 2) NaOH with SDS repeated extraction method. We found that our newly developed single step protein extraction method is the most sensitive method among the three methods. Furthermore, the construction of a human hair specific peptide mass spectral library, including previously reported GVPs, enables the evaluation of human hair proteome coverage and the effect of each protein extraction method on hair specific peptide identification and GVP detection.

Poster 079-WTT: Spray-Capillary: An Electrohydrodynamic Spray Assisted Device for Quantitative Ultra-Low Volume Extraction

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Analysis of ultra-low-volume samples provides invaluable insights in studying complex biological systems. In genomics, single-cell sequencing (SCS) has been advanced to study rare cell population, heterogeneity, etc. However, the proteomics or metabolomics analysis

of ultra-low-volume samples remains underachieved mainly because of lacking microsample handling and analysis techniques.

Efforts have been made to improve the sensitivity and throughput of ultra-low-volume sample analysis in MS-based omics, including advanced MS instrumentation, high-resolution separation, and efficient spray-MS interfaces. Although the sensitivity of such approaches has been dramatically improved, considerable sample losses during sample preparation largely limit the application of MS-based omics on ultra-low-volume samples, requiring the development of novel microsample handling techniques.

Herein, we proposed an electrohydrodynamic spray-assisted device for quantitative ultra-low-volume sample extraction, named Spray-Capillary. We utilized the ESI process for quantitative sample extraction through an etched long capillary (bare capillaries for proof-of-principle and coated CE capillaries for on-line separation and detection). Specifically, ESI voltage is applied to initiate the sample injection. The spray of droplets results in a small vacuum force in the capillary which drives the solution in the capillary move from the sample vial to the ESI emitter. We evaluated the reproducibility and accuracy of sample injection amounts using our Spray-Capillary by varying the ESI voltage, capillary ID, and length. Our results demonstrate that the Spray-Capillary allows reproducible, accurate, and quantitative sample injection ranging from 1 nL to 100 nL or higher. We further etched a LPA coated capillary for online CE-MS/MS of both peptides and protein mixtures.

The Spray-Capillary is a very simple quantitative sample handling device for ultra-low-volume samples. It can be directly integrated to a CE capillary for sensitive sample injection and separation without any additional devices, and therefore holds great potential for the high-throughput omics analysis of ultra-low-volume samples, such as single0cell mass spectrometry.

Poster 080-WTT: Total Solubilization of FFPE Samples for High throughput Clinical Proteomics

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Formalin fixed paraffin embedding (FFPE) is a decades-old sample preparation technique common in experimental research and medicine. FFPE embedded samples can be stored indefinitely at room temperature, resulting in an exceptionally large and rich worldwide collection. Despite its potential, proteomic analysis of FFPE samples has lagged. Traditionally, samples are first laboriously deparaffinized with often-toxic organic solvents. Subsequent protein extraction is extremely critical but no consensus has been reached as to an optimal protocol. Standardization is fully lacking.

Here, we present a one-pot solution which eliminates deparaffinization and which employs 5% SDS and S-Trap sample processing to exhaustively solubilize entire FFPE samples evaluated on human liver. Samples were paired and split, half flash-frozen and half fixed in formalin and paraffin embedded according to standard histopathology procedures. All samples were extracted with SDS using standard techniques (pulverization, syringe needles), probe sonication or Covaris AFA ultrasonication. SDS was removed by standard precipitation or S-Traps. Protein identification rates and reproducibility were evaluated after analysis on a Thermo QE HF-X or Fusion mass spectrometer.

Compared to standard procedures, the use of S-Traps resulted in significant increases in peptide (≥30%) and protein identification rate (≥20% increase) with greater reproducibility. The use of AFA decreased hands-on time, increased ID rates an additional 6% – 8% and significantly increased protein yield from FFPE samples (80% – ≥200%). The combination of S-Traps and AFA yielded ID rates comparable to those obtained from fresh frozen tissue (101%/ 97% ID rates for peptides/proteins) while eliminating toxic xylene and saving approximately 6 hrs in sample processing by avoiding deparaffinization.

Our system solves the problem of extraction bias and achieves the goal of reproducible standardized protein recovery from FFPE samples in a workflow suited to automated, high-throughput analyses. We anticipate this workflow will assist to usher in a new era of clinical proteomics.

Poster 081: Evaluation of a Novel LC System that Embeds Analytes in Pre-formed Gradients for Rapid, Ultra-Robust Proteomics

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Mass spectrometry-based proteomics and metabolomics are fast growing and powerful technologies, with the potential to revolutionize health care and precision medicine. However, available separation systems have so far limited throughput and robustness and thereby prevented omic technologies from being fully integrated and routinely applied in clinical settings. Here, we evaluate a conceptually novel liquid chromatography (LC) system that significantly increases robustness and sample throughput for cutting edge proteomic workflows.

The new system, called Evosep One, uses four low-pressure pumps in parallel to elute samples from a disposable and single use trap column while also forming a chromatographic gradient. The sample and gradient are moved into a storage loop that subsequently is switched in-line with a single high-pressure pump and an analytical separation column for separation.

We evaluated and benchmarked how to use the Evosep One in cutting edge LC-MS workflows to significantly increase overall performance and throughput and demonstrated the value of being able to increase the sample size in studies. The cross contamination (<0.07%), retention time stability and peak properties were monitored over 1500 HeLa runs and the short overhead time of approximately 2 min allows us to efficiently measure 300, 200, 100, 60 or 30 samples per day with corresponding gradient lengths of 3.2, 5.6, 11.5, 21 and 44 minutes, respectively. From fractionated HeLa cell lysates, deep proteomes covering more than 130,000 sequence unique peptides and around 10,000 proteins were rapidly acquired (18 h total instrument time). Using this data as a library for data independent acquisition, we demonstrate the quantitation of up to 5200 proteins in only 21 minutes.

We also demonstrate how this can be applied to clinical research workflows that require uninterrupted analysis of thousands of crude biological samples as well as routine applications such as doping and drug screening.

All posters will be displayed Monday and Tuesday.
Each day: odd-numbered posters present 1:30-2:15 pm; even-numbered posters present 2:15-3:00 pm.
ML = Mon. lightning talk; TL = Tues. lightning talk; WTT = Wed. tips & tricks talk.

Poster 082: PASEF on a TIMS-QTOF Instrument Is Reproducible, Sensitive and High-Throughput for Shotgun Proteomic Workflows

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In data dependent acquisition (DDA) experiments 20% of eluting peptide features are targeted for sequencing by MS/MS currently, due to limitations in sequencing speed, sensitivity and resolution. The previously introduced “Parallel Accumulation Serial Fragmentation” method (PASEF, Meier et al., JPR 2015) on a trapped ion mobility spectrometry quadrupole time of flight mass spectrometer has been shown to increase the sequencing speed and sensitivity of MS/MS scans at high resolution. We demonstrate instrument performance on low sample amounts (< 200 ng), offering unprecedented possibilities to investigate samples at high sensitivity, high throughput and high reproducibility.

We observed a sequencing speed > 100 Hz with a gain in sensitivity and no loss in resolution. In each 90 min gradient we could identify more than 5300 proteins (from 200 ng HeLa digest injected on column) and a total of 5672 proteins covering a dynamic range of 4-5 orders of magnitudes. Comparing protein identities between the triplicate analyses, we observed that more than 90% of the identified proteins were identified in each of them. This indicates high reproducibility with the PASEF method. Also, the reproducibility of the label-free intensities determined by MaxQuant is excellent with a $R^2 = 0.98$. To evaluate the accuracy of label free quantification we spiked in E.coli and yeast in two different concentrations into the HeLa proteome. All species could be nicely separated in the expected ratios of 1:2 and 1:5. Using very low sample amounts (<12 ng) of Hela digest and a 60 min gradient resulted in 20,000 unique peptides identified, corresponding to 2000 protein groups. Also using short gradient of 20 min and a sample amount of only 100 ng resulted in the identification of around 25,000 unique peptides and 3000 proteins groups.

Poster 083-WTT: 30 Second Analysis of Histone Post-Translational Modifications by Direct Infusion Mass Spectrometry

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Proteomics has become a fundamental and widely recognized discipline for basic science. The next frontier is its application in translational medicine. While much progress has been achieved, proteomics integration in diagnostics still has issues of throughput and repeatability (batch effects). These drawbacks are due to nano-liquid chromatography, as chromatographic columns have highly variable performance and require frequent replacement. We present a novel workflow for the analysis of histone post-translational modifications (PTMs) via direct injection in mass spectrometry (DI-MS) without the usage of liquid chromatography. Histone PTMs play fundamental roles in modulating DNA readout, including gene expression and DNA repair, but additionally, their disregulated abundance serve as drivers and potential diagnostic markers of many types of cancers.

We reduced the two-day sample preparation required to purify and digest histones into a 7 hour protocol (from tissue to ready-to-inject sample) which can be easily parallelized for ~750 samples per batch. The protocol includes a spike-in of a synthetic peptide that benchmarks the quality of sample preparation. Samples are eluted from desalting columns into 96-well plates and are ready to spray using dedicated equipment such as the NanoMate (Advion). Each injection replaces the tip used for pickup, solving the issue of carry-over and more importantly batch effects. Spectra are acquired using a multiplexed targeted selected ion monitoring (tSIM-MSX) combined with MS/MS events to discriminate isobaric forms, collecting the signal of >200 different histone PTMs in less than 30 seconds. Additionally, we have developed a new freely available computational software, EpiProfileLite, for raw file processing and peptide quantification. In conclusion, our workflow is capable of extracting and analyzing >1,000 histone samples per day, compared to the 15-20 we could with our previous state-of-the-art system. We believe this represents an important first step towards more robust analysis of epigenomes for clinical applications.

Poster 084-WTT: Kinetics of Acetone Precipitation: Optimizing Conditions to Efficiently Concentrate and Purify Protein Samples in Minutes with the ProTrap XG

Jessica Nickerson; Alan A. Doucette
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Introduction
Acetone precipitation is a widely used method of concentrating and purifying complex protein samples in top-down proteomic workflows [3]. Our group previously established that the addition of 1-30 mM salt facilitates protein recoveries >95% [2]. We later developed a filtration cartridge (ProTrap XG), which automates precipitation and SDS depletion [1]. The current bottleneck with acetone precipitation relates to the lengthy (overnight) incubation. In the interest of maximizing protein recovery, purity, and throughput, we investigated the kinetics of protein precipitation, optimizing conditions to achieve the most efficient protein recovery.
Methods
Aqueous protein samples (BSA, cytochrome c, and a yeast proteome) were combined with 4 volumes of acetone at a defined temperature (-20 to +37degC), either in conventional vials, or within the ProTrap XG, which contains a PTFE membrane to trap aggregated protein. The samples were incubated for times ranging from 2 minutes to 24 hours followed by centrifugation. The isolated supernatant was retained and residual protein was quantified by a Pierce BCA assay.
Results
It was found that increased temperature, protein and salt concentration provided the most efficient protein recovery. Under optimal conditions, >99% recovery was achieved after just 2 minutes of incubation. Kinetically slower conditions, such as low protein concentration, showed improved rates when additional salt was present or incubation temperature was increased.
Precipitated yeast proteins will be visualized by SDS PAGE (BioRad) and identified through a standard bottom-up LC-MS/MS approach.
Facilitated by the ProTrap XG and an improved understanding of precipitation kinetics, sample preparation ahead of top-down proteomic analysis is incredibly reliable and efficient.
References
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2. Crowell et al. (2013). *Analytica Chimica Acta*, *796*, 48-54.
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Poster 085: Size Dependence for Protein Precipitation: Optimized Conditions for Efficient Recovery of Low-Mass Proteins in the ProTrap XG

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Introduction: An essential consideration of any protein sample cleanup approach is maintaining high recovery of all sample components during purification. With organic solvent precipitation, low molecular weight proteins are generally less susceptible to

aggregation in organic solvent. A question remains as to what conditions enable maximum precipitation of low-mass proteins. Our group previously demonstrated the importance of salt in maximizing recovery of proteins during solvent precipitation [1]. We also demonstrated that aggregated proteins could be rapidly isolated in a two-stage filtration cartridge called the ProTrap XG [2]. We herein extend our previous work with an objective to establish precipitation protocols for the purification and recovery of low molecular weight proteins and peptides, suitable for both top-down and/or middle-down proteomic applications.

Methods: Pepsin-digested BSA was used to generate a mixture of low molecular weight protein fragments (<5 kDa). The sample was precipitated in the ProTrap XG by addition of organic solvent (acetone, acetonitrile, etc.) with inclusion of various concentrations of salt (NaCl, Na2SO4, ZnSO4, etc.). The pellet was isolated from the supernatant and subject to analysis by HPLC with UV quantitation, as well as SDS PAGE and LC-MS/MS to identify proteins/ peptides recovered in the resulting fractions.

Preliminary Results: Our findings indicate a strong dependence on the type of salt in the sample. The highest recoveries were determined using divalent ions (ZnSO4 > Na2SO4 > NaCl). SDS PAGE confirms the recovery of low molecular weight peptides (<5 kDa) in the pellet fraction with intensity similar to that of the control. Subsequent work will evaluate the size trends and properties of peptides recovered in the pellet vs. supernatant following optimized precipitation.

References:

1. Crowell et al., Analytica Chimica Acta, 796 (2013) 48–54.
2. Crowell et al., Journal of Proteomics, 118 (2015) 140-150.

Poster 086: Hands-off: Fully Automated & TMT-compatible Sample Preparation in Less than 4 Hours on the PreOn Platform Employing the iST-NHS Technology

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Sample preparation is a very important component of the overall mass spectrometry-based proteomics workflow and remains to be a limiting factor for high-throughput analyses. One method to increase the throughput is sample multiplexing using chemical labeling approaches such as iTRAQ or TMT. Here, we present a fully automated end-to-end solution for standardized sample processing, including cell lysis, digestion, TMT labeling and peptide cleanup within 4 hours.

In order to minimize sample loss and improve reproducibility, we sought to completely automate TMT sample processing from cell lysis to ready-to-measure labeled peptides. To facilitate this, we aimed to combine the NHS adaption of the iST technology (Kulak et al., 2014) with a newly developed automation platform called PreOn (HSE AG).

The workflow described here integrates robotic handling with chemical labeling of peptides in the very same reaction device used for cell lysis, protein denaturation, reduction, alkylation, digestion as well as the peptide cleanup. This workflow minimizes sample loss, overall hands-on time and the amount of required chemical labels by 50%. The PreOn platform features a built-in centrifuge, ultrasound liquid detection, deck load check and a drag-and-drop method selection for easy, fast and convenient user guidance.

We demonstrate successful labeling of several cancer cell lines using 11-plex TMT achieving >99% labeling efficiency and a reproducibility of R^2 = 0.98 for biological replicates (CVs <10%). Furthermore, we present data on more complex samples such as yeast or plasma and combination with peptide fractionation to increase proteome depth.

The described hands-off workflow enables sample processing and TMT-labeling of up to 11 samples in parallel in a fully automated fashion and in less than 4 hours, scaling from 1-100 µg of protein input material. We envision processing of tissue samples combined with chemical labeling to be implemented soon.

Poster 087: High-throughput and Robust Plasma Analysis with Capillary-flow LC and High-Resolution Accurate-Mass (HRAM) Mass-Spectrometry

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Analysis of blood and biofluids is a key step in clinical research. The different blood components (drugs, metabolites, electrolytes, proteins) are routinely measured in laboratories. The most widespread methods for quantification of individual proteins rely on antibodies. However, immunoaffinity methods have multiple limitations related to limited specificity and problems with multiplexing. In contrast, LCMS become a reliable technology that combines significant depth into the proteome and impeccable specificity including differentiation of post-translational modifications.

Limiting factors of LCMS adoption are high costs, low throughput, and insufficient robustness of typical nanoLCMS proteomics methods that are used in research. In this work, we developed a new capillary-flow LCMS method with a throughput of 100 samples per 24 hours. The UltiMate® 3000 RSLCnano system was coupled with Q Exactive® HF-X HRAM mass-spectrometer. Crude and top 12 depleted enzymatically digested plasma samples were separated on EASY-Spray® (ES800, 75 µm x 150 mm, 3 µm) column with flow rate 1 µL/min. Low gradient delay volume, fast sample loading (9 sec) and parallel washing of trap cartridge and analytical column allowed to achieve > 85% MS utilization. CapLCMS method was tested in two laboratories by analyzing of > 500 depleted and crude serum samples. The robust retention times (SD < 0.1 min), peak areas (RSD, % < 20), highly symmetric and sharp peaks (PWHM ca. 5 sec) confirmed that method is suitable for the analysis of large sample cohorts. More than 1500 peptide groups from plasma proteins which abundance cover 5 orders of magnitude can be identified in less than 15 min of analysis time.

Thus, capLCMS has a strong potential for providing a reliable, specific and multiplexed solution for identifying plasma protein fingerprints on a routine basis with low-costs per sample.

Poster 088: Quantifying Ubiquitination Signaling with a Chemical Proteomics Strategy

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Stoichiometric analysis of posttranslational modifications is an emerging strategy for absolute quantification of the modification's fractional abundance. In this study, we present a quantitative chemical proteomic workflow for stoichiometric analysis of ubiquitination, named Isotope-BAlanced Quantification of Ubiquitination (IBAQ-Ub). The strategy utilizes a new amine-reactive chemical tag (AcGG-NHS) for highly efficient labeling of amine residues. The new chemical tag is structurally homologue to the GG remnant of ubiquitin on modified lysine following trypsin cleavage and therefore enables the generation of structurally identical peptides from ubiquitinated and unmodified lysine residues following trypsin digestion and the secondary stable isotopic labeling. Quantitative analysis of labeled peptides with HPLC and high resolution mass spectrometry allows unbiased measurements of absolute site-specific ubiquitination stoichiometry. Using recombinant protein standards and isotopic serial dilution, we

showed that the strategy is highly robust, sensitive and accurate. We further designed a SILAC-based experiment to demonstrate the efficient labeling of lysine with the new AcGG tag in complex cell lysates. Application of the IBAQ-Ub workflow for the analysis of enriched histone fractions allowed us to measure the stoichiometry of histone H2B C-terminal ubiquitination. Comparative analysis of stoichiometric dynamics upon proteasome inhibitor treatments showed a dramatic decrease of histone H2B ubiquitination levels and a significant increase of polyubiquitination K48 linkage. Thus, this work provides an efficient chemical proteomics tool for quantitative stoichiometric analysis of ubiquitination signaling pathways in cells and diseases.

Poster 089-ML: Building an Antiviral Platform: Nuclear Protein Oligomerization as a Key Contributor to Innate Immune Response

Tim Howard; Krystal Lum; Catherina Pan; Ileana Cristea
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Innate immune responses to invading pathogens rely on the ability of specific host proteins to identify pathogen molecules and induce signals. The interferon-inducible protein IFI16 was shown to recognize pathogenic DNA after a variety of viral infections, including herpesviruses and HIV, as well as bacterial infections. Given the expression of IFI16 in numerous tissues relevant to host-pathogen interactions, including skin, nasal, and oral mucosa, this protein is thought to play a critical role in maintaining balanced immune responses for a healthy system. IFI16 has two functions in host defense against the dsDNA viruses herpes simplex virus type 1 (HSV-1) and human cytomegalovirus (HCMV). It induces antiviral cytokine expression and suppresses viral gene expression. At the core of these antiviral responses are its functional domains: a PYRIN domain (PYD) that mediates its oligomerization and two HIN-200 domains that bind pathogenic DNA. Our lab has previously demonstrated that PYD is required for IFI16 localization to incoming viral DNA at the nuclear periphery. However, how the PYD induces IFI16 oligomerization and the function of this nuclear aggregation in antiviral response remained unknown.

Here, we used an integrative approach of molecular biology, virology, microscopy, and quantitative proteomics to explore the role of IFI16 oligomerization during herpesvirus infection. We first discover solvent-exposed charged residues that regulate IFI16 oligomerization. This allowed us to generate IFI16 mutants that lack the ability to oligomerize. We find that cells expressing oligomerization-incompetent IFI16 permit more productive infections and result in increased virus protein production. Using immunoaffinity purifications of IFI16 mutants and parallel reaction monitoring (PRM) MS/MS, we determine that IFI16 oligomerization mediates interactions with other critical antiviral proteins. We further establish the necessity of IFI16 oligomerization for immune signaling. Altogether, we provide mechanistic insights into the contributions of PYD oligomerization in viral DNA sensing for innate immune response.

Poster 090-TL: Location, Location, Location: Using Spatial Proteomics to Uncover Functional Protein Translocations during Viral Infection

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Protein movement between organelles lies at the core of numerous essential cellular processes, such as gene expression, immune signaling, and apoptosis. As obligate intracellular parasites, viruses must co-opt these pathways, and consequently, viral infections induce diverse changes in proteome organization. Our lab has previously characterized the broad changes that occur in the protein composition of organelles during human cytomegalovirus (HCMV) infection. This led us to identify temporal alterations in protein localizations during infection, suggesting the existence of numerous, yet undiscovered, protein movement events. However, the knowledge of the global regulation of protein translocations during HCMV infection remains limited, and the analysis of proteins localized to multiple compartments remains challenging. Here, we designed a computational approach that is robust for multiply-localized proteins and can characterize protein movements between diverse cellular organelles during the progression of HCMV infection. Indeed, we uncovered translocations of both cellular and viral proteins, which we further confirmed and investigated using live cell microscopy. Additionally, this analysis allowed us to discover proteins that co-translocate with one another, pointing to putative functionally-related complexes. Consequently, these movements may contribute to biological processes that underlie either virus replication or host defense. For example, we found that the unconventional myosin, MYO18A, co-translocates with myosin motors and clathrin-associated adaptor proteins to sites of virus assembly. Follow-up functional analyses, including microscopy, virus genome and titer measurements, and knockdown assays, showed that MYO18A is required for virus production. Specifically, MYO18A is hijacked by the virus late in infection to aid the trafficking of viral particles for cellular egress. Overall, this project integrates spatial proteomics, computational biology, and molecular virology methods to gain new functional insights into the contribution of proteome organization to the intricate interplay between viruses and their hosts. Furthermore, this computational method can be applied to characterize translocating proteins during diverse biological processes.

Poster 091: Identifying the Molecular Mechanisms of Sex-Specific Severity of the Middle East Respiratory Syndrome Coronavirus (MERS-CoV) using Proteomics

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The novel Middle East Respiratory Syndrome Coronavirus (MERS-CoV) is a poorly understood pathogen causing high mortality rate (~35%) and surprising higher lethality in male vs female patients. The transgenic human DPP4 mouse is currently the most valuable model to study the poorly understood pathogenesis of MERS-CoV infection. We applied state-of-the-art mass spectrometry and unbiased non-parametric statistics in a transcriptomics/proteomics project to identify proteins modulated in response to MERS-CoV infection.

We analyzed 90 Trizol homogenates of lung tissue from male and female hDPP4 mice inoculated with high (10^5 TCID) and low (10^2 TCID) doses of MERS-CoV obtained on day 1, 2, 3, 4 and 5, along with mock controls. The quantification of the viral proteome showed that high dose inoculation led to similar viral load in the first 3 days, but then this dose decreased in females while it kept growing monotonically in males (Mann-Kendall test). Kaplan-Meier analysis of our mice models confirmed a longer survival rate for females than males. When comparing the proteomics response of high vs low dose of viral inoculation, we observed a remarkably different enrichment in protein functions between males and females; females translated mostly proteins related to defense response, while males “delayed” the response by activating first proteins related to generic translation. We identified remarkable differences in the abundance trends

(Kolmogorov-Smirnov goodness of fit test) of proteins involved in the B- cell receptor signaling between sexes (confirmed by transcriptomics analysis), showing that this pathway is activated at an earlier stage in females. Together, we have identified a critical difference in immune response in male vs female mice infected by MERS-CoV using non-parametric statistics on large proteomics data.

Poster 092-TL: Antiviral Function of Mitochondrial Sirtuin 4 during Human Cytomegalovirus Infection

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Sirtuins (SIRTs) are evolutionary-conserved enzymes, known to be ubiquitously expressed in mammalian cells and critical regulators of core cellular pathways. We previously discovered that SIRTs are broad-spectrum viral restriction factors, protecting host cells against DNA and RNA viruses. Among SIRTs, knockdown of mitochondrial SIRT4 led to the largest increase in replication of tested DNA viruses, including the β-herpesvirus human cytomegalovirus (HCMV). HCMV is a prevalent human pathogen, estimated to infect over half of the world's population. Although HCMV infection is typically asymptomatic, HCMV is a major concern for immunocompromised individuals and the leading infectious cause of congenital birth defects. Here we aimed to define mechanisms underlying SIRT4-mediated antiviral functions and their temporal regulation during HCMV infection. Although initially thought to be a deacetylase and ADP-ribosyltransferase, we discovered that SIRT4 is a more potent lipoamidase. SIRT4 removes the rare but essential posttranslational modification lipoylation from the pyruvate dehydrogenase complex (PDH), thereby inhibiting PDH activity. Therefore, SIRT4 functions as a critical regulator of cellular metabolism by modulating carbon entry from glycolysis into the TCA cycle. To determine the mechanism of SIRT4 antiviral function, we next used a multidisciplinary approach, integrating proteomics, microscopy, enzyme activity assays, and molecular virology. We monitored SIRT4 lipoamidase activity and the regulation of PDH lipoylation and PDH activity throughout the HCMV replication cycle. These findings were placed in the context of the requirement for upregulation of the TCA cycle and fatty acid biosynthesis during HCMV replication. Furthermore, characterization of SIRT4 protein interactions using mitochondrial enrichment and immunoaffinity purification-mass spectrometry led to the identification of a previously uncharacterized viral protein as a novel SIRT4 interaction. Follow-up functional assays via generation of virus strain mutants and enzymatic activity assays led us to demonstrate that this interaction represents a mechanism acquired by HCMV to inhibit the antiviral activity of SIRT4.

Poster 093: *Developing an Analysis Pipeline for PfEMP1s in Parasites Isolated from Children Presenting with Malaria*

Patricia Gonzales Hurtado[1]; Robert Morrison[1]; Jose M. C. Ribeiro[2]; Hussein Magale[1]; Oumar Attaher[3]; Bacary Diarra[3]; Almahamoudou Mahamar[3]; Amadou Barry[3]; Alassane Dicko[3]; Patrick Duffy[1]; Michal Fried[3]
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In 2017, an estimated 210 million cases of malaria occurred in the world and 435,000 died globally. *Plasmodium falciparum* is the deadliest of the four parasites that cause malaria and infects humans. The parasite proteins on the surface of infected erythrocytes are an important target for vaccines to prevent severe malaria. PfEMP1 which are surface proteins are important targets for developing a protective immunity to malaria caused by *P. falciparum*. The major challenge in *P. falciparum* proteomics studies is identifying these PfEMP1s at the protein level due to extensive antigenic variation. To identify these PfEMP1s using shotgun proteomics, we developed a pipeline that searches high resolution mass spectrometry spectra against a custom protein sequence database. We analyzed parasites from thirty-one *P.falciparum* infected Malian children. These parasites were grown in culture and then membrane proteins were extracted and electrophoresed followed by in-gel digestion with trypsin. The pipeline was first validated in the analysis of a laboratory strain with a known PfEMP1, then it was implemented on the analysis of parasite isolates from malaria-infected pregnant women and finally on the analysis of parasite isolates from malaria infected children.

Poster 094-TL: Dynamic Regulation of Mitochondria Morphology, Composition, Acetylation, and Function during Viral Infection

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Mitochondria govern a wide range of cellular processes, including metabolism, innate immunity, cell death, and signaling pathways. As viruses rely on the regulation of these cellular processes for their replication and spread, mitochondria shape and functions are known to be altered during viral infections. For example, the prominent herpesvirus, human cytomegalovirus (HCMV), induces fragmentation and increased motility of mitochondria as a means to regulate mitochondrial metabolism and inhibit apoptosis and immune response. Further highlighting the critical roles of mitochondria during infection, we have reported that the mitochondrial deacetylase sirtuin 3 (SIRT3) restricts HCMV, suggesting that protein acetylation status is involved in antagonizing viral infection. Here, we aimed to uncover the mechanisms underlying mitochondrial regulation and the role of mitochondrial acetylation during HCMV infection. We first defined the temporal acetylome during HCMV infection, which we placed in the context of protein abundance by using parallel acetyl-peptide enrichment and proteome analyses. Organelle-specific acetylomes were derived from integration of protein localization information based on density fractionation. We found the mitochondrial acetylome to be the most elevated by infection, when compared to other organelles. Mitochondrial protein acetylations were confirmed by mitochondria enrichment and parallel reaction monitoring (PRM). We next interrogated whether this is controlled enzymatically or non-enzymatically. We observed a decrease in mitochondrial pH during infection, indicating that non-enzymatic regulation is unlikely to be the driving force. We hypothesized that SIRT3 interactions contribute to these acetylation changes, and performed immunoaffinity purification to investigate SIRT3 associations with substrates during infection. Proteins involved in oxidative phosphorylation and mitochondrial dynamics displayed changes in both acetylation and SIRT3 association. Indeed, we determine that mitochondrial membrane potential decreased upon infection, and that SIRT3 normally functions to maintain it. Altogether, we provide insights into the regulation of mitochondrial composition and acetylation status during infection, and mechanisms underlying SIRT3 antiviral functions.

Poster 095: Quantitative Membrane Proteomics Analysis of Low Density and High Density Neutrophils from *Staphylococcus aureus* Infected Diabetic Mice

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Staphylococcus aureus infection is one of the major infections that are difficult to control diabetes pathogenesis. Neutrophils play an important role in defense against *S. aureus* infection by phagocytosis, oxidative burst and neutrophil extracellular traps (NETs) formation. Low density neutrophils (LDN), a subpopulation of neutrophils has

been implicated in many pathological conditions. Understanding distinct features of low (LDN) and high density neutrophils (HDN) in antibacterial defense is important to monitor the effect of therapeutic drugs in treatment of diabetic infections. We carried out quantitative membrane proteomic analysis to unravel the differentially expressed protein machinery in these cell types.

High density neutrophils (HDN) and low density neutrophils (LDN) were isolated from red blood cells and enriched for membrane proteins. Digested membrane proteins were labeled using 6-plex TMT reagents. Labeled peptides were further fractionated into 10 fractions using basic reversed-phase fractionation on Oasis HLB material. LC-MS/MS analysis of TMT labeled peptides was carried out on an Orbitrap Fusion Tribrid™ (Thermo Fisher Scientific). Each fraction was also analyzed in multinotch MS/MS/MS mode. Mass spectrometry data was analyzed using Proteome Discoverer 2.2 (Thermo Fisher Scientific) software with search engines Mascot (version 2.6.0).

We have identified ~2,330 membrane associated proteins from neutrophils subtypes at 1% false discovery cutoff. Principal Component (PCA) analysis revealed distinct differentially expressed proteins in three LDN and HDN groups. Proteins encoded by Akt2 Ppp2r5d, Ptpa and Tfrc were significantly down-regulated in LDNs. In contrast, proteins encoded Tlr2, Cxcr2, Rab2a, Itm2b, Cd9, Cd177, C5ar1, Fpr2 were up-regulated in LDNs. Pathway analysis revealed protein changes associated with NET formation, AKT activation and central role of GSK3β in neutrophil function. The data from this study helped to understand the contribution of LDNs in *S. aureus* in diabetic mice model.

Poster 096-TL: Quantitative Proteomics Reveals Host Protein SLFN5 as a Target of HSV ICP0-Mediated Ubiquitination and Degradation

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Viruses have evolved mechanisms to hijack the host ubiquitin system in order to control ubiquitination of host proteins and thereby modify the cellular proteome and subvert host immune responses. Herpes simplex virus 1 (HSV) encodes “infected cell protein 0” (ICP0), a viral E3 ubiquitin ligase that is necessary for efficient infection. ICP0 is known to ubiquitinate and degrade host anti-viral factors, but the extent to which HSV utilizes ubiquitination during infection is not well understood. In order to identify host proteins that associate with the viral genome during infection, we used a quantitative proteomics approach that combines the “isolation of proteins on nascent DNA” (iPOND) technique with mass spectrometry (MS). We hypothesized that iPOND-MS data generated during wildtype or ΔICP0 HSV infection would identify potential ICP0 substrates by quantifying ICP0-dependent changes in the proteome associated with the viral genome. Using a PCA-based clustering strategy, we identify SLFN5 as a host protein that exhibits abundance patterns similar to known ICP0 substrates during infection, suggesting that SLFN5 is a potential ICP0 substrate. Additionally, di-glycine remnant profiling, combined with whole cell proteomics data, show that SLFN5 is ubiquitinated by ICP0 and decreased during infection. Subsequent experiments confirm the proteasomal degradation of SLFN5 during HSV infection and show that transcription of viral genes is enhanced by SLFN5 knockdown. These data suggest that SLFN5 is a restriction factor of HSV that inhibits viral gene transcription. Our study demonstrates that identification of the proteome associated with viral genomes during infection with mutant viruses can reveal host proteins that are degraded or mislocalized as the virus counteracts intrinsic host defenses. We used quantitative proteomics to identify the host protein SLFN5 as a novel substrate of HSV ICP0.Our data provide the first indication that SLFN5 is a restriction factor for DNA viruses and is degraded by a viral activator of transcription.

Poster 097: Quantitative Crotonylome Analysis Expands the Roles of p300 in the Regulation of The Lysine Crotonylation Pathway

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We recently discovered nine chemically distinct lysine modifications that can occur on histone proteins, including lysine crotonylation (Kcr) [1,2]. We demonstrated that lysine crotonylation can be enzymatically installed by acetyl-transferase, p300. p300-catalyzed histone Kcr is associated with active gene expression[3,4]. Nevertheless, the substrates regulated by p300 remain largely unknown, hindering efforts to establish other potential mechanistic links between Kcr and p300-madiated phenotypes. Here we used quantitative proteomics to characterize the p300-regulated lysine crotonylome in wildtype (WT) and in p300 knockout (KO) cells. A total of 816 unique endogenous crotonylation sites were identified across 392 protein, with 88 sites from 69 protein dynamically decreasing by 0.7-fold (log2 <-0.5) in response to p300 KO and 31 sites from 17 proteins increasing by 1.4-fold (log2>0.5) in response to p300 KO. The most significantly altered crotonylome pathways in the p300 KO cells are centered around key components of nonsense-mediated decay, infectious disease pathways, and viral/eukaryotic translation pathways. Further network analysis highlights the complexity and interconnectivity of the p300-targted Kcr substrates. Moreover, we reveal that some p300-targeted Kcr substrates are potentially linked to diseases such as cancer. Taken together, our results reveal the p300-regulated lysine crotonylome, shedding light on the role for lysine crotonylation in the regulation of diverse cellular processes, and providing the foundation for the discovery of new mechanistic roles for p300. References: **1.** Tan, M., Wysocka, J., Ye, Y., Khochbin, S., Ren, B., Zhao, Y. (2011). *Cell*, *146* (6), 1016-28. **2.** Bhanu, N., Yuan, Z., Garcia, B. (2018) H2AV Lysine Crotonylation: An Epigenetic Switch During Human Myogenic Differentiation. World HUPO. **3.** Sabari, B. R., Huang, H., Zhao, Y., Roeder, R. G., Allis, C. D. (2015). *Molecular cell*, 58(2), 203-15**. 4.** Li, Y., Sabari, B. R., Huang, H., Tang, Z., Zhao, Y., Roeder, R. G., Shi, X., Allis, C. D., Li, H. (2016). *Molecular cell*, *62* (2), 181-193.

Poster 098: Development of Mass Spectrometry-Compatible Peptide Biosensors to Detect Kinase Activity

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Protein kinases are responsible for attaching phosphate groups to other molecules as part of a signaling pathway. Kinases are strictly regulated, and can cause cancer when mutated; this is exemplified by the Philadelphia chromosome, a translocation mutation which renders Abl kinase constitutively active and causes chronic myeloid leukemia (CML). Mutations in other kinases, such as Janus kinase 2 (Jak2), have also been implicated in CML. Treatment of CML was progressed following development of kinase inhibitor imatinib (Gleevec®); unfortunately, a significant portion of patients are resistant to the inhibitor, rendering this therapy futile. Because of the importance of kinase response to inhibitor treatment, it is advantageous to quickly measure kinase activity in response to treatment. This can be accomplished through the use of a biosensor-a small peptide phosphorylated by the kinase and ideally analyzed by mass spectrometry (MS), due to the sensitivity of this method to analyze

clinically-relevant concentrations of cell samples. The goal of this project is to use KINATEST-ID, an in silico kinase substrate generator, to create a biosensor compatible with MS analysis methods to detect kinase activity. Biosensors were generated by KINATEST-ID, and then run through machine-learning algorithms in CONSeQuence to screen for predicted MS ionization efficiency, an indicator of MS detection compatibility. These substrates were then synthesized and purified, and used in an in vitro kinase assay with enzyme-linked immunosorbent assay (ELISA) analysis to determine biosensor specificity; these experiments were then repeated with other kinases to test for biosensor selectivity. We plan to continue analyzing ionization efficiency with synthesized Jak2 substrates, and will move forward by developing and analyzing substrates for Src family and Syk kinases.

Poster 099-ML: cGAS is Regulated by Phosphorylation and Acetylation during Infection with Herpes Simplex Virus 1

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Cyclic GMP-AMP synthase (cGAS) is a DNA sensor critical for initiating innate immunity in response to diverse types of pathogen infections. cGAS detects pathogenic DNA and induces the host expression of Type I interferons and other antiviral cytokines upon infection with both cytoplasmic- and nuclear-replicating DNA viruses, as well as upon bacterial infections. cGAS binds double stranded DNA and synthesizes cyclic GMP-AMP (cGAMP) to activate the STING-TBK1-IRF3 pathway and induce the cytokine expressions. With this central immune signaling axis defined, focus now starts to be placed on understanding the regulation of cGAS. What triggers cGAS activation and localization to pathogenic DNA? Although posttranslational modification (PTMs) has emerged as an important facet of cGAS regulation, only few cGAS modifications are known to date. Here, we aimed to acquire a better understanding of PTMs that can regulate cGAS during either cellular homeostasis or active immune signaling. We enriched cGAS via immunoaffinity purification and used quantitative mass spectrometry to characterize the cGAS modification status in uninfected primary human fibroblasts and upon sensing viral DNA. We compared cGAS PTMs upon infection with herpes simplex virus type 1 (HSV-1) and detection of cytoplasmic vaccinia virus DNA. These analyses led us to discover novel cGAS phosphorylation and acetylation sites during active immune signaling. We further validated and quantified infection-induced changes in these PTMs using parallel reaction monitoring (PRM). To characterize the function of these modifications, we generated a series of cGAS mutants that either mimic the acetylation or phosphorylation or an unmodified state. As a positive control, we compared these mutations to the mutation of the previously characterized S305 phosphorylation site. This allowed us to identify cGAS PTMs that impact cytokine induction, providing new insights into the dynamic regulation of cGAS during active immune signaling.

Poster 100: Mapping Disulfide Linkages Without Having to Detect Disulfide-linked Peptides

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Disulfide bonds are important structural elements of proteins; biological activity depends on their correct formation, and their assignment and verification are essential to the development and production of protein therapeutics such as monoclonal antibodies. Assigning disulfide connectivity is, however, a frequently difficult task, primarily because of the need to detect and perform tandem mass spectrometry on the crosslinked peptides. These can be large, sometimes multiply branched, and generally different in their chromatographic and mass spectrometric behaviors from the reduced and alkylated peptides routinely analyzed in the proteomics field. We therefore took an alternative approach that enables disulfide mapping on fully reduced and alkylated peptides that are chemically tagged in such a way that information about their original linkages is preserved.

Proteins were treated with dithiothreitol (DTT), varying time or temperature to yield partial reduction of the disulfides. Treatment with an alkylating reagent (e.g. iodoacetamide) marked all cysteines that were in their reduced forms. Proteins were then fully reduced and all remaining cysteines labeled with a second alkylating reagent (e.g. n-isopropyl iodoacetamide or acrylamide). Alternatively, isotopically labeled alkylating reagents such as deuterated versions or Iodo-TMT can be employed. Enzymatic digestion (typically tryptic) was performed and conventional reverse phase chromatography and tandem mass spectrometry were used to detect the now-fully reduced and alkylated peptides. The fraction of each cysteine alkylated with each reagent was used to calculate the amount of each cysteine that was in its reduced form after partial reduction. Symmetry requires that cleavage of a disulfide bond releases the involved cysteines to identical degree, so pairs of cysteines with the same extent of reduction are inferred to have been disulfide-linked. Examples of therapeutically important proteins including human growth hormone and monoclonal antibodies demonstrate that disulfide linkages can be assigned without directly detecting the disulfide-linked peptides.

Poster 101-ML: Ethionine, Produced by Commensal *Lactobacillus reuteri*, Is Immunomodulatory, Proteogenic, and Leads to Ethylation of Human Proteins

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Colonization of the human gut by immunomodulatory *Lactobacillus reuteri* strains reduces the risks for inflammatory diseases and colorectal cancer. The underlying molecular mechanisms, however, are not fully understood. Our previous research showed that the immunomodulation is dependent on *L. reuteri's* folate metabolism. Mass spectrometric (MS) analysis of *L. reuteri* strain 6475 folyl-polyglutamates revealed a novel folate derivative, corresponding to 5,10-methenyl tetrahydrofolate that carried an additional methyl group. It was identified by MS fragmentation and NMR to be 5,10-ethenyl tetrahydrofolate (Et-THF). Isotopic labeling of folic acid precursors and substrates of the folate cycle demonstrated that the two ethenyl carbons originated from acetate, thereby identifying acetate as a novel carbon source for the folate cycle in this commensal bacterium. Moreover, Et-THF not a dead-end product, but a substrate of the folate cycle. It enables the transfer of ethyl groups instead of methyl groups onto homocysteine, leading to the production of ethionine. The unconventional amino acid ethionine has immunomodulatory functions: Treatment of human monocytic THP-1 cells with ethionine, resulted in reduced proliferation and LPS-induced immune activation. Mass spectrometric analysis of histones of ethionine-treated THP-1 cells revealed a reduction of lysine methylation. Furthermore, we detected partial ethylation of histone tail lysine residues as a novel posttranslational protein modification. Ethionine was also incorporated into proteins in place of methionine, demonstrating the proteogenic potential of this uncommon amino acid. Our study implicates human microbiome as capable in modifying key human host proteins involved in gene regulation.

All posters will be displayed Monday and Tuesday.
Each day: odd-numbered posters present 1:30-2:15 pm; even-numbered posters present 2:15-3:00 pm.
ML = Mon. lightning talk; TL = Tues. lightning talk; WTT = Wed. tips & tricks talk.

Poster 102-TL: A Novel Method of Quantifying Protein Methylation Utilizing DIA-MS

Aaron Robinson[1]; Ronald Holewinski[1]; Vidya Venkatraman[1]; Jose Mato[2]; Shelly Lu[1]; Jennifer Van Eyk[1]
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Protein methylation of Arginine and Lysine is an important post-translational modification in disease but little is known about its effect in-vivo. Global protein methylation analysis has recently been enabled by the development of peptide immunoprecipitations against methylated Lysine and Arginine and subsequent mass spectrometry. However, methyl-enrichments require large amounts of sample and multiple antibodies to cover all methyl forms, the antibodies contain a sequence bias masking a portion of the methylome, and total protein quantification needs to be done separately from a methyl enrichment inhibiting the ability to study site occupancy. We have developed a method to differentiate an unmodified peptide from a peptide containing mono-, di- or tri-methylated Lysine or Arginine through a data independent acquisition approach utilizing small precursor mass windows. We then applied our method to complex cellular lysate from differentially methylated in-vivo non-alcoholic steatohepatitis mouse models. By first creating a biologically hyper-methylated peptide assay library to which each experimental sample is compared, we were able to identify and quantify protein methylation without the need to enrich each sample. In conclusion, our method provides the framework needed to study global protein methylation, as well as total protein quantification from the same data independent acquisition.

Poster 103: Method Development for Phosphorylation and Glycosylation Detection using Orbitrap Fusion Lumos

Susanne B. Breitkopf; Jeffrey A. Culver; Michelle F. Clasquin; Bei Betty Zhang; Mara Monetti
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Nearly all proteins undergo post-translational modifications (PTMs) which are crucial for function, structure, activity, expression of the proteins and protein interactions. Particularly glycosylation and phosphorylation are involved in pathways critical for signaling, and are often altered in disease states. Mass spectrometry (MS) is a powerful tool for the identification of protein glycosylation and phosphorylation due to its sensitivity of detection and its ability to analyze complex mixtures. Combining multiple MS fragmentation techniques (i.e. HCD, ETD, etc) allows for a comprehensive structural characterization of modified proteins.

Phosphorylation occurs on serine, threonine, and tyrosine residues. Typical methods for enrichment include immobilized metal affinity chromatography (IMAC) or titanium dioxide (TiO2) beads, which have selective affinity and interact with phosphopeptides. Comparing both methods we identified more unique phosphosites with IMAC compared to TiO2 enrichment. MS fragmentation techniques are also crucial for successful identification of PTMs and the most phosphosites were identified with IT-(ion trap)-HCD fragmentation using a top speed method (scan cycle with a maximum of 3 seconds).

Glycosylation can occur on asparagines (N-linked) or on serine or threonine residues (O-linked). Enrichment strategies such as TiO2, which is selective for sialic acid-containing glycopeptides, and lectins, with their ability to bind glycoconjugates, are often used. In our comparison the lectin enrichment exceeded the TiO2 strategy. In addition lectin enrichment on Filter Aided Sample Prep (FASP) filters is preferable for O-linked glycopeptides, lectin enrichment on agarose beads is superior for N-linked glycosylation. Similarly to phosphorylation, we compared different MS fragmentation techniques and identified the IT-ETD_OT (orbitrap)-HCD as the method that provides the most complete protein glycosylation coverage.

Poster 104-TL: ELTA-MS: Labeling, Enrichment and Identification of ADP-ribosylated Peptides by Mass Spectrometry

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The post-translational modification of polypeptides is a ubiquitous mechanism by which cells control the function, localization, and stability of cellular proteins. The modification of proteins with monomers or polymers of the small molecule ADP-ribose is known as ADP-ribosylation and is implicated in a wide variety of cellular processes including DNA repair and antiviral responses. In many cases the identities of ADP-ribosylated substrates and specific sites of conjugation are, however, unknown. In recent years, liquid chromatography-tandem mass spectrometry (LC-MS/MS) has proven to be an unparalleled tool for the global identification of PTMs on substrate proteins. Because ADP-ribosylation is not an abundant modification, the modified substrates must be enriched before LC-MS/MS analyses. Existing approaches utilize immobilized chemical groups and protein domains with an affinity for the ADP-ribose moiety to enrich for ADP-ribosylated peptides. These protocols are capable of identifying hundreds of sites of ADP-ribosylation. However, there is incomplete overlap between the different data sets, suggesting that the complete ADP-ribosylome has yet to be uncovered and highlighting the need for an approach with higher sensitivity. Recently our group has developed a novel method called ELTA for labeling ADP-ribosylated substrates with chemical groups, including tags such as biotin and clickable functional groups. Here we have applied this approach to optimize a workflow (ELTA-MS) for the specific labeling and functionalization of ADP-ribosylated peptides with biotin followed by selective enrichment and identification by mass spectrometry.

Poster 105: Detection of BTK Activity using a Terbium-Chelating Peptide Biosensor

Naomi Widstrom[1]; Minervo Perez[1, 2]; John Blankenhorn[1]; Lindsay Breidenbach[1]; Hannah Peterson[1]; Laurie L. Parker[1]
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Protein tyrosine kinases are involved in many crucial cell signaling pathways. Due to this, tyrosine kinases frequently exhibit altered activity in many types of cancer, making them excellent targets for cancer therapeutics. Efficient and effective assays are needed to test candidate tyrosine kinase inhibitors. To address this need, we develop peptide biosensors that are both good substrates and selective for the kinase of interest. In addition, several biosensors have been designed to selectively chelate to terbium. Terbium binds with stronger affinity to the phosphorylated tyrosine and has a longer luminescence lifetime, allowing a clear distinction between the phosphorylated and unphosphorylated forms of the peptide. We have illustrated this concept using a biosensor for Bruton's tyrosine kinase (BTK), a nonreceptor tyrosine kinase implicated in B-cell leukemias. Preliminary work identified sequences preferentially phosphorylated by BTK using phospho-proteomics. These sequences were run through the in-silico pipeline KINATEST-ID to generate a library of putative BTK substrates. Of these substrates, BTK artificial substrate D (BAStide-D) was designed to selectively chelate to terbium. We demonstrated that the percentage of phosphorylated peptide present can be determined using time-resolved luminescent detection. Kinase assays demonstrated that BAStide-D is rapidly phosphorylated by BTK, indicating it is an efficient substrate. We aim to further characterize BAStide-D by characterizing the kinetics of the biosensor

and kinase interaction. Additionally, we aim to use this assay to evaluate the response of BTK to inhibitors. This efficient antibody-free assay can be used to screen potential BTK inhibitors.

Poster 106: Human Breast Cancer Cell Line Phosphoproteome Revealed by an Automated and Highly Selective Enrichment Workflow

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Background
Immobilized metal affinity chromatography (IMAC) using a nitrilotriacetic acid (NTA) chelating ligand functionalized with Fe(III) is one of the most widely adopted phosphopeptide enrichment techniques for LC/MS applications. Agilent AssayMAP Bravo platform is able to provide a fully automated and highly selective phosphopeptide enrichment workflow using high capacity Fe(III)-NTA cartridges. In this study, we evaluated how the ratio of total peptide sample amount to affinity resin affects the performance of the enrichment results.
Methods
The Agilent AssayMAP was used for automated phosphopeptide enrichment with Fe(III)-NTA cartridge. Human MCF7 breast cancer cell line tryptic digest was loaded onto the cartridges with four different sample to resin ratios. With a 90-min nanoLC gradient, 6550 iFunnel Q-TOF was used for phosphopeptide discovery. MRM analysis of 20 light and heavy pre-spiked phosphopeptide standards was performed on 6495B QQQ to measure their overall recovery from the enrichment procedure.
Results
Prior to enrichment, about 1.5% of peptides were identified as phosphopeptides. After enrichment, phosphopeptide selectivity was routinely above 90% across all 12 samples. Injecting 1/5 of the final enriched sample, Spectrum Mill was able to identify about 1,200 to 1,500 total number of phosphopeptides from the 4 ratios of sample. About 55% of all phosphorylation sites were assigned. The recovery rates were consistent for all the standards across the 4 ratios of sample.
Conclusions
AssayMAP Bravo provides fully automated, highly selective and reproducible enrichment for phophopeptides. Nanodapter effectively converts UHPLC to nanoflow UHPLC which allows users to have both standard flow and nanoflow LC in one system. Together with nanoESI source, 6550 Q-TOF offers the ultimate sensitivity for maximum number of phosphopeptide identification. 6495B Triple Quadrupole offers fast, accurate and robust MRM based peptide quantitation.
Keywords: AssayMAP, Fe(III)-NTA, phosphopeptide enrichment, peptide quantitation

Poster 107: Ischemic Stress to Kidneys from SIRT5 Mice Is Mitigated by Succinylation Response

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Oxidative stress and kidney dysfunction are relevant pathological consequences due to ischemia/reperfusion-induced acute kidney injury (AKI). In previous studies we observed protection against AKI in SIRT5 knockout (KO) mice. Here, we investigated the effect of ischemic stress on the kidney succinylome in SIRT5KO versus wild-type (WT) mice.

In WT and SIRT5KO mice (N=3), one kidney was subjected to 22 minutes of ischemia while the other kidney served as uninjured control. The protein succinylome was determined in whole kidney. Following tissue lysis and tryptic digestion, succinylated peptides were enriched with the PTMScan succinylation affinity enrichment kit (CST). Samples were subjected to data-independent acquisitions (DIA) on a TripleTOF 6600 system. Succinylation sites were quantified using Skyline.

Data-independent acquisitions for succinyl-lysine from mouse kidney yielded overall over 1000 succinylation sites. We identified more unique succinylated sites (731) in injured WT than uninjured WT (528); however, we detected fewer unique sites in injured Sirt5KO (891) versus uninjured SIRT5KO (989). Quantitative comparisons between the different conditions revealed mostly robust up-regulation of succinylation levels in KO vs. WT, both from uninjured and injured kidneys. More than 95% of the succinylation sites were on mitochondrial proteins. Pathway analysis indicated that the most significantly affected pathway was mitochondrial fatty acid oxidation (FAO). Interestingly, for many of the Sirt5-regulated sites on FAO enzymes and respiratory chain targets, the fold changes between KO and WT were lower in the injured kidneys than in the uninjured kidneys. We also quantified 50 sites that were significantly less succinylated in injured KO kidneys compared to injured WT kidneys, while in uninjured KO kidneys only hypersuccinylation was observed. We believe that the total succinylation load increases during ischemic injury in WT but not KO kidneys, possibly due to higher mitochondrial metabolism in the WT driving succinyl-CoA formation thus leading to higher protein succinylation levels.

Poster 108: Adenoviral Proteins E1B55K and E4orf6 Use Non-Degradative Ubiquitination to Regulate Viral Late Protein Expression

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During the early phase of infection, viruses establish favorable conditions for late phase infection by co-opting host signaling processes and countering host antiviral defenses. Adenovirus is a DNA virus important both for its role in human diseases and for its archetypal insights in understanding virus-host interactions. Two early adenoviral proteins, E1B55K and E4orf6, associate with the host Cullin-5 ubiquitin ligase complex and redirect substrate recognition in order to promote an environment conducive for viral replication. Mutating these viral genes impairs production of late viral proteins and reduces viral progeny. Previous work has identified a limited number of substrates of the E1B55K/E4orf6 complex which are ubiquitinated and degraded upon infection. However, these known targets do not account for the decrease in late viral protein production. Here we identify new targets of the viral ubiquitin ligase that may account for the late phase defects in the E1B55K-deficient virus. We transduced HeLa cells with E1B55K and E4orf6, enriched for ubiquitinated proteins over a timecourse of E1B55K/E4orf6 expression, and applied mass spectrometry to identify potential substrates. We combined these results with proteomic analysis to normalize ubiquitin levels against protein abundance. In total, we identified >6000 proteins and >4000 modified peptides. Our integrative analysis reveals that ubiquitination occurs both on proteins that decrease over time, characteristic of degradative signaling, and on proteins whose abundances are unchanged, characteristic of non-degradative signaling. Our non-degraded ubiquitination targets are enriched in RNA-binding proteins, with hnRNP-C and RALY among the most abundantly modified. We show that these homologous proteins are specifically ubiquitinated by the E1B55K/E4orf6 complex and that they play a functional role in viral late protein production in an E1B55K-dependent context. Overall, we provide the first known case of viral-

mediated non-degradative ubiquitin signaling affecting protein production.

Poster 109-ML: Lysine Benzoylation is a Histone Mark Regulated by SIRT2

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Metabolic regulation of histone marks is associated with diverse biological processes through dynamic modulation of chromatin structure and function[1]. Here, we report the identification and characterization of a histone mark, lysine benzoylation (K_{bz})[2]. Our study identifies 22 K_{bz} sites on histones from HepG2 and RAW cells. This histone mark can be stimulated by sodium benzoate (SB), an FDA-approved drug and a widely used chemical food preservative, via generation of benzoyl-CoA. ChIP-seq and RNA-seq analysis demonstrate that histone K_{bz} marks are associated with gene expression and have physiological relevance distinct from histone acetylation. In addition, we demonstrate that SIRT2, an NAD^+-dependent protein deacetylase, removes histone K_{bz} both in vitro and in vivo. This study reveals a new type of histone mark with potential physiological relevance and identifies possible non-canonical functions of a widely used chemical food preservative. References: **1.** Sabari, B. R., Zhang, D., Allis, C. D., & Zhao, Y. (2016). Metabolic regulation of gene expression through histone acylations. *Nature reviews. Molecular cell biology*, *18* (2), 90-101. **2.** Huang, H., Zhang, D., Wang, Y., Perez-Neut, M., Han, Z., Zheng, Y. G., Hao, Q., … Zhao, Y. (2018). Lysine benzoylation is a histone mark regulated by SIRT2. *Nature communications*, *9* (1), 3374. doi:10.1038/s41467-018-05567-w

Poster 110: Response to Variables for Mice on Caloric Restriction Experiments

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Caloric restriction experiments show that dieting leads to more stable and functionally folded proteins. The purpose of this study is to determine the effects other variables have on caloric restricted mice. Mice are divided into two groups. Group 1 are put on a diet, limiting their food intake. Each day they are fed at a specific time with a specific amount of food. Group 2, the control group, have continuous access to food. Both groups are evaluated for: weight change, movement/activity level, O2consumption, and quantitative proteomics. Movement/activity levels are measured through the use of a laser beam system. The laser system attaches to the outside of the cage and sends beams through the cage. Movement is recorded each time the light beam is broken inside the cage. The mice's weight is recorded routinely every seven days. O2consumption is measured through blood gas samples. A mass spectrometer gathered quantitative proteomics data. The determining variables that are analyzed include: mice strain, company purchased from, and the length of time on the caloric restriction diet prior to proteomic analyzation. Data gathered would demonstrate the effects different variables have on the weight change, movement/activity levels, O2consumption, and quantitative proteomics.

Poster 112-TL: Acetylation of the Lamina Promotes the Integrity of the Nuclear Periphery and Inhibits Virus Production

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The regulation of the nuclear shape, composition, and function is at the core of numerous types of viral infections. For example, HIV induces perforations in the nuclear lamina, while herpesviruses induce nuclear swelling. One of the most striking alterations of nuclear shape occurs during infection with human cytomegalovirus (HCMV), a widely-spread pathogen that infects 50-90% of the global population and for which there are no effective treatments or vaccine. As HCMV is a nuclear-replicating virus, its capsids are assembled within the nucleus. However, as the viral capsid is too large to exit via nuclear pores, the lamina at the nuclear periphery must be disrupted, forming infoldings that allow capsid budding through the nuclear membrane. Additionally, the nucleus acquires a kidney-like shape to facilitate the formation of an assembly complex juxtaposed to the nucleus where the viral capsid undergoes maturation before cellular egress. Here, we report our discovery that acetylation is a critical regulator of viral capsid egress. Using acetyl-peptide enrichment and quantitative mass spectrometry, we find acetylations on lamina proteins that are temporally regulated during the progression of infection. Among these, K134 acetylation on lamin B1 (LMNB1) displayed one of the largest increases late in infection. To determine the function of acetylation, we generated acetyl-mimic (K-to-Q) and charge-mimic (K-to-R) LMNB1 mutants, and found that acetylation induces reductions in both infectious extracellular and intracellular virus. Live cell microscopy showed that LMNB1 acetylation induced the accumulation of viral capsids within the nucleus. Moreover, cells expressing LMNB1 acetyl mimic had reduced nuclear infoldings and curvature. Altogether, we demonstrate that LMNB1 K134 acetylation acts in host defense by stabilizing the nuclear periphery and inhibiting viral capsid nuclear egress. As K134 is conserved among mammalian species, we predict this acetylation can serve as a general regulator of nuclear morphology under different biological conditions.

Poster 113: Global PTM Analysis to Study Cross-Talk between Lysine Acetylome and Tyrosine Phosphoproteome in EGFR TKI Resistant Human Lung Adenocarcinoma

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Osimertinib, a 3rd generation epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) has been approved to treat lung cancer patients harboring $EGFR^{T790M}$; unfortunately, all patients eventually develop acquired resistance. Here, we employed a mass spectrometry (MS)-based proteomic approach to identify and quantify post-translational modifications (PTMs) to understand the crosstalk between tyrosine phosphorylation (pY) and lysine acetylation (lysAc) in osimertinib-sensitive lung adenocarcinoma cell line, PC-9, that harbor the $EGFR^{Del746-750}$ mutant, and its isogenic resistant counterpart, PC-9-OsiR-NCI-1. We used stable isotope labeling in cell culture (SILAC) and dual PTM immunoprecipitation to quantify the pY and lysAc sites. The enriched PTM peptides were analyzed with data dependent acquisition on a tandem MS (Q-Exactive-HF). The raw file library search was performed by MaxQuant and PEAKS. In total, we quantified 1064 pY and 475 lysAc sites, of which 191 pY sites were up-regulated and 53 sites were down-regulated in the resistant cells; 43 lysAc sites were upregulated and 7 sites were down-regulated in the resistant cells compared to the sensitive cells. Interestingly, we discovered that EGFR phosphorylation is down-regulated at Y1197, Y1172, and Y772 residues in the resistant cells indicating that EGFR signaling may be dampened in osimertinib-resistant cells. LysAc of the tumor suppressor protein p53 (TP53) significantly decreased at K120 postition in the resistant cells. Interestingly, 54 proteins were modified both at pY and lysAc sites. For example, lysine acetylation and phosphorylation at cyclin-dependent kinase 1 (CDK1)-K33 lysAc and pY15 sites were significantly reduced but pY19 phosphorylation was marginally increased in the resistant cells. We hypothesize that the

lysAc and pY of CDK1 may be co-regulated to modulate CDK1 function in osimertinib acquired resistance. CDK1 may be a potential drug target to overcome resistance. Further experiments are underway to validate these findings and assess the efficacy of CDK1 inhibition in overcoming osimertinib resistance.

Poster 113-ML: Tissue-Specific Protein Sulfhydrome Analysis in Mice as a Function of Age, Sex, and Diet

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Endogenously produced hydrogen sulfide (H2S) is a redox reactive metabolite that serves as a vasorelaxant and signaling molecule. One potential mechanism of action for endogenous H2S is through posttranslational modifications of protein thiols, termed sulfhydration. Sulfhydration, similar to other posttranslational modifications, alters a protein's structure, function, localization, stability, and/or interactions with other macromolecules. Despite the importance of sulfhydrated proteins in various physiological processes, global sulfhydrome profiles in multiple mammalian tissues as a function of age, sex, and diet, which are known modulators of endogenous H2S levels and production, are yet to be determined. We first hypothesized that caloric restriction (CR), one of the best studied interventions to extend lifespan, improve stress resistance, and boost endogenous H2S production in a number of tissues, increases and/or diversifies tissue-specific sulfhydrome profiles. Male C57BL/6J mice were fed ad libitum or 50% CR for one week prior to tissue harvest followed by a combinatorial sulfhydration proteomics approach utilizing an optimized biotin-thiol pulldown assay with LC-MS/MS quantitative proteomics and bioinformatics. We identified 1006, 1107, 474, 450, 905, and 165 individual sulfhydrated proteins in the liver, kidney, heart, muscle, brain and serum, respectively. 50% CR enriched for sulfhydrated proteins in the liver, kidney, muscle, brain and serum, but not in the heart. Pathway enrichment analysis indicated CR promoted sulfhydration of proteins involved in redox homeostasis, metabolism, and biosynthesis pathways. In addition to diet, we determined age and sex act as important biological variables impacting the global sulfhydrome. Aged mice had increased numbers, but altered pathway enrichment, of sulhydrated proteins in the brain while female mice had similar numbers, but different pathway enrichments, of sulhydrated proteins compared to male mice. In summary, we identified tissue-specific sulfhydrated proteins and the impact of diet, age, and sex on their individual abundance with implications in multiple biological pathways.

Poster 114-TL: Systems-Level Identification of PKA-Independent Vasopressin Signaling in Renal Epithelial Cells

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Vasopressin signaling in the renal collecting duct is believed to be mediated predominantly by the activation of protein kinase A (PKA). The recent generation of mouse cell lines ("PKA-null" cells), derived from mpkCCD cells, in which both PKA catalytic subunit genes have been deleted via CRISPR-Cas9 affords us an opportunity to identify PKA-independent signaling. Here, we used SILAC coupled protein mass spectrometry to profile proteome-wide phosphorylation changes in response to vasopressin (dDAVP, 0.1 nM, 30 min) in PKA-null cells and in control mpkCCD (PKA-intact) cells. Experiments were done in three different PKA-null lines and three different PKA-intact cell lines.

Overall, >14,800 unique phosphopeptides were quantified in both PKA-null and -intact cells. In PKA-intact cells, 452 distinct phosphorylation sites were altered in abundance in response to dDAVP. In contrast, in PKA-null cells, only 61 phosphorylation sites were altered in abundance, indicating that a large component of the vasopressin response is PKA-dependent. Notably, phosphorylation of Ser256 of AQP2 (thought to be a PKA site) increased in response to dDAVP in PKA-null cells, indicating that other kinases can phosphorylate this site. The upregulated phosphorylation sites in PKA-null cells mapped to an R-X-N-(S/T)*-X motif, consistent with activation of other basophilic kinases. An expanded list of vasopressin-regulated phosphorylation sites were obtained as a byproduct of this approach. Interestingly, cAMP measurements showed that baseline cAMP levels in PKA-null cells were ~10-fold higher than in PKA-intact cells, although both PKA-null and -intact cells showed significant increases in cAMP levels in response to dDAVP. This indicates that there is likely to be a PKA-dependent feedback on some component of the Avpr2-G_{α}s-Adcy6 signaling pathway responsible for cAMP generation.

Poster 115: Quantitative Protein Expression Biomarker Feasibility in Advanced Ovarian Cancer

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This study quantitates the differentially expressed proteins from tumor tissue derived from ovarian cancer patients treated with Vigil, an expressive bi-shRNA-furin and GM-CSF DNA transfected autologous tumor vaccine. Briefly, tumor tissue was removed and transfected with Vigil. Pre- and post-Vigil transfected samples were lysed and equal concentrations of protein were trypsin digested, and run on a Synapt G2-Si high definition mass spectrometer. Protein quantitation statistics was run using Progenesis-QIP software and IPA to provide more detailed information regarding the pathways in which the target genes were involved. Proteomic analysis by mass spectrometry of two blinded samples split from the same patient (#046) pre- and post-Vigil transfection revealed that furin and downstream signal pathway protein TGFβ1 and TGFβ2 were significantly downregulated post-bi-shRNA-furin knockdown DNA transfection. These results are consistent with reduced TGFβ1 (32 pg/10^6 cells to below detection) from prior ELISA of same sample. Additional samples were assayed from patient #046 who had a poor response to Vigil (overall survival (OS) < 1 year) and compared to patient #1017 who had a good response to Vigil (OS > 4 years). Proteins (109) were identified as significantly downregulated and 161 as significantly upregulated between the two patients. Two relevant protein signals, endoglin (167-fold) and retinoid x receptor (RXR) gamma (15600-fold) were identified as dramatically upregulated in the good response patient. Interestingly, patient #1017 also had much more activity by ELISA of knockdown related to TGFβ1 (TGFβ1 pre-transfection 709 pg/10^6 cells to post 0 pg/10^6 cells). These studies demonstrate the feasibility of quantifying differentially expressed proteins from tumor tissue derived from ovarian cancer patients treated with Vigil, and support the applicability of proteomics towards new biomarker discovery.

Poster 116-TL: Comparing Personalized Profiles of Host-Expressed Proteins and Microbes in Human Stool Reveals Complementary Inter-Subject Distinction

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Measuring host stool proteins through noninvasive stool-based assays opens new avenues for characterizing states of gastrointestinal health. However, the extent to which these proteins vary over time and between healthy subjects is poorly characterized.

Here, we investigate how host stool protein measurements quantitatively and qualitatively depend on technical and biological sources of variability when measured by mass spectrometry. We further compare these measurements to parallel gut microbiota surveys. Examining the proteomes and microbiomes measured from five self-reported healthy individuals –each sampled eight times over four weeks, let us investigate four sources of measurement variance. We find that mass spectrometry-based identification and label-free quantification of stool proteins was consistent over technical and preparative replicates, supporting this assay's utility for biomarker measurement. Although several human stool proteins tended to vary significantly over time within individuals, we observed far more protein variation between subjects. Both proteome and microbiome data sets consistently distinguish different subjects. Host-proteins measured from stool unambiguously point to clear innate and adaptive immune response mechanisms which varied over time and between subjects. Inter-subject differences were further observed for canonical IBD biomarkers such as fecal calprotectin (subunits S100A8 and S100A9). We conclude that host-centric protein stool measurements indicate a surprisingly wide range of "healthy" protein variation, adding a valuable functional complement to high throughput microbiota surveys. For example, one subject's proteins support a chronic sub-clinical inflammation state – an observation which would have been difficult to make solely with microbe-derived data.

Poster 117: Development of mitoTurboID to Unveil Components of the Mitochondrial Signaling Hub Following Innate Immune Activation

Clinton Bradfield
SSS, LISB, NIAID,NIH, Bethesda, MD

Macrophage innate-immune signaling utilizes receptor-ligand interactions to incite antimicrobial defenses, rewire transcription, and promote activation of the adaptive immune system. In the case of the classical toll-like receptor 4 (TLR4) response to lipopolysaccharide (LPS), distinct spatial utilization of signaling adaptors Myd88 and Trif leads to discrete transcriptional responses. This differential utilization of plasma-membrane and endosomal TLR signaling highlights the need to understand organelles as dynamic signaling hubs. While it is well appreciated that many transcriptional responses following TLR stimulation rely on alterations of mitochondrial physiology, namely through the glycolytic shunt, mechanistic understanding of how these changes occur and how they promote altered transcriptional responses remain understudied. Herein we utilized a new proximity-labeling system (mito-TurboID) expressed on the outer membrane of the mitochondria to dynamically assess proteins associated with the mitochondrial signaling scaffold following immune stimulation.

Poster 118: Protein ADP-ribosylation and Its Impact on Protein Complex Formation and Release in the LPS Activated Human Macrophage

Casey Daniels[1]; Pauline Kaplan[1]; Clinton Bradfield[2]; Iain Fraser[2]; Aleksandra Nita-Lazar[1]
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Protein complex formation and release regulates many cellular processes, including the immune response to lipopolysaccharide (LPS). Protein ADP-ribosylation, a polymeric post-translational modification (PTM), is known to serve as a scaffold for complexes and the quick formation and release of this polymer by Poly(ADP-ribose) Polymerases (PARPs) and Glycohydrolases (PARGs) is an understudied mechanism for protein complex regulation. Here we have separated protein complexes from their monomeric subunits by Size Exclusion Chromatography (SEC), using mass spectrometry to detect proteins as they co-elute with smaller and larger macromolecules. Time course application of this method to human macrophages stimulated with LPS has revealed several PARP proteins moving between fractions as macrophages are activated, as well as proteins whose movement into or out of complexes upon LPS stimulation is reversed by PARP inhibition. Use of our powerful, in-house ADP-ribose site identification method has allowed for detection of ADP-ribosylation events during macrophage LPS activation, and detection of PTMs which vary between monomeric and complexed proteoforms. This work sheds light on an understudied aspect of both LPS signaling and PAR proteomics. This research was supported by the Intramural Research Program of NIAID, NIH.

Poster 119-ML: Composition of the Myddosome during the Innate Immune Response

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Critical for the innate immune response to PAMPs, including viral RNA, the myddosome is a complex of proteins characterized by the presence of myeloid differentiation factor 88 (Myd88). The myddosome acts to transfer signals from the toll-like receptor (TLR) proteins to TNF receptor-associated factor 6 (TRAF6). In addition to Myd88, the myddosome contains multiple copies of the interleukin-1 receptor associated kinases (IRAK) 2 and 4 and is theorized to form following stimulation of the TLR proteins. Using affinity purification – mass spectrometry analysis (AP-MS), we identified the Myd88-associated proteins in mouse immortalized bone marrow-derived macrophages before and after lipopolysaccharide (LPS) treatment. Prior to LPS treatment, we found the stable association of Myd88 to Tripartite motif-containing protein 21 (TRIM21) and other inhibitory factors. Following LPS treatment, the inhibitory factors were lost and Myd88 associated to the downstream effector proteins, including IRAK1, IRAK4, and TRAF6. Using stable isotope labeling by amino acids in cell culture (SILAC), we found that the association of Myd88 to the IRAK proteins changes as the LPS response progresses. Because of this, we are examining the mechanism(s) that regulate the activity of the myddosome. We are currently using AP-MS followed by phosphopeptide enrichment with MS/MS analysis to identify possible phosphorylation sites. Using these data, we will evaluate the role of phosphorylation on myddosome activity. This research was supported by the Intramural Research Program of the NIH, National Institute of Allergy and Infectious Diseases.

Poster 120: Identification of Dynamic Nuclear Receptor Co-Regulatory Protein Complexes by Reverse Phase Protein Array and Mass Spectrometry

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Nuclear hormone receptors (NHR) are a family of ligand-dependent transcription factors regulating expression of important gene networks. NHRs bind specific DNA sequences and recruit transcriptional co-regulators (CoRs) that are a dynamic complex of proteins .

In this study we combined mass spectrometry (MS) and reverse phase protein array (RPPA) proteomic platforms to identify the changes in composition of CoRs complexes associated with progesterone receptor (PR) in response to physiological stimuli. Nuclear proteins from mouse brain tissues or human cancer cells were incubated

respectively with mouse or human PR (A or B isoforms) bound to bead-immobilized DNA harboring hormone response elements. Assembled DNA-bound PR-CoR complexes were treated with progesterone (P4) or ATP, eluted and analyzed by MS profiling and RPPA, the later with > 200 validated antibodies to various protein signaling pathways including a group of known CoRs.

In mouse brain tissues, a total of 50 proteins were identified by MS and RPPA in isolated PR-CoR complexes in ligand dependent manner. Among them, 33 were identified by MS and 17 by RPPA. The majority of proteins detected by RPPA were not detected by MS including the well known steroid receptor coactivators 1,2 (SRC-1, -2), regulatory proteins involved in signal transduction and metabolism and phosphorylated forms of MAPK1 and src tyrosine kinase.

In isolated human PR-CoR complexes from cancer cells, a total of 45 proteins were detected by MS and RPPA. Since the RPPA platform contains > 70 antibodies to phosphorylated forms of proteins, it further identified recruitment of 19 phosphorylated proteins to the PR-CoR complex by ATP treatment that was not possible by MS profiling.

Conclusion: Combining MS profiling and targeted RPPA enabled a more comprehensive identification of known and novel protein interactions indicating that these are complementary proteomic platforms for analysis of the dynamics of cellular protein complexes and interactomes.

Poster 121: Determination of Host Cell Proteins in Antibody Preparations using PASEF on the timsTOF PRO

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The analysis of host cell proteins (HCP) at the ppm level is critical. ELISA is currently the gold standard for QC applications, but the advantages of mass spectrometry are abundant. We show how PASEF (parallel accumulation and serial fragmentation), as implemented on the timsTOF PRO QTOF, can be applied to HCP analysis for highly sensitive detection with enhanced data quality.

NISTmAb Reference Material 8671 and the Universal Proteomics Standard (UPS1, Sigma) were reduced, and digested. Peptides were separated on an Intensity Solo 2 1.8µm C18 column using an Elute UHPLC coupled to a timsTOF Pro ion mobility QTOF mass spectrometer (all Bruker Daltonics). A 150 minute gradient was used. For nanospray, a nanoElute UHPLC was fitted with an IonOpticks C18 column using a 210 minute gradient.

The UPS1 standard was used to make a 5-step 1:3 dilution series in a constant background of NIST mAb over a concentration range from 0.3 to 934 ppm. PASEF enabled the detection of UPS1 proteins down to low single digit ppm concentrations in the presence of 25 µg NIST mAb. Linear response for the UPS1 protein beta-2-microglobulin in the concentration range from 132 ppm to 1.6 ppm was observed. The dilution series indicated that PASEF enabled detection of HCPs in the range of 1 to 100 ppm of the therapeutic protein. Nano UHPLC was also evaluated for its suitability for HCP identification. This setup has already been established as the new benchmark for bottom-up proteomics applications [1, 2]. More than 200 HCPs were identified in 1.5 µg NIST mAb, including expected and previously unreported HCP proteins. The depth of HCP coverage and sequence coverage was further extended to >280 HCP identifications by employing an alternate digestion method in which only the HCPs are digested allowing the mAb to be removed prior to analysis.

Poster 122: Multiplexed Interactomics Reveals Coordination of Proteostasis Network Remodeling and Mechanisms of Protein Quality Control

Madison T. Wright; Lars Plate
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Proteins are folded and transported to the appropriate subcellular locations with the guidance of various protein folding and trafficking factors, which together comprise the proteostasis network. Coordinated activity of these factors governs protein quality decisions ensuring correct folding and localization of properly matured client proteins while degrading unstable, misfolded proteins. Imbalances in proteostasis activity resulting from genetic defects, stress or aging are associated with etiologically-diverse gain- and loss-of-function protein misfolding diseases, due to decreased protein quality control. Consequently, remodeling of the proteostasis network has emerged as a promising therapeutic strategy to correct the underlying quality control defects for diverse proteins associated with such disorders. However, it often remains difficult to define the underlying molecular mechanisms responsible for the protein quality control defects that lead to improper partitioning of destabilized, disease-associated proteins between folding, trafficking and degradation. Protein quality control is dictated by transient interactions between client proteins and individual proteostasis network components and mass spectrometry-based interactomics can globally profile changes among these interactions. Here, we discuss integration of tandem mass tag (TMT)-based multiplexed quantitative interactomics as a method to further elucidate dynamic proteostasis network processing in order to define altered protein recognition associated with destabilized protein variants responsible for aggregation diseases. Furthermore, we determine how remodeling of the endoplasmic reticulum (ER) proteostasis network can alter client protein engagement to influence trafficking and degradation decisions. Our results provide mechanistic insight how remodeling of the ER proteostasis network can improve quality control decisions for aggregation-prone protein variants, specifically clients associated with amyloid or loss-of-function protein misfolding diseases. The work highlights the utility of multiplexed quantitative interactomics to define mechanisms of aberrant protein quality control that can be exploited for therapeutic intervention in protein misfolding diseases.

Poster 123: Influence of Post-Translational Modifications on Protein Stability

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Recent studies demonstrate that imbalances in the quality and concentration of most proteins in each cell (proteostasis) are at the core of many of today's devastating diseases. The causes in these imbalances are poorly understood. Thus, it is important to create diagnostic methods that can detect changes in the quality and concentration of many proteins within a patient's proteome. We recently identified changes in structure and post translational modifications of abundant proteins in the blood serum samples of rheumatoid arthritis (RA) patients relative to healthy controls. These results suggest that either protein modification or changes in secondary structure could influence disease etiology.

Our long-term goal involves understanding the role of disease specific post-translational modifications on protein turnover and protein stability. We observed differences in protein denaturation curves between serum samples of RA and otherwise "healthy" patients.

Subsequent proteomics studies confirmed a change in secondary structure of abundant blood proteins. Based on this preliminary data, we hypothesize that modifications of protein folding are related to disease. Our first objective focuses on the use of chemical probes to monitor protein unfolding energies across the proteome by modifying surface exposed AA sites during denaturation. We are optimizing use of tyrosine, tryptophan, and methionine. These modifications are introduced at different degrees of protein unfolding, and measured with mass spectrometry. Use of these three probes should result in the ability to monitor 98% of the proteome. This will allow us to test how protein homeostasis changes with RA development. This understanding will also lead to greater knowledge of the control of protein homeostasis during development and aging.

Poster 124-TL: Quantitative Interactomics to Determine Protein Quality Control Mechanisms Dictating Thyroglobulin Secretion

Madison Wright; Lars Plate
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The proteostasis network (PN) is composed of folding, trafficking, and degradation factors that facilitate protein quality control (PQC). Perturbations to the PN caused by environmental, age-related, or genetic alterations are linked to a variety of disease states, including protein aggregation and amyloid diseases that are often difficult to treat. These detrimental states are a result of diminished PQC capacity, leading to aberrant processing of client proteins during biogenesis and downstream handling. Modulation of PN components or entire pathways has shown promise as a potential therapeutic strategy to ameliorate such protein misfolding diseases. In the current study, we utilize thyroglobulin (Tg), the 330 kDa thyroid prohormone, as a model to elucidate mechanisms of PQC directed by PN pathways. Tg maturation relies heavily on PN components for proper domain assembly, trafficking, and ultimately secretion due to its large size and extensive requirements for post-translational modification (PTM). Once folded, Tg is secreted and further processed to create T3 and T4 hormones that are critical for fetal development and utilized in adulthood to regulate intermediary metabolism. Many patient-derived Tg mutations associated with congenital hypothyroidism lead to aberrant processing, resulting in decreased or complete loss of Tg secretion and subsequent hormone production. We have implemented a multiplexed quantitative affinity purification – mass spectrometry (AP-MS) method to delineate the wild-type Tg interactome from pathological variants in order to identify PN dynamics facilitating aberrant processing of these clinical mutations. An improved understanding of the PQC defects for Tg variants provides new therapeutic strategies directed at recovering Tg secretion and hormone production, and may be applicable to other protein misfolding diseases.

Poster 125: Reference Materials for Proteomic Investigations

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High-throughput, bottom-up proteomic experiments typically involve complex experimental designs, both for sample preparation and for instrumental analysis. The complex workflows frequently used in proteomics are difficult to optimize and trouble-shoot, impacting the quality of the proteomic investigative results. NIST has developed two reference materials which were designed to support the quality of proteomic results: RM 8323 Yeast Protein Extract and RM 8321 Peptide Mixture for Proteomics.

RM 8323 (Yeast Protein Extract) is a frozen aqueous solution of extracted *Saccharomyces cerevisiae* yeast proteins. The *S. cerevisiae* yeast proteome is an attractive material for proteomic reference materials for several reasons including its proteome complexity and availability. RM 8323 can be utilized in the design and optimization of proteomics-based methodologies from sample preparation to data analysis.

In contrast to RM 8323, RM 8321 (Peptide Mixture for Proteomics) is composed from approximately 440 synthetic peptides in a frozen aqueous solution. Selection of the constituent peptides was based on several factors including, their identification as “proteotypic” peptides in several archived MS/MS databases and chromatographic retention. The reference material was formulated with several tiered peptide concentrations in order to evaluate a LC-MS/MS platform’s performance for a range of peptide concentrations in a single analysis. Both discovery-based and targeted LC-MS/MS methods were used to identify the peptides present and a confidence-ranking system was developed to combine results of peptide identities from these methods.

We provide examples of how NIST RM 8323 and RM 8321 can be used as a quality control material for method optimization in common proteomics-based workflows to improve measurement quality. These reference materials were used to develop digestion metrics, to optimize LC-MS/MS parameters, and to evaluate the search engines used in discovery-based proteomics.

Poster 126: SASP Atlas: A Comprehensive and Unbiased Proteomic Database of the Senescence Associated Secretory Phenotype

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Introduction: The senescence-associated secretory phenotype (SASP) has recently emerged as both a driver of -- and promising therapeutic target for -- a multitude of chronic age-related conditions, ranging from neurodegeneration to cancer. The complexity of the SASP has been greatly underappreciated and a small set of factors cannot explain the diverse phenotypes it produces in vivo. Here, we present ‘SASP Atlas’, a comprehensive proteomic database of SASPs, including a novel exosome SASP phenotype, driven by multiple inducers of senescence in different human cell types. We also propose that SASP proteins are promising biomarkers to assess senescent cell burden in aging and disease.
Methods: Secretomes of senescent cells were characterized by comparing the secreted soluble (sSASP) and exosome (eSASP) proteins of irradiation-, oncogenic RAS-, or HIV drug atazanavir-induced senescent human fibroblasts to non-senescent controls. Secreted proteins and exosomes were obtained from the medium of cells cultured for 24 hours in serum-free conditions. Data-independent acquisitions (DIA) were performed on a TripleTOF 6600.
Results: While 172 proteins increased in all inducers and 53 proteins increased in all inducers and cell types, the SASP of each inducer and cell type were largely distinct, totaling over 1000 unique proteins. CXCL1, MMP1, and STC1 were consistently among the top increased proteins in response to all inducers. Senescent cells, on average, secreted larger exosomes containing a highly distinct set of proteins compared with the soluble secretome, including proteins involved in G-protein and RAS signaling, prostaglandin regulation and the complement system.
Discussion: Together, the data demonstrate that the SASP is a complex and highly diverse set of secretory phenotypes, including a unique exosome SASP. This resource will aid in identifying the proteins that drive senescence-associated phenotypes and provide comprehensive catalogs of potential biomarkers that can be used to assess the burden and origin of senescent cells in vivo.

POSTER ABSTRACTS

All posters will be displayed Monday and Tuesday.
Each day: odd-numbered posters present 1:30-2:15 pm; even-numbered posters present 2:15-3:00 pm.
ML = Mon. lightning talk; TL = Tues. lightning talk; WTT = Wed. tips & tricks talk.

Poster 127: Physical Activity Associated Proteomics of Skeletal Muscle: Being Physically Active in Daily Life Protect Skeletal Muscle from Aging

Ceereena Ubaida-Mohien; Marta Gonzalez-Freire; Alexey Lyashkov; Ruin Moaddel; Chee Chia; Eleanor Simonsick; Ranjan Sen; Luigi Ferrucci
NIA/NIH, Baltimore, MD

Introduction
Muscle strength declines with aging and exercise is the only intervention known to attenuate this decline. In order to adequately investigate both preventive and therapeutic interventions against sarcopenia, a better understanding of the biological changes that are induced by exercise in aging skeletal muscle is required.
Method
In an effort to determine the effect of physical activity on the skeletal muscle proteome, we utilized liquid-chromatography mass spectrometry to collect quantitative tandem mass tag proteomics data on human skeletal muscle biopsies obtained from 60 well-characterized healthy individuals (20-87 years) who reported heterogeneous levels of physical activity (not active, active, moderately active, and highly active).
Results
Over 4000 proteins were analyzed, and higher self-reported physical activity was associated with substantial overrepresentation of proteins associated with mitochondrial, TCA cycle, structural and contractile muscle, and genome maintenance. Conversely, proteins related to the spliceosome, transcription regulation, immune function, apoptosis, DNA damage, and senescence were underrepresented with higher self-reported activity. These differences in observed protein expression were related to different levels of physical activity in daily life and not intense competitive exercise. In most instances, differences in protein levels were directly opposite to those observed with aging. These data suggest that being physically active in daily life has strong beneficial effects on muscle.

Poster 128-TL: Multifaceted Proteomic Evaluation of Lysosome Dysfunction In Neurodegeneration via Human iPSC-derived Neurons

Ling Hao[1]; Ryan Prestil[1, 2]; Michael Fernandopulle[1, 2]; Stewart Humble[1]; Daniel Lee[3]; Saadia Hasan[1]; Maia Parsadanian[1]; Richard Youle[1]; Michael Ward[1]
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Lysosome dysfunctions have been associated with neurodegeneration, but the molecular mechanisms remain unclear. Here, we developed novel proteomic approaches in iPSC-derived neurons to study the molecular correlations between lysosomal biology and neurodegeneration. **1)** To characterize protein turnover in neurons, I **developed a dynamic SILAC labeling method to calculate protein half-lives** in iPSC-neurons. **2)** To characterize **lysosome interactions** with other organelle and cytosolic proteins, I **developed a proximity labeling method** by stably expressing Lamp1-APEX tag to biotinylate proteins within ~20 nm of lysosomes in living neurons. **3)** We then applied these two methods to evaluate the changes of lysosome functions when **knocking out genes that cause neurodegenerative diseases**. The first gene we studied is GRN, whose mutations lead to the deficiency of progranulin, a lysosomal glycoproteins, and cause frontotemporal dementia. Loss of PGRN impairs lysosomal activity, but the precise function of PGRN remains unknown.

By dynamic SILAC proteomics, I found that the neuronal protein degradation curve follows the 1st order kinetics. I developed a multi-time point and a single time point methods to calculate protein half-lives, both yielded reproducible and consistent results. Neuronal proteins showed longer half-lives compared to other cell types. Histones and nucleoporins have super long half-lives (<300 hrs) in neurons, and proteins related to neuron projection and ubiquitin proteasome pathways have much shorter half-lives. Lamp-Apex proteomics captured the transient lysosomal interacting proteins in living neurons, and 1548 out of 4413 proteins were truly interacted with lysosome after comparing to the cytosolic-Apex as a spatial control. Knocking out GRN resulted dramatic changes in protein half-lives and lysosomal interacting proteins in neurons, which are related to the regulation of hydrolase activity and vesicle-mediated transport (crucial lysosomal functions). PGRN deficiency also reduced recruitment of multiple annexins to the lysosome, in particular annexin A11 whose mutations cause autosomal dominant neurodegeneration.

Poster 129: Effects of Oxidized Lipoprotein (oxLDL) on the Proteome in Retinal Pigment Epithelial Cells

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Retinal pigment epithelium (RPE) are specialized support cells essential for vision. Loss of these cells contributes to development of age-related macular degeneration (AMD). Oxidative stress and ensuing molecular and cellular damage are major contributors to the pathological mechanisms of AMD. Oxidatively modified low-density lipoprotein (oxLDL), formed from LDL in a pro-oxidant milieu, induces oxidative stress and is cytotoxic to the RPE. In the sub-RPE space, oxLDL is found in extracellular deposits (drusen), which are a prominent clinical feature of AMD. Information on how the RPE respond to exposure to oxLDL is therefore a critical element in the mechanistic understanding of RPE dysfunction and death in the context of AMD. The purpose of this study was to determine protein expression changes in the RPE proteome induced by exposure of the cells to sub-lethal levels of oxLDL. ARPE-19 cells were treated with oxLDL (100 µg/mL) for 24 h. Proteins extracted from control and oxLDL-treated cells were digested and analyzed with high-resolution LC-MS/MS on a Synapt G2-Si system in the data-independent acquisition mode (MSE) and using ion mobility. Protein identification and quantification was performed with Progenesis QIP. With this workflow, close to 3,000 RPE proteins were identified. Treatment with oxLDL resulted in expression changes of 300 proteins (fold change ≥ 1.5, q value < 0.05). Functional analysis of the differentially expressed proteins with DAVID and STRING tools showed that upon oxLDL uptake the RPE cells mount a complex molecular response affecting multiple biological processes. This response involves upregulation of proteins related to autophagy, and of anti-oxidative stress proteins whose expression is controlled by the transcription factor Nrf2. Among the proteins down-regulated by oxLDL treatment are ribosome components and proteins involved in translation initiation, which suggests that the mechanisms through which RPE cells respond to oxLDL exposure include inhibition of protein synthesis.

Poster 130: Calorie Restriction Conditions May Modulate Aging Rates by Altering Ribosomal Maintenance and Quality

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One of the hallmarks of aging is decreased proteome quality, leading to increased risk of age-related diseases. Calorie restriction is the gold standard intervention for increasing lifespan and healthspan in laboratory animals; decades of study suggest that these benefits result from an overall improvement in protein quality and maintenance. However, the mechanisms by which the proteome control machinery is adjusted to do so remain incompletely understood. Using cohorts of mice placed on calorie restriction with varying dietary perturbations, we performed an analysis of kinetic and quantitative proteomic data combined with RNA-sequencing data to investigate nodes of

proteome control. Our data show that a general slowdown of protein turnover results from calorie restriction; additionally, the metabolic flux of ribosomal proteins shifts, suggesting significant changes in translational quality and ribosomal maintenance. Further, the data suggest that this slowdown in protein translation may be accomplished by alterations to the peripheral cellular protein translational machinery. Improvements in ribosome quality leading to increased translational fidelity, along with secondary effects such as increased time for co-translational protein folding, are the expected positive effects on overall proteome quality due to slower translation rates. These data provide evidence for a model in which the phenotypic benefits of calorie restriction arise from the cell actively perturbing translational controls to slow down proteome turnover.

Poster 132: Characterization of Changes in the Insolublome with Aging and Age-related Diseases

Xueshu Xie; Dipa Bhaumik; Kathleen Dumas; Manish Chamoli; Anja Holtz; Suzanne Angeli; Renuka Sivapatham; Julie Andersen; Gordon Lithgow; Birgit Schilling
Buck Institute for Research on Aging, Novato, CA

We and others have shown that increased protein insolubility is a feature of normal aging. The proteins that enter the insolublome are enriched for lifespan determining functions, but it is not clear how normal aging processes and Alzheimer's disease (AD) processes are related. To investigate SDS insoluble protein aggregates, we are using data-dependent acquisitions (DDA) for protein discovery and identification, and data-independent acquisitions (DIA or SWATH) for comprehensive sampling of protein aggregates or the 'insolublome'. Taking advantage of the powerful genetic tools available in C. elegans models, we have assessed and compared conditions, such as young and old (for N2 and Ju775 strains), but also 'human A-beta' expressing worms vs. control. We routinely identify and quantify 500-1000 proteins in the insoluble protein fractions. Significantly regulated proteins observed and increased in aggregates from old vs young worms were compared to insoluble proteins significantly regulated in 'human A-beta' expression models vs control. Additional time courses throughout aging were assessed monitoring dynamic changes over time in the protein insolublome.

Poster 133: Changes in Skeletal Muscle Proteins: A Story of Splicing, Mitochondria, and Immune Function

Ceereena Ubaida-Mohien[1]; Alexey Lyashkov[1]; Marta Gonzalez-Freire[1]; Ravi Tharakan[1]; , Michelle Shardell[1]; Ruin Moaddel[1]; Richard Semba[2]; Chee Chia[1]; Ranjan Sen[1]; Luigi Ferrucci[1]
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Introduction:
Muscle degeneration is a substantial cause of weakness and frailty in older persons, but the cause of such degeneration remains unknown and no previous study assessed the human skeletal muscle proteome over the course of aging. We have examined the proteome of human skeletal muscle utilizing a Tandem Mass Tag (TMT) based protein quantification approach in very healthy persons dispersed over a wide age-range.
Methods:
Skeletal muscle biopsies were collected from 60 healthy human donors ranging in age from 22 to 87 years. TMT 6plex was used for relative protein quantification. Skeletal muscle proteins were extracted, trypsin digested, reduced and alkylated. MS/MS peaks from the samples were searched and analyzed. For final representation, spectra were normalized by median polish and median sweep, protein identifications were quantified and annotated. Relationships of protein levels with aging were examined by the linear regression model.
Results:
Several functional classes of proteins were underrepresented or overrepresented with aging. Proteins related to energetic metabolism were underrepresented in older participants, including those related to the TCA cycle, respiratory and electron transportation chain, mitochondria respiratory complex assembly and glycolysis. Several proteins that play important roles in innate and adaptive immunity were overrepresented with older age. Similarly, proteins of the major spliceosome complexes were also overrepresented with older age and this was paralleled by a global change in alternative splicing. Our analysis indicates that the skeletal muscle proteome undergoes substantial changes with healthy aging, particularly evident in the areas of energy metabolism, inflammation, proteostasis, and splicing.
Conclusion:
Our preliminary analysis shows that the skeletal muscle proteome undergoes substantial changes with healthy aging, indicating profound changes in energy metabolism and spliceosome complex.

Poster 134-TL: Using Protein Stability as a Matric of Protein Quality in Protein Homeostasis

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Brigham Young University, Provo, UT

The lifespan of US population has been increased from 45 in 1913 to 77 in 2015. People lives longer, in the same time age-related disease, such as cancer, heart diseases, Parkinson's disease, and Alzheimer's disease, also becomes more common. Slowing down the onset of these diseases may be accomplished by understanding the biochemical mechanisms of aging. It has been thought that aging is related to the decline of protein homeostasis (proteostasis), which is the decline of the functional balance in proteome. However, what contributes to the proteostasis decline remains unclear. We are creating a system scale model of proteostasis which includes synthesis, folding, and degradation, together to describe the phenomenon of proteostasis. The model we propose utilizes protein concentration, protein turnover rate and protein stability to understand how protein population and quality has changed overtime and between different condition. Using mouse models we have measured changes in proteostasis during periods where the aging rate is modified. The literature and our preliminary data suggests that during slow aging, the protein turnover slows down and the protein stability decreases. Here we investigate how this change in proteome scale turnover affects protein quality by comparing changes of protein turnover verses the protein fold stability across multiple protein groups and mouse models.

Poster 135: Identifying ApoE Isoform-Dependent Changes for Protein Turnover in the Brain

Joseph Creery; Russell Denton; John Price
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It is known that a significant contributor to proteopathy in late-onset Alzheimer's disease (AD) is the genetic variation of Apolipoprotein E (ApoE). There is evidence to support that relative to isoform 3, isoform type 4 is implicated in increased AD risk, and isoform 2 protects against AD. It is also known that many specific protein-protein interactions contribute, even accelerate disease progression, including the Tau effect and amyloid-creating-peptidase proteins. Although this is known, there is no clear picture of total protein homeostasis and turnover in the presence of the different isoforms of ApoE. Comparing transgenic mice (C57BL/6), expressing one of the three isoforms of human ApoE, we can observe differences in relative concentrations of proteins that do not directly interact with ApoE on a protein-protein level. The differences in protein concentrations of these seemingly unrelated proteins have been observed by others as well as ourselves. We hypothesize that there are systematic responses to the ApoE isoform differences to compensate for a lack of control in the ApoE dominion or subsection of the system. From the combination of protein turnover and concentration data, we can

determine whether a change in synthesis or degradation is controlling for the change of protein concentration for each of the proteins in the proteome.

Poster 136-ML: Proteomic Approaches to Study Cell Death Mechanisms in Human Stem Cell-Derived Retinal Ganglion Cells

Joseph Mertz; Xitiz Chamling; Ah Young Lee; Xiaoli Chang; Byoung-Kyu Cho; David Clark; Cynthia Berlinicke; Hui Zhang; Donald Zack
Johns Hopkins Medical School, Baltimore, MD

Glaucoma is characterized by progressive loss of retinal ganglion cells (RGCs) and while reasonable therapies exist – primarily to lower intraocular pressure – they are not always effective. To date, there are no FDA-approved neuroprotective agents that directly halt vision loss. Our goal is to increase our understanding of RGC neuroprotective signaling networks during axonal injury using global and phosphoproteomics in human stem cell-derived RGCs (hscRGCs), a highly disease-relevant model. Our primary focus is on the dual leucine zipper kinase (DLK)/leucine zipper kinase (LZK)/c-Jun N-terminal kinase (JNK)/stress activated protein kinase (SAPK) pathway and the phosphorylation events that bring about cell death upon axonal injury.

Human ES cells expressing the murine cell-surface protein THY1.2 under *BRN3B* promoter control were differentiated into RGCs for 35 days, immunopurified through THY1.2, and cultured as a purified population for 7 days. Axonal injury was induced by the microtubule destabilizer colchicine for 5 minutes to 48 hours with a subset simultaneously subjected to pharmacological DLK inhibition by VX-680. Viability, morphology, and SAPK pathway activation under these conditions were assessed, samples prepared for LC-MS/MS analysis including phosphopeptide enrichment by Fe^{3+} immobilized metal affinity chromatography (IMAC), and LC-MS/MS performed on Orbitrap instruments.

At 48 hours, 1μM colchicine significantly decreased cell viability (13.1±0.9%vs ctl) and neurite length (6.9±2.1% vs ctl), and engaged SAPK signaling (increased phospho-cJun) after only 30 minutes. DLK inhibition by VX-680 strongly suppressed all of these effects (87.1±2.3% survival, 47.5±6.4% neurite length, and no detectable phospho-cJun). Global and phosphoproteome samples have been prepared and LC-MS/MS analysis is underway. We expect to quantify over 9,000 proteins and 10,000 phosphorylation sites.

Multi-tiered proteomic analysis of signaling networks involved in axon injury-induced cell death in conjunction with pharmacological modulation of these networks will provide insights into key signaling events in RGC death, and hopefully lead to improved therapeutic approaches.

Poster 137: Body-wide Dynamics of Organ Proteomes for Health and Diseases

Bingyun Sun
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Body-wide organs and tissues are closely related and function as an integrated network. Pathological response of one organ often has effects to other organ and tissue types. Some of these relationships are well established such as the dysregulated insulin secretion in pancreas of diabetic patients causes complications in cardiovascular, urinary, and nervous systems. Nevertheless, in many other pathological conditions, multiorgan responses have been overlooked, such as in drug induced toxicity. For instance, in acetaminophen overdose, whole-body histology has been performed in animal models, from which several extraheptic organs besides the liver shown toxic response. Using organ specific proteins, multiorgan responses had been detected in the blood of over dosed mice. We here further demonstrate that by studying the proteome of different organs during the course of the acetaminophen overdose, it is possible to infer the trend of muliorgan response observed at histology and blood proteome level. When superimpose the organ proteome dynamics caused by drug perturbation to the proteome of different in-bred strains, we can also start to reveal the pathological phenotypes of different mouse strains. Our results were summarized from proteomes of upto 13 major organs in four in-bred strains of mice, and some of the organ proteomes had been further characterized for their changes in response to half lethal dose of acetaminophen.

Poster 138-WTT: *Burkholderia* Rewires Its Proteome to Lower Antibiotics Sensitivities and to Support Biofilm Formation

Mohd M. Khan[1, 5]; Supaksorn Chattagul[2]; Bao Q. Tran[3, 6]; Jeffrey A. Freiberg[4]; Aleksandra Nita-Lazar[5]; Mark E. Shirtliff[4]; Rasana W. Sermswan[2]; Robert K. Ernst[4]; David R. Goodlett[3]
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The melioidosis causing opportunistic pathogen *Burkholderia pseudomallei*, a tier 1 overlap select agent by the Centers for Disease Control and Prevention (CDC), imparts a significant public health burden in Southeast Asia and northern Australia. Unfortunately, *Burkholderia* antibiotics sensitivities are lowered in biofilm forms while treating drug-resistant strains is still a challenge. Further, lack of an effective vaccine that protects against *B. pseudomallei* infection poses a severe threat to public health, particularly for populations that are at risk of naturally acquired melioidosis infections. Earlier, using transcriptomics and reverse phase liquid chromatography tandem mass spectrometry (LC-MS/MS), we have shown that biofilm proteome expression follows a transcript-decoupled mechanism. Given biofilm forming bacteria exhibit distinct phenotypic attributes compared to their planktonic states, for example with regard to antibiotic susceptibility, here we report the temporal proteomic profiling of multiple planktonic and biofilm states of *B. pseudomallei* 's surrogate model *Burkholderia thailandensis*. Using a continuous bio-flow apparatus for biofilm formation, the early (1 day), maturing (3 days) and late (6 days) biofilm proteome samples were analyzed and compared with different planktonic (early-exponential, mid-exponential, early-stationary and mid-stationary) phases' proteomes. Key results from global proteomic analysis revealed *Burkholderia* down-regulates metabolic proteins while up-regulating stress-related proteins presumably aiding survival in biofilms in part by decreased antibiotic susceptibility.

Poster 139: Rapid Diagnosis of New and Relapse Tuberculosis by Quantification of a Circulating Antigen in HIV-infected Adults

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Background

HIV-associated immune defects inhibit tuberculosis (TB) diagnosis, promote the development of extrapulmonary TB and paucibacillary pulmonary TB cases with atypical radiographic features, and increase TB relapse rates. We, therefore, assessed the diagnostic performance of a novel assay that directly quantitates serum levels of the Mycobacterium tuberculosis (Mtb) virulence factor protein to overcome limitations associated with detecting Mtb bacilli in sputum or tissue biopsies.

All posters will be displayed Monday and Tuesday.
Each day: odd-numbered posters present 1:30-2:15 pm; even-numbered posters present 2:15-3:00 pm.
ML = Mon. lightning talk; TL = Tues. lightning talk; WTT = Wed. tips & tricks talk.

Methods
This study analyzed HIV-positive adults enrolled in a large, population-based TB screening and surveillance project, the Houston Tuberculosis Initiative, between October 1995 and September 2004, and assigned case designations using standardized criteria. Serum samples were trypsin-digested and immunoprecipitated using a customized anti-Mtb-specific peptide that was quantified by liquid chromatography-mass spectrometry for rapid and sensitive TB diagnosis.
Results
Among the 1053 enrolled patients, 110 met all inclusion criteria; they included 60 tuberculosis cases (12 culture-negative TB), including 9 relapse TB cases, and 50 non-TB controls, including 15 cases with the history of TB. Serum Mtb antigen levels diagnosed 89.6% (77.3–96.5) and 66.7% (34.9–90.1) of culture-positive and culture-negative TB cases, respectively, and exhibited 88% (75.7–95.5) diagnostic specificity in all non-TB controls. Serum antigen detection and culture, respectively, identified 85% (73.4–92.9) and 80.0% (67.3–88.8) of all 60 TB cases.
Conclusions
Quantitation of the Mtb virulence factor in serum samples of HIV-infected subjects diagnosed active TB cases with high sensitivity and specificity and detected cases missed by the gold standard of Mtb culture. These results suggest that serum Mtb antigen quantitation holds great promise for the rapid diagnosis of suspected TB cases in patients who are HIV-infected.

Poster 140: Development of a Quality Control Standard for Tandem Mass Tags (TMT) Workflows

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Quantitative proteomics strategies using Tandem Mass Tags (TMT) enable sample multiplexing and precise measurement of protein abundance. However, successful execution of this workflow includes multiple steps that may require optimization including chromatography, mass spectrometry (MS), and data analysis. *Paulo et al.* (JASMS, 2016) created a yeast triple gene knockout (TKO) TMT9plex standard to access ratio distortion using different instrument methods, as co-isolated ion interference can suppress accurate ratio quantification and thereby mask true differences in protein abundance across biological systems. Therefore to be able to detect co-isolation interference, enable MS method optimization and validation, we extended above mentioned TKO sample and developed the Thermo Scientific® Pierce® TMT11plex yeast digest standard. We demonstrate that the TMT11plex standard can be used an LC-MS system suitability standard to measure and optimize protein/peptides identification, acquisition, and data analysis methods to limit co-isolation interference, while also diagnosing MS instrument status by monitoring mass accuracy, ion injection time, and reporter ion signal to noise. We then used the TMT11plex standard to establish a standardized workflow including two LC methods (50min for QC assay or 120min gradients for method development) for a variety of nano-spray liquid chromatography setups, optimized MS acquisition settings for Hybrid or Tribrid® Orbitrap mass spectrometers, and data analysis including statistics in Proteome Discoverer 2.3 software. As expected, synchronous precursor selection (SPS) based methods provided the best accuracy and precision as compared to MS^2 methods. The TMT11plex yeast digest standard provides users a tool to measure the accuracy, precision and dynamic range assessments for different mass spectrometry approaches, and is an excellent quality control assay to assess the LC and MS instrument status in a standardized workflow.

Poster 141-ML: Statistical Testing using Multiple Levels of Quantitative Information in DIA Experiments

Ting Huang[1]; Roland Bruderer[2]; Jan Muntel[2]; Olga Vitek[1]; Lukas Reiter[2]
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In data-independent acquisition (DIA) experiments, both MS1 precursor intensity data and MS2 fragment ion intensity data can be acquired for statistical testing. MS1 and MS2 measurements of a peptide are independent but provide complementary information. However, very few DIA quantification methods utilize MS1 data due to the interferences in MS1 caused by other isotope envelopes or by fragments from coeluting peptides. Recent progress in MS instrumentation enables higher resolutions MS1 scans at faster speeds (up to 1,000,000 on the orbitrap platform). Thus, quantification methods which can take advantage of the high quality MS1 information are expected.

In this study, we developed a statistical approach using both MS1 and MS2 information to detect the significant changes in protein abundances in DIA experiments. We applied this testing approach to four published controlled DIA datasets recorded on various instruments. Additionally, we prepared another DIA dataset where the controlled differential abundance was spiked into a background with biological variance. The results of these analyses were compared with those of the same testing approach, but applied to only MS1 data or MS2 data. We detected a consistent improvement of the statistical power when using both MS1 and MS2 information, judged by AUC value, sensitivity and specificity. We also generated a set of four controlled DIA datasets recorded with different MS1 resolutions to benchmark the contribution of MS1 and MS2 information. The influence of the MS1 data quality is apparent and enables generation of optimal DIA methods for statistical testing based on MS1 and MS2 data. Finally, we applied the testing approach to a study with 24 cancer and control samples and combining MS1 and MS2 data shows a higher sensitivity by detecting more differentially abundant proteins. Our work demonstrates the practical benefits of properly acquiring and utilizing MS1 data for statistical testing in DIA experiments.

Poster 142: Quantitative Analysis of the Fetal Tissue Translatome by Mass Spectrometry Reveals Temporal and Tissue-Specific Regulatory Networks *in utero*

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[1]University of Pennsylvania School of Medicine, Philadelphia, PA; [2]Children's Hospital of Philadelphia, Philadelphia, PA

During development, multicellular, complex organisms differentiate from a single zygote into different cell types in a highly ordered and reproducible manner. Precise spatial and temporal regulation of the gene expression program is crucial for normal development. Therefore, determining the precise timing of protein expression in tissues is necessary for understanding the complex regulatory networks involved during development. In this study, we developed a method for quantifying the embryonic tissue translatome, i.e., protein translation rates of various tissues at different stages of mouse fetal development. Using this technique, we relate changes in tissue specific translation rates to activation of various signaling pathways. To determine developmental stage-specific protein translation rates, fetal mice were administered a single pulse of isotopic amino acids via the vitelline vein. This minimally invasive procedure bypasses the need to administer labeled amino acids in the diet of the pregnant mouse and allows for the precise timing of developmental stage-specific protein labeling. Isotopic amino acids circulate throughout the

fetus and are transported to developing organs where they are used by the translational machinery for protein synthesis. At specific time points after amino acid introduction, fetal tissue is harvested and subsequently analyzed using quantitative mass spectrometry. Analyzing multiple time points allows us to quantify protein synthesis rates in various tissues including the brain, heart, kidney, liver, and lung. We quantified newly synthesized proteins of the mouse fetal liver and compared E13, E14, E16, and P0 gestational ages and find that the developing fetus reprograms the proteome in a tissue and stage specific manner.

Poster 143: Model-Free SILAC Data Analysis is Possible, Reproducible, and Essential

David Chiang; Patrick Chu
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Using SILAC as a case study, we aim to solve this paradox: How proteomics — an analytical science with high-accuracy data — can produce irreproducible results.

One factor is that labs rely on complex models (search engines, discriminant scores, posterior probabilities) within often-opaque software to interpret m/z data. Increased complexity generally means narrower applicability. Because mass spectrometry data have wide signal-to-noise, any complex model is prone to fitting problems for some data subset while depriving researchers of data insight.

For SILAC differential quantitation, a conventional workflow uses a statistical formula to calculate a “probability” (actually p-value) to evaluate a peptide ID, then fit a Gaussian area-under-curve model to quantify light and heavy peptides to derive their ratio. It’s simple in concept but tricky in practice.

Experienced users often find that while 90% or more of the protein ratios are generally correct (if imprecise), a small portion (but often the most critical) have essentially random ratios.

Two susceptibilities explain this: (1) Over-fitted p-values randomizes IDs and (2) under-fitted areas can randomize quantitation. A Gaussian fit requires at least one data-point near the maximum. In any large dataset, some small percentage would lack such points to yield random ratios.

Counter to complexity bias, a robust foundation requires direct analysis of raw m/z data with model-free peptide significance and SILAC quantitation.

Specifically, each peptide-sequence match (PSM) can be distilled to (1) number of matched fragments and (2) their average RMS error. For SILAC quantitation, each precursor scan is essentially an independent ratio-sampling experiment whose median approximates the true ratio. We believe this model-free approach is novel and fundamentally solves irreproducibility.

Poster 144-TL: Bringing KINATEST-ID to Everyone: A Pipeline for Studying Tyrosine Kinases

John Blankenhorn; James Johnson; Laurie Parker
UMN, Minneapolis, MN

Previous work in the Parker Lab has led to the creation of data-mining tools which can be used to discover a preferred substrate motif from an *in vitro* generated dataset from an enzymatically active tyrosine kinase. In short, by using phosphoproteomics and mass spectrometry, a large dataset of phosphopeptides specific to a kinase of interest can be established. Then using data mining and statistical tools, those peptide sequences can be used to discover the kinase of interest’s preferred substrate motif. Now these tools are being published to a broad audience using the GalaxyP platform, an open-source way for any researcher to use bioinformatic programs created by others. R scripts and Java programs instantiating these tools have been deposited in the UMN GalaxyP toolshed, where they are available to be used as part of the KINATEST-ID workflow, and thereby get used by other researchers to discover the preferred substrate motifs of their kinases of interest. This may especially prove useful for orphan kinases about which little is currently known.

Poster 145: Achieving Robust Deep Proteome Coverage on 2D Multiplexed Samples with the Evosep One LC System while Reducing Analysis Time

Jonathan Krieger[1]; Leanne Wybenga-Groot[1]; Jiefei Tong[1]; Nicolai Bache[2]; Ming Tsao[3, 4]; Michael F. Moran[1, 4]
[1]The Hospital for Sick Children, Toronto, Canada; [2]Evosep, Odense, Denmark; [3]Princess Margaret Cancer Centre, Toronto, Canada; [4]University of Toronto, Toronto, Canada

Balancing the comprehensiveness of proteome coverage and amount of mass spectrometry time is a challenge the field of proteomics. Multidimensional fractionation strategies to analyze multiplexed samples have significantly increased the quality of data and depth of coverage obtained from large proteomic studies, however, these strategies also result in significant increases in mass spectrometry time.

In order to reduce required data acquisition time, without compromising depth of coverage, we incorporated the Evosep One liquid chromatography system into our multidimensional multiplexing workflow for quantitative analysis of TMT-labelled non-small cell lung carcinoma patient-derived xenograft samples.

Using Evosep One technology attached to an Orbitrap Fusion Lumos Tribrid mass spectrometer, we identified >12000 proteins while only requiring 48 hours of instrument time. Comparatively, 67.5 hours of instrument time was required to achieve the same depth of coverage from the same samples using our conventional approach, where fractions collected in the first dimension are concatenated and run on our EASY-nLC system. Furthermore, even shorter runs on the Evosep One allowed us to decrease instrument time by another 50% while only losing 10% of protein identifications.

Thus, combining the Evosep one with two-dimensional fractionation and tandem mass tagging results in equal or increased depth of proteome coverage while greatly reducing instrument load.

Poster 146-TL: Global Quantification of Proteome and Phosphoproteome Revealed Novel Cellular Signaling Mechanisms Responsive to Hypoxia and Iron Deficiency

Luke Erber; Yao Gong; Maolin Tu; Phu Tran; Yue Chen
University of Minnesota, Minneapolis,

Micronutrient sensing is critical for cellular growth and differentiation. Iron and oxygen deficiencies are common features in pathophysiological conditions such as ischemia, neurological diseases and cancer. Cellular adaptive responses to starvation include repression of mitochondrial respiration, promotion of angiogenesis, and cell cycle control. We applied a systematic proteomics analysis to identify and quantify the dynamics of the global proteome and phosphorylation signaling in response to acute hypoxia, chronic and acute iron deficiency. Our analysis identified over 8600 proteins in nearly 5000 protein groups and about 16000 phosphorylation sites. At least ten percent of the phosphorylation sites

increased by two folds under each treatment conditions. In addition to the regulation of pyruvate dehydrogenase activity and fatty acid beta-oxidation, phosphorylation targeted DNA repair functions, cell cycle activity and ubiquitin ligases under oxygen starvation. Specifically, phosphorylation of proteins involved in iron sequestration, glutamate metabolism, and histone methylation pathway were found to be highly sensitive to iron deficiency. The dynamics of the phosphorylation response to micronutrient starvation provides a missing link in the global view of cellular regulation.

Poster 147: Quantitative Proteomic Profiling of a Model MCF10A-KRas^{G12V} Cell Line Reveals Dysregulated Pathways

Jian-Jiang Hao[1]; Mason Tao[1]
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Activating mutations in the KRAS oncogene are highly prevalent in tumors. To better understand the molecular mechanisms that KRAS mutant cells rely upon for their growth, we employed a comparative proteomic analysis of MCF10A cell line transfected with constitutively activated KRasG12V and native MCF10A cells transduced with an empty vector using isobaric tandem mass tag (TMT)-based quantitative mass spectrometry. 6605 proteins were quantified and 770 proteins that presented a 1.5-fold change were considered a KRasG12V proteomic signature. A significant pattern of changes in protein abundance was uncovered, a significant elevation of proteins responsible for cell proliferation, chromatin modification, gene expression and DNA replication and damage repair, and a decreased expression of proteins responsible for mitochondria functions and metabolisms including Carbohydrate metabolism, Oxidative phosphorylation, and lipid metabolism. Specifically, the imbalance of between the elevated proteins involved in cell proliferation and attenuated proteins involved in metabolisms reflects the consequences of oncogenesis promoted by KRasG12V. Altogether, our proteomic analysis reveals the molecular consequences of active KRAS mutant and provide new insights on the role of KRAS mutants in human cancers.

Poster 148-TL: Quantitative Top-Down Proteomics in Complex Samples using Protein-Level Tandem Mass Tag (TMT) Labeling

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The isobaric chemical tag labeling (*e.g.*, iTRAQ and TMT) has been widely applied for the quantification of peptides and proteins in bottom-up proteomics. However, until recently, successful top-down proteomics using this method has been limited, and it has not yet been applied to labeling and quantifying complex intact protein samples. One major challenge with intact complex sample labeling is that proteins tend to precipitate and "crush" out of solution because organic solvents used in the reaction are known extraneous protein precipitants that lower the solubility of proteins.

In this study, we report a TMT top-down MS platform for identifying and quantifying intact proteoforms less than 35 kDa in complex biological samples. To remove large proteins in the complex sample, we developed a filter-SEC approach that combines a MWCO step with a high-performance size exclusion chromatography (SEC). Our results indicate that the filter-SEC approach increases the protein sample solubility for efficient labeling. In addition, reaction solvent and protein-to-reagent ratios were optimized to increase the labeling efficiency in our intact protein-level TMT labeling experiments. To our knowledge, it represents the first high-throughput isobaric chemical tag labeling based quantitative top-down MS analysis of complex biological samples.

A total of 408 intact proteoforms from 95 proteins were confidently identified and quantified from two LC-MS/MS runs after manual evaluation. Among them, 303 were completely labeled (both at the N-terminus and at all lysine residues), while 64 were labeled on all lysine residues but were missing a label at the N-terminus. We further coupled our optimized TMT top-down MS platform to a 2D pH RP/RPLC-MS/MS platform for deeper quantification on Hela cell lysate. In our preliminary result, we identified 2931 proteoforms from 224 proteins before labeling.

Poster 149-ML: Proteomic Characterization of the Spemann Organizer in *Xenopus laevis* (frog) Embryos

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Development of neural tissues in vertebrate embryos is dependent on molecules produced by specialized tissues, such as the Spemann organizer (SO) in the frog *Xenopus laevis*. However, our current understanding of molecules implicated in this tissue has been limited to transcripts and some abundant proteins due to current limitations in mass spectrometry (MS) sensitivity. To fill this technology-knowledge gap, we here revised a bottom-up proteomic method for the discovery characterization of the developing SO in *X. laevis*. The approach began with visualization of SO tissue in the embryo via fluorescent cell lineage tracing. The fluorescently labeled SO was dissected and collected alongside the remaining tissue from the embryo. The samples were yolk-depleted and processed for bottom-up proteomics. The resulting peptides were barcoded with tandem mass tags and combined for relative quantification. The peptides were fractionated using high pH C18 spin columns, separated using low-pH nano-liquid chromatography, and ionized by a nanoelectrospray source. The peptide ions were detected by high resolution tandem MS and quantified using multi-notch MS^3 (Orbitrap Fusion Lumos, Thermo). Approx. 4,700 protein groups were identified (<1% FDR at peptide and protein levels), including 18 transcription factors. Furthermore, the quantitative dataset revealed 47 proteins to be differently expressed by at least 2-fold (Student's t-test, $p < 0.05$) between the SO and the rest of the embryo, providing a list of candidate proteins for functional experiments. HRMS-based proteomics raises a potential to expand our knowledge of basic molecular mechanisms underlying vertebrate embryonic development.

Poster 150: Processed by Angiotensin-Converting Enzyme: A Plethora of Non-Angiotensin-I Peptide Substrates and Products

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The dicarboxypeptidase Angiotensin converting enzyme (ACE) converts angiotensin I into the potent vasoconstrictor angiotensin II, which leads to the elevation of blood pressure. In addition, ACE activity is also involved in a number of other physiological processes, including fibrosis, male fertility, atherosclerosis, renal development, Alzheimer's disease, immune display, and resistance to cancer. These non-conventional effects of ACE are presumably mediated through processing of peptides unrelated to angiotensin. The goal of this study was to identify novel natural substrates and products of ACE by mass spectrometric peptidomic experiments. We compared the ACE-treated and untreated plasma peptidome of ACE knockout (KO) mice, validated the results with a set of synthetic peptides, and finally conducted a quantitative *in vivo* study of ACE substrates in mice with

distinct genetic ACE backgrounds. The plasma peptidome of mice treated with the ACE inhibitor Ramipril served as a control. 244 natural peptides were identified *ex vivo* as possible substrates or products of ACE, demonstrating high promiscuity of the enzyme. Several of the novel ACE substrates have known or predicted biological activities, including a fragment of complement C3, the spasmogenic C3f, which was processed by ACE *ex vivo* and *in vitro*. Analyses with N-domain inactive (N-KO) ACE allowed us to determine domain selectivity towards substrates. The *in vivo* ACE substrate concentrations in WT, transgenic ACE KO, N-KO, and C-KO mice correspond well with the *in vitro* observations, in that higher levels of the ACE substrates were observed when the processing domain was knocked out or ACE was inhibited by Ramipril. This study highlights the vast extent of ACE promiscuity and provides a valuable platform for further investigations of ACE functionality. As such our method is now well suited to study the unknown off-target effects of ACE inhibitors used for the treatment of human hypertension in the future.

Poster 151: Targeted Proteomics-Driven Computational Modeling of the Mouse Macrophage Toll-like Receptor Signaling Pathway

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The Toll-like receptor (TLR) signaling pathway in macrophages is essential for generating an effective innate immune response. Subtle variation in the concentration, timing, and structure of the stimuli (e.g., lipopolysaccharide (LPS)) is known to affect TLR signaling and the resulting immune response. Tight regulation is essential to avoid acute tissue damage and chronic inflammation. In general, accurate pathway modeling is needed to understand how signaling networks function in time and space in response to stimuli.

RNA-seq was used to identify transcripts, and targeted mass spectrometry was used to measure the absolute abundance of the TLR pathway proteins (using two quantified internal peptide standards per protein) within basal and LPS-stimulated mouse macrophages. The resulting values are being used as pathway model parameters. Molecular interaction rates were estimated using structural modeling using TransComp and Simulation of Diffusional Association. Rule-based pathway modeling is being performed using the Simmune software suite. Phosphosite absolute quantification using internal phosphopeptide standards and parallel reaction monitoring is being performed to produce data to be used for model training (via parameter scanning), testing, and validation. Previously published proteomics, phosphoproteomics, and flow cytometry data will also be used.

Preliminary protein absolute quantification data was produced for unstimulated and stimulated (100 ng/ml LPS for 30 min) macrophages. Abundance decreases were observed for proteins known to be targeted for degradation in response to TLR pathway activation. In addition, association rate constants were estimated for 256 protein-protein interactions using high performance computing.

This research was supported by the Intramural Research Program of the National Institute of Allergy and Infectious Diseases, NIH.

Poster 152-TL: Mechanical Stimulation Induces Rapid Phosphorylation-dependent Signaling in *Xenopus* Embryos

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Mechanical forces are essential drivers of numerous biological processes, notably during development. Although it is well-recognized that cells sense and adapt to mechanical forces, the signal transduction pathways that underlie mechanosensing have remained elusive. Here, we investigate the impact of mechanical force on phosphorylation-mediated signaling in *Xenopus laevis* embryos. For this, we designed force stimulation workflows to assess responses to both centrifugation and compression in *Xenopus laevis* embryos. We performed whole proteome analyses, phosphopeptide enrichments for phosphoproteome characterization, and targeted MS analysis (PRM) for validation, in conjunction with pathway perturbation using kinase inhibitors. Over 8,500 phosphopeptides were quantified in response to temporal force stimulation analysis. By monitoring these temporal phosphoproteome and proteome alterations in response to force, we discover and validate elevated phosphorylation on focal adhesion and tight junction components, leading to several mechanistic insights into mechanosensing. First, we determine changes in kinase activity profiles during mechanoresponse, identifying the activation of basophilic kinases. Pathway interrogation using kinase inhibitor treatment uncovers a crosstalk between the focal adhesion kinase (FAK) and protein kinase C (PKC) in mechanoresponse. Second, we find the LIM domain 7 protein (Lmo7) as upregulated upon centrifugation, contributing to mechanoresponse. Third, we discover that mechanical compression force induces a mesenchymal epithelial transition (MET)-like phenotype. Altogether, this study provides insights into mechanisms underlying mechanical force sensing pathways, as well as proteome and phosphoproteome resources in *Xenopus laevis*.

Poster 153-ML: Systems-Wide Hijacking of Host Cells during Herpes Simplex Virus (HSV-1) Infection

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Herpes simplex virus (HSV-1) successful infection is the result of global modulation of the host cell proteome. This includes harnessing host protein complexes for viral replication and manipulating host chromatin to prevent cell apoptosis and antiviral responses. These fine-tuned events are still mostly unknown, especially when considering how they synergistically act together to trick a eukaryotic cell into producing abundant viral progeny. We present a system level characterization of proteome dynamics during the HSV-1 lytic infection of human foreskin fibroblast cells. We specifically employ an integrative perspective that links cell signaling with changes in chromatin state during virus invasion. Our study includes identification and quantification of the host and viral proteomes, phosphoproteomes, chromatin bound proteomes and post-translational modifications (PTMs) on cellular histones during the time course of infection. Globally, we accurately quantified more than 4,000 proteins, 200 differently modified histone peptides and 9,000 phosphorylation sites on cellular proteins. In addition, we identified 67 viral proteins and quantified 571 phosphorylation events on viral proteins, which is currently the most comprehensive map of the HSV-1 phosphoproteome. This multi-omics dataset was combined by assessing trends in protein/PTM abundance resolved over a time-course, and networking the chromatin associated proteome with modifications on histones. Results highlighted that stress-activated protein kinase activity and histone acetyltransferase complexes were globally upregulated during viral infection. These observations were

validated by quantifying a steady increase of histone H3 acetylation and phosphorylation marks that are indicators of open chromatin. For example, the relative increase in abundance of the histone mark H3K9acS10ph highlighted signaling activities activated in cells during infection. Together, our epi-phospho-proteomics data provide an unprecedented systems overview to investigate cross-talking events of the human and viral proteome dynamics during infection. These data reveal potential candidates for biomarker targets that can be inhibited to arrest viral infection and spread.

Poster 154-TL: Towards Elucidation of Muscle-Specific Receptor Tyrosine Kinase (MuSK) Signaling Pathway by Differential Agonists

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Muscle-Specific receptor tyrosine Kinase (MuSK) is essential for neuromuscular junction (NMJ) formation. MuSK is activated upon binding to Lrp4 co-receptor in complex with agrin, a motor neuron signaling molecule. In response to MuSK activation, acetylcholine receptors begin clustering on muscle surface to guide neuronal attachment. Molecular players involved in propagation of MuSK signaling are not yet fully defined. We employed mass spectrometry-based quantification by TMT to elucidate ubiquitination- and phosphorylation-mediated signaling pathways activated in response to treatment with agrin or MuSK agonist antibody. We observed that majority of MuSK signaling was mediated by phosphorylation. Significant increases in phosphotyrosine levels were detected on MuSK and its adaptor protein Dok7 upon treatment with each agonist. GO Biological Process enrichment analysis highlighted signaling through ERK1/2, small GTPases and cytoskeleton rearrangements that would be required for NMJ formation. Several AchR subunits were detected with increased phosphorylation, in line with previously reported observations that phosphorylation plays an important role in AchR clustering. KGG profiling revealed that proteins with functions in clathrin-mediated endocytosis are regulated by ubiquitination events downstream of MuSK. Overall results point at a significant overlap in signaling processes initiated by MuSK natural agonist agrin and MuSK agonist antibody.

Poster 155: Applications of Mass Spectrometry Targeted Assays for Quantitative Analysis of Cancer Signaling Proteins

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Background: Many genetic alterations in cancer cells modify the protein expression from AKT, RAS and TP53 pathways. Highly accurate monitoring of cancer signaling pathway proteins has not been achieved, due to poor reproducibility, unreliable quantitation, and lack of standardized methods and reagents. We have optimized a multiplex immunoprecipitation to targeted mass spectrometry (mIP-tMS) workflow to develop the SureQuant™ pathway panels, achieving simultaneous enrichment and absolute quantitation of multiple total and phosphorylated proteins from the AKT pathway, RAS, and TP53. These assays can quantitate multiple proteins, post-translational modifications (PTMs) and interacting partners, which creates new possibilities for a broad range of applications, including cancer diagnosis and prognosis, drug development, and precision medicine.
Method: The SureQuant AKT pathway (total or phospho), RAS, or TP53 panels include a multiplex IP module (antibodies and lysate), MS sample prep, absolute or relative quantitation modules (AQUA Ultimate peptides standards), and software. Serum-starved, inhibitor-treated (LY294002/NVP-BEZ235/Rapamycin) HCT116, A549, and MCF7 cells were stimulated with hIGF-1. SureQuant AKT pathway or RAS panels were used to determine the absolute concentration of target peptides using targeted MS analysis. The panels were benchmarked against Western blotting (WB) using three unstimulated, hIGF-1 stimulated or inhibited cell lysates, as well as several tissue lysates.
Results: Previously, we showed the feasibility of optimized mIP-tMS assays to quantitate AKT and RAS pathway proteins across 2 cancer cell lines ± LY294002. The SureQuant™ multiplex AKT pathway panels allowed absolute quantitation of multiple total and phosphorylated targets across three unstimulated, hIGF-1 stimulated and inhibited cell lysates as well as tissue/xenograft lysates. Benchmarking of mIP-tMS assays demonstrated moderate correlation relative to WB.
Conclusion: SureQuant pathway panels allowed simultaneous absolute quantitation of AKT pathway, RAS proteins and PTMs in a streamlined, standardized workflow.

Poster 156-TL: Global Immunoproteomic Profiling of Endotoxin-Stimulated Macrophages Uncovers Specifics of TLR4- and Caspase11- mediated Recognition

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The innate immune system is the body's first line of defense against pathogens and protects them against infectious diseases. On host myeloid cells surface, Gram-negative bacterial outer membrane component lipopolysaccharide (LPS) is sensed by the toll like receptor 4 (TLR). Intracellularly, LPS is recognized by caspase11 through non-canonical inflammasome and intracellular LPS induce an inflammatory form of death pyroptosis. TLR4-mediated signaling perturbations results in secretion of cytokines and chemokines that help clear infection and facilitate the adaptive immunity. Although the signaling events and associated proteins for TLR4 signaling pathway are well established, the specifics and deciphering the differences in complex signaling events and associated proteins of non-canonical inflammasome pathway remain challenging. Here we deduce the temporal differences in global protein secretion for TLR4- and caspase11- mediated protein secretion in macrophages using mass spectrometry (MS)-based quantitative secretomics. Using an integrated strategy spanning functional activity assays, top-down structural elucidation of endotoxins and secretomics of stimulated macrophages, we identified differences in TLR4- and caspase11-mediated protein secretion in response to two different Gram-negative bacterial endotoxins.

Poster 157: Epidermal Growth Factor-induced Phosphorylation Responses in Rat Inner Medullary Collecting Duct

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Epidermal growth factor (EGF) is known to antagonize the effect of vasopressin to increase water transport in the renal collecting duct. To understand the physiology of EGF's action, we carried out a large-scale phosphoproteomic study in rat inner medullary collecting duct (IMCD). IMCD suspensions were treated with 1 μM EGF or vehicle for 30 min. Three biological replicates were analyzed by LC-MS/MS after TMT isobaric labeling and phosphopeptide enrichment. 12260 phosphopeptides with unique phosphorylation sites were quantified, with 78 peptides increased and 100 peptides decreased in phosphorylation based on stringent statistical criteria. Sequences of upregulated phosphopeptides are consistent with activation of proline-directed kinases. Both ERK1 and ERK2 showed increases in

activation loop phosphorylation. Bioinformatic analysis of the significantly changed proteins showed "ErbB" as the prominent KEGG signaling pathway, consistent with the conclusion that EGF activates ERK via the canonical Ras-Raf-MEK pathway. This response is in general the opposite of phosphorylation changes in this pathway seen in response to vasopressin. Several regulated phosphoproteins were found to be associated with the PI3k-Akt-Mtor pathway. There were concomitant changes in proteins associated with regulation of translation (Ago2, eIF2ak3, eIF2ak4, eIF4ebp1, Eef2, Eef2k, Fbxo6, Pdcd4). Regulated phosphoproteins also showed a significant association with the Plcγ phosphoinositide calcium signaling pathway (Plcg1, Plcb4, Itpr2, Camk2g, Ppp3cb and Nos1). EGF had no significant effect on vasopressin-regulated phosphorylation sites of the aquaporin-2 (AQP2) water channel (S256, S261, S264, S269) or the UT-A1 urea channel (S84, S486, S499). However, several modulators of cytoskeleton rearrangement (Cfl1, Fnbp1, Macf1, Parva, Plec, Sipa1l1, Specc1l, Trip10) and trafficking proteins (Dnajc5, Epn3, Mvb12a, Stx6, Ston2, Sytl5, Trak2) underwent phosphorylation changes, consistent with roles in AQP2 trafficking regulation. These data provide a framework for further studies to understand how EGF and vasopressin regulate water transport in the renal collecting duct.

Poster 158: Single-cell Mass Spectrometry for Proteomic Analysis of Patch-clamp Electrophysiology Identified Dopaminergic Neurons

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Direct measurement of proteins in single neurons promises to advance the understanding of molecular mechanisms responsible for brain development. However, due to sensitivity limitations, proteomics by current high-resolution mass spectrometry (HRMS) technology is typically only applicable to large populations of cells. We and others have recently advanced capillary electrophoresis electrospray ionization (CE-ESI) for HRMS to enable the characterization of proteins from limited populations of neurons. Here we advance the sensitivity of our microanalytical approach to single identified neurons in the mouse brain. Using patch-clamp electrophysiology and microscopy, dopaminergic neurons were identified in the substantia nigra. Immediately thereafter, a small portion (<10 nL) of the neuronal soma was microaspirated using the patch-clam capillary. The aspirated cell content was processed for microscale bottom-up proteomics, and the resulting peptides were analyzed using a custom-built CE-ESI-HRMS instrument. Approximately 140 protein groups were identified against the mouse proteome between 5 biological measurements (<1% FDR). Most of the identified protein groups corresponded to neuronal markers and neurotransmitters that are known to be expressed in dopaminergic neurons, thus validating the approach. The combined application of single-cell HRMS and patch-clamp electrophysiology provides a new tool to study neuronal development and homeostasis in the brain.

Poster 159-ML: Proteoform Family Identification and Quantification using Proteoform Suite

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Cellular functions are performed by a vast and diverse set of proteins. Proteoforms are the specific forms of proteins produced as a result of processes such as genetic variation, RNA splicing, and post-translational modifications (PTMs). In top-down analyses, many proteoforms are observed in MS1 spectra but are not fragmented due to limited instrument time over the course of data acquisition. This fragmentation data is required by most proteoform identification software programs to match to theoretical spectra, so proteoforms not selected for fragmentation or with low-quality fragmentation data cannot be identified. We have developed an open-source and freely available software program, Proteoform Suite, to identify and quantify proteoforms using exact masses obtained from deconvoluting MS1 spectra of intact proteins. Proteoform Suite can be integrated into top-down proteomic workflows to increase the number of proteoform identifications and determine proteoform-level abundance changes. Proteoform Suite was used to increase the number of identified proteoforms by ~40% in standard top-down analyses of yeast and mouse mitochondria samples by identifying non-fragmented proteoforms. Proteoform Suite also quantified proteoforms and determined 129 proteoforms with statistically significant abundance changes across mitochondria from mouse myoblasts (undifferentiated muscle cell precursors) and myotubes (differentiated muscle cells). We used Proteoform Suite to analyze a published MS1-only dataset of human heart tissue proteins fractionated by a novel serial size exclusion method. Proteoform Suite identified 478 proteoforms below 50 kDa and 8 proteoforms >50 kDa that were unidentified in the original study. Finally, we describe how Proteoform Suite facilitates targeted top-down MS/MS analyses by revealing unidentified proteoforms with significant abundance changes across conditions or proteoforms with unlocalized biologically interesting modifications. Proteoform Suite is a versatile software tool that can increase the number of proteoform identifications and determine proteoform abundance changes.

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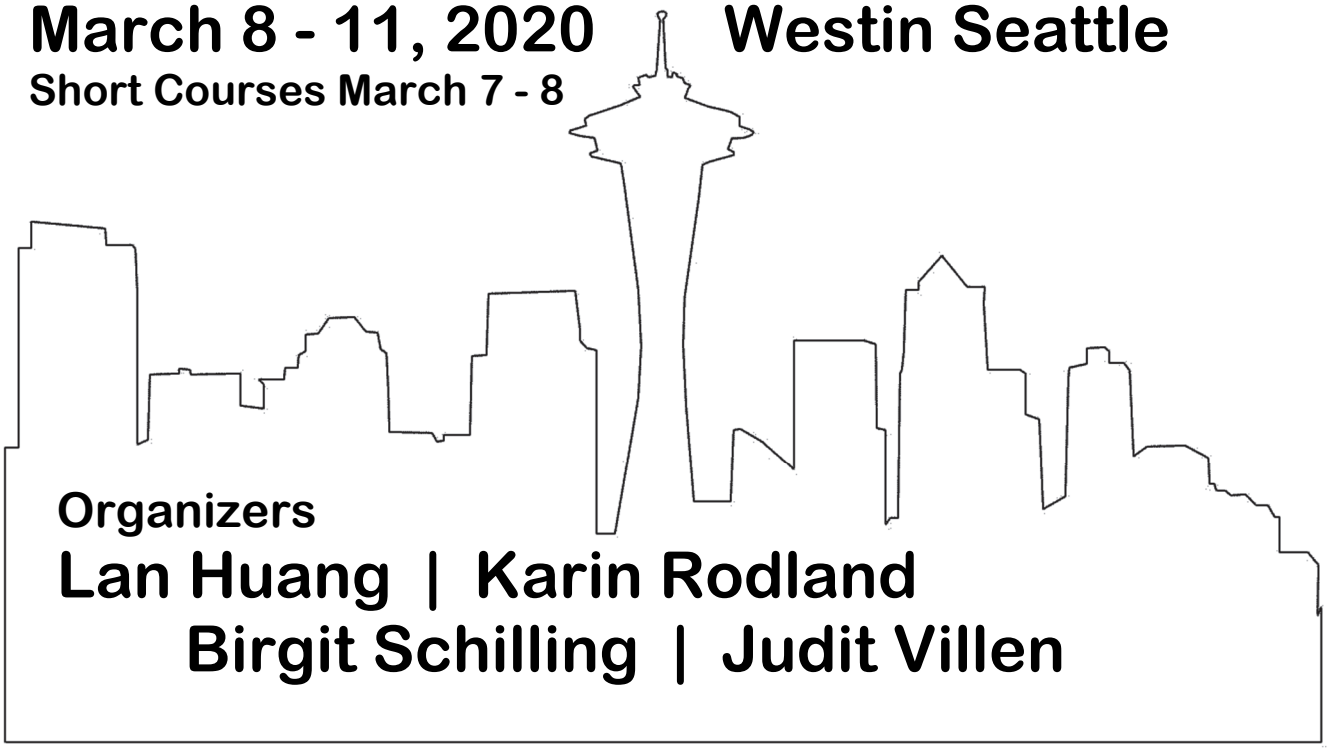

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