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TABLE OF CONTENTS

SUN, MARCH 11	MON, MARCH 12	TUES, MARCH 13	WED, MARCH 14
8 am - 7 pm **Registration** *Lobby Level* **Short Courses** **9:00 am - 4:00 pm** Full-Day Course, Day 2 **Design and Analysis of Quantitative Proteomics Experiments** *Deer Lake* **9:00 am - 12:00 pm** Morning Short Course **Cross-Linking Mass Spectrometry** *Maple-Birch Lake* **1:00 – 4:00 pm** Afternoon Short Course **Stable and Transient Protein-Protein Interactions** *Maple-Birch Lake*		**7:00 – 7:45 am** Zumba Participants go to Conf Reg Desk for directions to class!	
	8:00 – 8:30 am Early Morning Coffee *Exhibits & Posters, Ballroom 3-4*	**8:00 – 8:30 am** Early Morning Coffee *Exhibits & Posters, Ballroom 3-4*	**8:00 – 8:30 am** Early Morning Coffee *Exhibits & Posters, Ballroom 3-4*
	8:30 – 9:20 am **JPR Distinguished Achievement in Proteomics Award Lecture** Donald F. Hunt *Ballroom 2*	**8:30 – 9:20 am** **Award Presentations + Talks** Hannes Röst and Leslie Hicks *Ballroom 2*	**8:30 – 9:20 am** **Tips & Tricks Talks** *Ballroom 2*
	9:20 – 9:50 am Coffee Break *Exhibits & Posters, Ballroom 3-4*	**9:20 – 9:50 am** Coffee Break *Exhibits & Posters, Ballroom 3-4*	**9:20 – 9:50 am** Coffee Break, *Foyer near Registration*
	9:50 – 11:10 am Parallel Sessions **Microbiome** *Ballroom 2,* **Signaling & Protein Interactomics** *Ballroom 1*	**9:50 – 11:10 am** Parallel Sessions **Quanitative Proteomics II** *Ballroom 2* **Intact Protein Structural Analysis** *Ballroom 1*	**9:50 – 11:10 am** Parallel Sessions **NextGen Clinical MS-Based Proteomics** *Ballroom 2* **Integrative 'Omics: Multi-Omics** *Ballroom 1*
	11:10 am – 12:00 pm Plenary Session **Lightning Talks – Round I** *Ballroom 2*	**11:10 am – 12:00 pm** Plenary Session **Lightning Talks – Round II** *Ballroom 2*	**11:10 am – 12:00 pm** **Plenary Lecture** Emma Lundberg *Ballroom 2*
	12:15 – 1:30 pm **Lunch Seminars** Bruker, *Deer-Elk Lake* Biognosys, *Maple-Pine Lake*	**12:15 – 1:30 pm** **Lunch Seminar** Thermo, *Deer-Elk Lake* SCIEX, *Maple-Pine Lake*	
	1:30 - 3:00 pm **Poster Session** *Exhibits & Posters, Ballroom 3-4*	**1:30 - 3:00 pm** **Tuesday Posters** *Exhibits & Posters, Ballroom 3-4*	
	3:00 – 4:20 pm Parallel Sessions **Informatics: Emerging & New Approaches** *Ballroom 2* **Proteomics of Cancer & Disease** *Ballroom 1*	**3:00 – 4:20 pm** Parallel Sessions **Metabolism & Disease** *Ballroom 2* **Immunopeptidomics** *Ballroom 1*	
	4:30 – 5:50 pm Parallel Sessions **Quantitative Proteomics I** *Ballroom 2* **Proteomics of Aging** *Ballroom 1*	**4:30 – 5:50 pm** Parallel Sessions **PTMs: New Approaches & Applications** *Ballroom 2* **Glycomics for Clinical Applications** *Ballroom 1*	
6:00 – 7:00 pm **Opening Session Plenary Lecture** Ruedi Aebersold *Ballroom 2*	**5:50 – 6:30 pm** **Mixer** with Exhibitors *Poster-Exhibits* Munchies & Drinks All are welcome! Grab something to eat before the workshop.	**6:00 – 7:30 pm** **Career Development Event** sponsored by Bio-Techne *Deer-Elk Lake*	
7:00 – 8:30 pm **Opening Reception** *with Exhibitors,* Food & Drinks All are welcome!	**6:30 – 8:00 pm** Evening Workshops **LINCS** *Ballroom 2*	**7:30 – 9:00 pm** **Social Event** Supper event featuring live music. Free to all attendees. *Atrium*	

GENERAL INFORMATION

VENUE. All meetings, sessions, and exhibits are on the fourth floor of the Marriott City Center Hotel.

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 Ballroom adjacent to the session rooms. Monday posters should be mounted between 8-8:30am on Monday, Tuesday posters should be mounted between 8-8:30 am on Tuesday. 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 in the Ballroom on fourth floor of hotel.

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 (Deer-Elk Lake and Maple-Pine Lake) near Registration. 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 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.

ZUMBA. Tuesday morning come to Registration at 7:00 am. The Zumba class will be in nearby meeting room. Class will conclude by 8:00 am. Free and open to all. It may be cold outside but we will heat things up with our early morning dance moves! Anticipate that almost everyone will be a Zumba newbie so there are no excuses not to join in on the fun.

CONFERENCE T-SHIRTS. Custom t-shirt is quick-dry jersey and appropriate for exercise or everyday wear. Advance online purchases ($5) will be accommodated first, but there are extra available for purchase onsite ($10). Come to registration for purchase or pick-up of your pre-order.

CAREER DEVELOPMENT EVENT ON TUESDAY. A special program coordinated by a committee of young researchers. All are invited, but the target participants are graduate students, post-docs, and other early career researchers. The program will feature a panelist poster session followed by a reflection exercise and discussion.

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

MARRIOTT CITY CENTER HOTEL – FOURTH FLOOR
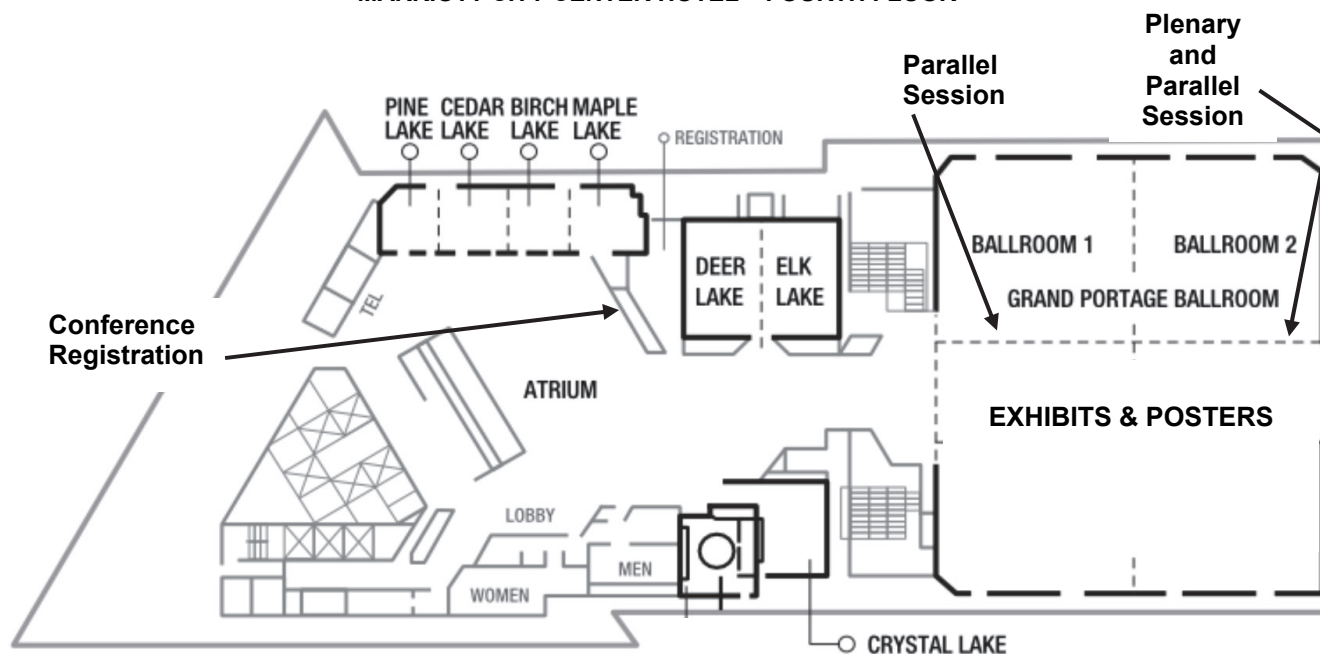
EXHIBITORS

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	BIOGNOSYS NEXT GENERATION PROTEOMICS
BSi Bioinformatics Solutions Inc.	BSG BIOTECH SUPPORT GROUP Sample Prep that Matters	BRUKER
EVOSEP	PBI Pressure BioSciences Inc.	Proteome Softw
RANDOX	SCIEX	SHIMADZU Excellence in Science
Silantes Stable Isotope Labeled Biomolecules	ThermoFisher SCIENTIFIC	veritomyx Accuracy matters
Waters THE SCIENCE OF WHAT'S POSSIBLE.™		

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, *Deer-Elk Lake* BRUKER	**Thermo Scientific**, *Deer-Elk Lake* ThermoFisher SCIENTIFIC
Biognosys, *Maple-Pine Lake* BIOGNOSYS NEXT GENERATION PROTEOMICS	**SCIEX**, *Maple-Pine Lake* SCIEX

AWARDS

US HUPO is pleased to announce a new award sponsored by the *Journal of Proteome Research* (JPR), an ACS Publication. This new award recognizes distinguished achievement in the field of proteomics. The award will continue annually with JPR's support. Recipients of this award will deliver a plenary lecture at the US HUPO annual conference and receive a cash prize of $2,500. Nomination deadline for 2019 is November 30. Please visit www.ushupo.org / about us hupo/ awards for details..

JPR and US HUPO honor Professor Donald F. Hunt with the inaugural 2018 award.

JPR Distinguished Achievement in Proteomics Award presented at US HUPO

2018 Recipient: **Donald F. Hunt**
University of Virginia
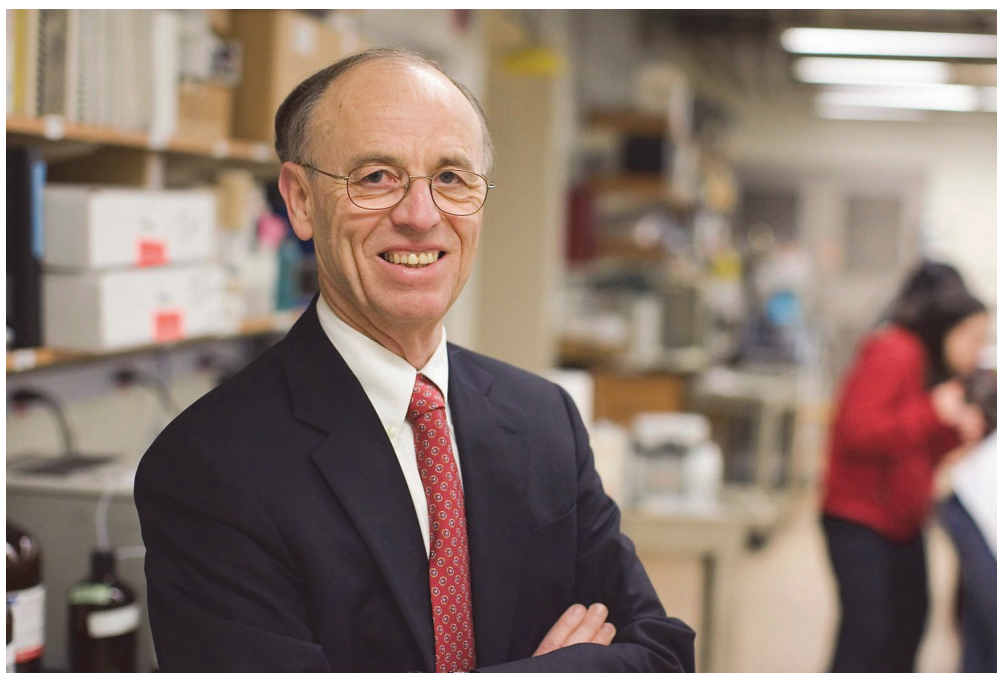
Professor Hunt pioneered the development of mass spectrometry-based methods and instrumentation that set the standard for ultrasensitive detection and characterization of proteins and peptides. His contributions to measurement science helped create the booming field of proteomics, which has amassed almost a million citations since 1995 (WoS 988,678 citations). These methods have had a dramatic impact on research in immunology, cell signaling, cell migration, chromatin biology and cancer and have led to a better understanding of many disease mechanisms as well as basic biology. Hunt's development of tandem mass spectrometry for protein sequencing together with software tools developed in the field for rapid interpretation of peptide spectra were so disruptive to the field of protein chemistry that the former state of the art Edman Degradation protein sequencing method is no longer used. Because of Hunt's work, today it is hard to imagine analyzing proteins and peptides by any other method than tandem mass spectrometry

AWARDS

US HUPO supports these two awards created to honor the name and contributions of leaders in the field and of the Society. Recipients of these two awards share a plenary session and each receive a $2,500 prize. Deadline for 2019 nominations is November 30. Please visit www.ushupo.org / about us hupo / awards for details.

GILBERT S. OMENN COMPUTATIONAL PROTEOMICS AWARD

2018 Recipient: **Hannes Röst**
University of Toronto

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.

Professor Hannes Röst is recognized with the 2018 Computational Proteomics Award for his development of the "OpenSWATH" software pipeline which is able to produce a comprehensive, targeted analysis of SWATH-MS data and align multiple such runs to produce an accurate and complete proteomics data matrix. The software pipeline is described in two award winning journal articles (Röst et al., 2014, *Nature Biotechnology*; Röst et al., 2016, *Nature Methods*) and integrated into the OpenMS software platform (Röst et al., 2016, *Nature Methods*). The work for this software pipeline was performed during Röst's PhD work at ETH Zürich under the supervision of Prof. Ruedi Aebersold (2010 to 2014.) In these three papers, Röst describes the development of a novel software approach to analyze single SWATH-MS runs using a targeted approach, a strategy to align multiple such runs into an experiment-wide data matrix and finally a collaborative, multi-lab C++ software framework which implements these software pipelines.

The OpenSWATH software pipeline is widely used and has sparked a large number of subsequent implementations and further research on SWATH-MS data. The novel ideas presented in the software, the open nature of the source code and integration with OpenMS have allowed other researchers to freely use, adopt and modify the software in a truly collaborative fashion.

ROBERT J. COTTER NEW INVESTIGATOR AWARD

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

2018 Recipient: **Leslie Hicks**
University of North Carolina, Chapel Hill

Professor Hicks started her appointment as Assistant Professor at UNC in 2013. She completed her PhD at the University of Illinois (Neil Kelleher) and went on to the serve as Director of Proteomics & Mass Spectrometry at the Danforth Plant Center (Washington University at St. Louis) prior to her academic appointment at UNC. The footprint of her science is widely recognized by mentors, collaborators, and members of her group.

The Hicks laboratory seeks to understand how metabolic control is achieved in photosynthetic cells to regulate the flow of fixed carbon from basic functions like cell proliferation to alternative pathways such as production of storage compounds, and specifically how intracellular signaling governs these processes.

In addition to addressing basic biological questions via proteomics in photosynthetic organisms, the Hicks lab is actively involved in bioactive peptide natural product discovery and characterization with our PepSAVI-MS pipeline. PepSAVI-MS (Statistically-guided bioactive peptides prioritized via mass spectrometry) achieves increased efficiency by implementing a hybrid approach combining the power of whole-cell bioassays and top-down peptidomics, whereby peptides are analyzed intact and contain all necessary modifications, such that we directly target and characterize only those species contributing to the bioactivity. Significant challenges associated with botanicals including complex matrices due to plant cell structure and complex secondary metabolism are addressed by our approach, promoting robust exploration of the plant bioactive peptide repertoire. The pipeline can easily be changed to screen for active components from any natural source, as well as test against multiple physiological targets of various cell lines or organisms, including fungi, viruses, protozoans, and cancer cells. The high efficiency and throughput of the pipeline allows for the rapid identification of multiple lead compounds.

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            Welcome to the Opening Session
  TECHNOLOGY ACCELERATING DISCOVERY
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Session Chair: Tim Griffin (University of Minnesota)

6:00 – 6:10 pm   Opening Remarks

6:10 – 7:00 pm   **Ruedi Aebersold**; *ETH Zurich*

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

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

MONDAY, MARCH 12

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

8:30 - 9:20 AM: **JPR Distinguished Achievement in Proteomics Award Lecture**, *Ballroom 2*
Session Chair: John Yates (The Scripps Research Institute)

8:30 - 9:20 am   **Proteomics in the Hunt Lab: A Brief History of Instrumentation and Methods Development for the the Sequence Analysis of Peptides and Intact Proteins**; Donald F. Hunt; *University of Virginia*

Dr. Hunt is the recipient of this inaugural award funded by JPR and presented at US HUPO. This honor will be awarded annually at future US HUPO conferences.

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

9:50 – 11:10 AM: PARALLEL SESSION
MICROBIOME PROTEOMICS, *Ballroom 2*
Session Chair: Bob Hettich (Oak Ridge National Laboratory)

9:50-10:15 am   **Metabolic Activity Within the Gut Microbiome and Its Impact on Human Health**; Kelsey Veldkamp; Kimberleigh Romano; Federico Rey; Daniel Amador-Noguez; *University of Wisconsin, Madison, WI*

10:15-10:40 am   **Interrogation of Gut Microbiomes with Chemical Probes and MS Proteomics**; Dennis Wolan, *The Scripps Research Institute*

10:40-10:55 am   **Reproducible, Robust Quantitative Functional Analysis of Metaproteomes Using the Galaxy Platform**; Caleb Easterly[1]; Joel Rudney[1]; James Johnson[1]; Carolin Kolmeder[2]; Andrea Argentini[3]; Thomas Mcgowan[1]; Bjoern Gruening[4]; Praveen Kumar[1]; Subina Mehta[1]; Lennart Martens[3]; Tim Griffin[1]; Pratik Jagtap[1]; *[1]University of Minnesota, Minneapolis, MN; [2]Max Planck Institute for Developmental Biology, Tuebingen, Germany; [3]Ghent University, Ghent, Belgium; [4]University of Freiburg, Freiburg, Germany*

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]; Zuo-Fei Yuan[1]; Kevin Janssen[1]; Yemin Lan[1]; Nathaniel Snyder[2]; Gary Wu[1]; Benjamin Garcia[1]; *[1]University of Pennsylvania, Philadelphia, PA; [2]AJ Drexel Autism Institute, Drexel University, Philadelphia, PA*

9:50 – 11:10 AM: PARALLEL SESSION
SIGNALING & PROTEIN INTERACTOMICS, *Ballroom 1*
Session Chair: Fernando Bazan (Bio-Techne)

9:50-10:15 am   **From RNA Granules to Signaling Networks: A Proximity Map of a Human Cell**; Anne-Claude Gingras; *University of Toronto, Toronto, Canada*

10:15-10:40 am   **Proteome-Scale Profiling of the Human Protein Interaction Landscape**; Edward Huttlin; *Harvard Medical School, Boston, MA*

10:40-10:55 am   **Integrating Virology and Proteomics to Define Mechanisms of Cellular Innate Immune Signaling during Herpesvirus Infection**; Krystal Lum; Benjamin Diner; Catherina Pan; Timothy Howard; Ileana Cristea; *Princeton University, Princeton, New Jersey*

10:55-11:10 Am   **Proteomic Analysis Reveals Novel Hydroxyproline-Dependent Cellular Pathways in Cancer Cells**; Luke Erber; Tong Zhou; Yue Chen; *University Of Minnesota, Minneapolis, MN*

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

Presentation Order

Mon 01   **A Portable Pathway for Processing Peptide Array Data in KNIME with an Interest in Protein-Protein Interactions;** Lee Parsons; *Univ of Minnesota, Plymouth, MN. **See Poster 18.***

Mon 02   **Development of a Novel Strategy for Measurement of the Neurotoxin Beta-N-Methylamino-L-Alanine in Environmental Samples;** Kaylie Kirkwood; Joshua Beri; Michael Bereman; David Muddiman; *North Carolina State University, Raleigh, NC. **See Poster 31.***

Mon 03   **Adaptation of KALIP for the Development and Prediction of Artificial Peptide Substrates to Monitor FMS-Like Tyrosine Kinase 3 (FLT3) Activity;** Minervo Perez[1]; W. Andy Tao[3]; Laurie L. Parker[2]; *[1]University of Minnesota, Minneapolis, MN; [2]University of Minnesota Twin Cities, Minneapolis, MN; [3]Purdue University, West Lafayette, IN. **See Poster 49.***

Mon 04   **Novel High-Throughput Metabolomic Techniques and Mainstream Tools for the Discovery of Drug Mechanism of Action**; Akos Vertes[1]; Albert-Baskar Arul[1]; Andrew R. Korte[1]; Hang Li[1]; Peter Avar[1]; Peter Nemes[1]; Lida Parvin[1]; Sylwia Stopka[1]; Sunil Hwang[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 Chem., The George Washington University, Washington, DC; [2]SRI International, Menlo Park, CA; [3]GE Global Research, Niskayuna, NY. **See Poster 34.***

Mon 05   **Changes in Glycan and Protein Expression in Aging Brain Nigrostriatal Pathway for Adeno-Associated Virus Mediated Gene Therapy in Parkinson's Disease**; Rekha Raghunathan[1]; Nicole Polinski[2]; Joshua Klein[1]; John D. Hogan[1]; Chun Shao[1]; Kshitij Khatri[1]; Deborah Leon[1]; Mark McComb[1]; Caryl Sortwell[2]; Joseph Zaia[1]; *[1]Boston University, Boston, MA; [2]Michigan State University, Grand Rapids, MI. **See Poster 54.***

Mon 06   **Approach to the Confident Determination of Elemental Compositions in Mass Spectrometry Imaging Using IR-MALDESI**; Sitora Khodjaniyazova[1]; Milad Nazari[1]; Kenneth Garrard[1]; Mayara Matos[2]; Glen Jackson[2]; David Muddiman[1]; *[1]North Carolina State University, Raleigh, NC; [2]West Virginia University, Morgantown, WV. **See Poster 30***

Mon 07   **Determining Peptide Substrates for Bruton's Tyrosine Kinase Biosensors via the KALIP Process**; Lindsay Breidenbach[1]; Minervo Perez[2]; John B[3]; Laurie L. Parker[4]; *[1]University of Minnesota, Maple Grove, MN; [2]University of Minnesota, Minneapolis, MN; [3]UMN, Minneapolis, MN; [4]University of Minnesota Twin Cities, Minneapolis, MN. **See Poster 64.***

Mon 08   **Application for Nanofluidic Devices Towards Single-Cell Proteomics: A Study with Xenopus Laevis .Embryos;** Anumita Saha-Shah[1]; Melody Esmaeili[1]; Peter Klein[1]; Benjamin A. Garcia[2]; *[1]University of Pennsylvania, Philadelphia, Pennsylvania; [2]University of Pennsylvania School of Medicine, Philadelphia, PA. **See Poster 65.***

Mon 09   **Galaxy-Based Multi-Stage Two-Step Database Searching Pipeline for Improved multi-omics analysis**; Praveen Kumar[1]; James Johnson[1]; Thomas McGowan[1]; Matthew Chambers[2]; Mohammad Heydarian[3]; Subina Mehta[1]; Caleb Easterly[1]; Joel Rudney[1]; Pratik Jagtap[1]; Timothy Griffin[1]; *[1]University of Minnesota, Minneapolis, MN; [2]Vanderbilt University, Nashville, TN; [3]Johns Hopkins University, Baltimore, MD. **See Poster 78.***

Mon 10   **Proteome-Wide Structure-Based Function Prediction Reveals Roles of Proteins Responsible for *E. coli* Fitness**; Chengxin Zhang; Mehdi Rahimpour; Peter Freddolino; Yang Zhang; *University of Michigan, Ann Arbor, MI.* **See Poster 66.**

Mon 11   **Applying Proteomics and Metabolomics to Understand the Role Sphingolipid Signaling in Pancreatic Cancer and Drug Sensitivity;** Max Jones; Monique Speirs; Connor Holman; John Price; *Brigham Young University, Provo, UT.* **See Poster 07**

Mon 12   **iOmicsPASS: Integrative-Omics Approach for Predictive Analysis with Subnetwork Signatures in Breast and Colorectal Cancer Subtypes;** Hiromi Wai Ling Koh[1]; Hyungwon Choi[1, 2]; [1]*National University of Singapore, Singapore;* [2]*Institute of Molecular Cell and Biology, A*STAR, Singapore.* **See Poster 22.**

Mon 13   **Predicting Cell-Line Specific Protein and Phospho-protein Abundances in Cancers**; Hongyang Li; Gilbert Omenn; Yuanfang Guan; *University of Michigan, Ann Arbor, MI.* **See Poster 24.**

Mon 14   **Traumatic Brain Injury Proteomics Guides Novel KCC2-Targeted Therapy**; Pavel N. Lizhnyak; Demisha D.L. Porter; John T. Povloshock; Andrew K. Ottens; *Anatomy & Neurobiology, Virginia Commonwealth Univ, Richmond, VA.* **See Poster 36.**

Mon 15   **Inhibition of KDM4C Leads to Histone Tail Clipping in a Triple Negative Breast Cancer Context;** Shawn Egri[1]; Guillermo Peluffo[2, 3]; Malvina Papanastasiou[1]; Kornelia Polyak[2, 3]; Jacob Jaffe[1]; [1]*The Broad Institute, Cambridge, Massachusetts;* [2]*Dana Farber Cancer Institue, Boston, MA;* [3]*Harvard Medical School, Boston, MA.* **See Poster 10.**

Mon 16   **New Protocols to Monitor Proteome Dynamics in the Developing Chordate Heart;** Burcu Vitrinel; Lionel Christiaen; Christine Vogel; *New York University, New York, NY.* **See Poster 09.**

Mon 17   **Statistical Model for dDtecting dDfferentially Abundant Proteins in Isobaric Labeling-Based Protein Quantification Experiments;** Ting Huang[1]; Meena Choi[1]; Manuel Tzouros[2]; Nikhil Pandya[2]; Balazs Banfai[2]; Tom Dunkley[2]; Olga Vitek[1]; [1]*Northeastern University, Boston, MA;* [2]*Roche Innovation Center Basel, Basel, Switzerland.* **See Poster 83.**

Mon 18   **Dynamics of Proteomics Changes in Presence of ER Stress in ALS Relevant Mouse Neuronal Model;** Shuvadeep Maity[1]; Disi An[2]; Justin Rendleman[1]; Esteban Mazzoni[1]; Christine Vogel[1]; [1]*Center for Genomics and System Biology, NYU, New York, NY;* [2]*Department of Biology, NYU, New York, NY.* **See Poster 61.**

Mon 19   **Impact of Three Different Mutations in Ehrlichia Chaffeensis in Altering the Global Gene Expression Patterns**; Chandramouli Kondethimmanahalli; Roman Ganta; *Kansas State University, Manhattan, KS.* **See Poster 46.**

Mon 20   **Finding Proteins Connected to Rheumatoid Arthritis**; Marcus Hadfield[1]; David Parkinson[1]; Stephen Ames[1]; Brad Naylor[1]; Lavender Lin[1]; Colette Quinn[2]; Lee Hansen[1]; John Price[1]; [1]*Brigham Young University, Provo, UT;* [2]*TA Instruments, Draper, UT.* **See Poster 50**

MONDAY LUNCH SEMINARS
<u>Open to all attendees</u>, 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 new timsTOF Pro, a novel QTOF instrument with a Trapped Ion Mobility Spectrometry (TIMS) front end, using the Parallel Accumulation Serial Fragmentation (PASEF) method will be described. In addition, exciting new applications in shotgun proteomics enabled by the technology described in the first talk will be discussed.

Highest Sensitivity, Highest Speed and Robust DDA Shotgun Proteomics with the timsTOF Pro Powered by PASEF
Gary Kruppa, *Ph. D., Vice President Proteomics, Bruker Daltonics Inc., Billerica MA*

Applications of PASEF on the timsTOF Pro for High Sensitivity Proteomics, Proximity Ligation Workflows and Multiplexed Analysis
Chris Adams, *Ph. D., Director of Proteomics, Stanford University Mass Spectrometry (SUMS)*

12:15 – 1:30 PM: **Biognosys**, *Maple-Pine Lake*

Taking a Leap to Quantifying the Proteome

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

Next generation proteomics based on data independent acquisition (DIA) enables deep and reproducible quantification of thousands of proteins in a single measurement experiments (Bruderer et al., 2015, 2017).

DIA workflows overcome the technical limitation of sampling speed of mass spectrometers by isolating broad ranges of peptide ions in parallel-- because of this, powerful data deconvolution algorithms must be utilized. This enables DIA technology to become only limited by the sensitivity of the detector, and not the sequential speed of the mass spectrometer.

Strategies and Challenges for Large-Scale SWATH-MS Dataset Generation and Analysis for Quantitative Discovery Proteomics
Dr. Mukul K. Midha, *Institute for Systems Biology, Seattle*

Latest Advancements in Data Independent acquisition (DIA) Using Spectronaut Pulsar
Dr. Florian Marty, *Biognosys AG, Schlieren, Switzerland*

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

3:00 – 4:20 PM: PARALLEL SESSION
INFORMATICS: EMERGING & NEW APPROACHES, *Ballroom 2*
Session Chair: Bobbie-Jo Webb-Roberstson (PNNL)

3:00-3:25 pm	**Alexey Nesvizhskii;** *University of Michigan, Ann Arbor, MI*
3:25-3:50 pm	**Next Generation MaxQuant: Machine Learning Enhanced Quantitative Proteomics**; Juergen Cox; *Max-Planck Institute*
3:50-4:05 pm	**Improved Protein Inference for Multiple Protease MS Data Using a Single Database Search**; Rachel Miller[1]; Connor Hoffmann[1]; Gloria Sheynkman[2, 2]; Robert Millikin[1]; Stefan Solntsev[1]; Anthony Cesnik[1]; Michael Shortreed[1]; Lloyd Smith[1, 3]; *[1]University Wisconsin-Madison, Madison, Wisconsin; [2]Center for Cancer Systems Biology (CCSB), Boston, MA; [3]Genome Center of Wisconsin, Madison, WI*
4:05-4:20 pm	**A Sampling of Perceptions in the Field of Mass Spectrometry Data Processing Software**; Rob Smith; *University of Montana, Missoula, MT*

3:00 – 4:20 PM: PARALLEL SESSION
PROTEOMICS OF CANCER & DISEASE, *Ballroom 1*
Session Chair: Lingjun Li (University of Wisconsin-Madison)

3:00-3:25 pm	**Proteomic Analysis of Stalled Replication Forks**; Anja Bielinsky[1]; Ya-Chu Chang[1]; Rebecca Rivard[2]; Yee Mon Thu[1]; Susan Kaye Van Riper[1]; LeeAnn Higgins[2]; Todd Markowski[1]; Katarzyna Kulej[2]; Jack Hedberg[1]; Luke Erber[1]; Yue Chen[1]; Eric Brown[2]; *[1]University of Minnesota, Minneapolis, MN; [2]University of Pennsylvania, Philadelphia, PA*
3:25-3:50 pm	**Hunting Circulating Mediators of Systemic Vascular Dysfunction after Carbon Nanotube Exposure**; Andrew Ottens; *Virginia Commonwealth University, Richmond, VA*
3:50-4:05 pm	**Polycomb Loss Enhances Oncogenesis But Leads to Therapeutic Vulnerabilities in Malignant Peripheral Nerve Sheath Tumors**; John Wojcik[1, 2]; Dylan Marchione[1, 2]; Simone Sidoli[1, 2]; Ben Garcia[1, 2]; *[1]University of Pennsylvania, Philadelphia, PA; [2]University of Pennsylvania School of Medicine, Philadelphia, PA*
4:05-4:20 pm	**A Proteomics Approach to Understand the Role of Autophagy in Colorectal Cancer and Enhance Chemosensitivity**; Monique Speirs; Emily Cannon; John Price; *Brigham Young University, Provo, Utah*

4:30 – 5:50 PM: PARALLEL SESSION
QUANTITATIVE PROTEOMICS I, *Ballroom 2*
Session Chair: Sarah Parker (Cedars-Sinai Medical Center)

4:30-4:55 pm	**Drug Target Identification by Label-Free Differential Mass Spectrometry**; Nathan Yates; *University of Pittsburgh, Pittsburgh, PA;*
4:55-5:20 pm	**Targeted Peptide Quantification Using Data Independent Acquisition**; Michael MacCoss; *University of Washington, Seattle, WA*
5:20-5:35 pm	**FlashLFQ: Ultrafast Label-Free Quantification of Peptides in Proteomics**; Robert J. Millikin; Stefan K. Solntsev; Michael R. Shortreed; Lloyd M. Smith; *University of Wisconsin, Madison, WI*
5:35-5:50 pm	**Optimization of Experimental Parameters in Data-Independent Mass Spectrometry Significantly Increases Depth and Reproducibility of Results**; Florian Marty; Roland Bruderer; Lukas Reiter; *Biognosys AG, Schlieren, Switzerland*

4:30 – 5:50 PM: PARALLEL SESSION
PROTEOMICS OF AGING & AGE-RELATED DISEASE, *Ballroom 1*
Session Chair: Birgit Schilling (Buck Institute)

4:30-4:55 pm	**How Protein PTMs Inspired Pharmacological Chaperones and Crosslinkers That Minimize Dead-End Modifications**; Jeff Agar; *Northeastern University, Boston, MA*
4:55-5:20 pm	**Understanding the Influence of Aging on Host Response to Infection**; Renä Robinson; *Vanderbilt University, Nashville, TN*
5:20-5:35 pm	**Proteome-wide Modulation of Degradation Dynamics in Response to Growth Arrest**; Tian Zhang[1, 2]; Kevin Welle[2]; Jennifer Hryhorenko[2]; Sina Ghaemmaghami[2]; *[1]Harvard Medical School, Boston, MA; [2]University of Rochester, Rochester, NY*
5:35-5:50 pm	**Identifying ApoE isoform Dependent Changes for Protein Turnover in the Brain**; Joseph Creery; Joshua Chamberlain; Russell Denton; John Price; *Brigham Young University, Provo, UT*

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

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

EVENING WORKSHOPS
All attendees are invited to participate in Monday evening workshops.

6:30 – 8:00 PM: PARALLEL EVENING WORKSHOP

LINCS WORKSHOP, *Ballroom 2*

Workshop Organizers: Andrea Matlock (Cedars-Sinai Medical Center), Mike MacCoss (University of Washington), and Jenny Van Eyk (Cedars-Sinai Medical Center)

Workshop Chair: Mike MacCoss

The NIH program **Library of Network-Based Cellular Signatures** (LINCS) presents benefits and challenges of large proteomic data sets, current efforts to generate a comprehensive library of signatures representative of chemical and genetic perturbations in cellular systems, as well as analysis tools and platforms for integration across different data types. Paving the road to a shared vision of powerful biological knowledge gains from large proteomic data sets queried individually or combined with omic integration studies, now and into the future.

TUESDAY, MARCH 13

7:00 AM: ZUMBA!!

Have a little early morning fun, join us for a 45-min Zumba session. Come by Registration, sign a quick waiver and we will direct you to the US HUPO Zumba class. There is a free class for US HUPO attendees only. Look for US HUPO President David Muddiman (a Zumba afficionado) to be among the intrepid!

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

8:30 – 9:20 AM: PLENARY SESSION
AWARD PRESENTATIONS AND LECTURES, *Ballroom 2*
Gil Omenn, presiding

8:30 – 8:55 am   **Gilbert S. Omenn Computational Proteomics Award: Hannes Röst**, *University of Toronto*
Award presentation followed by 20 minute talk.

8:55 – 9:08 am   **Robert J. Cotter New Investigator Award: Leslie Hicks**, *University of North Carolina, Chapel Hill*
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
QUANTITATIVE PROTEOMICS II, *Ballroom 2*
Session Chair: Yue Chen (University of Minnesota)

9:50-10:15 am   **Quantitative Multidimensional Characterization of Protein-Protein Interactions in Cancer**; Danielle Swaney[1]; Minkyu Kim[1]; Margaret Soucheray[1]; Kyumin Kim[1]; Fan Zheng[2]; Jennifer Grandis[1]; Trey Ideker[2]; Nevan Krogan[1]; *[1]University of California, San Francisco, San Francisco, CA; [2]University of California, San Diego, San Diego, CA*

10:15-10:40 am   **Exploring Protein Interactions Networks with High-Throughput Quantitative Proteomics**; Wilhelm Haas; *Massachusetts General Hospital, Boston, MA*

10:40-10:55 am   **Quantifying Protein Synthesis Rates During Fetal Development Reveals Temporal and Tissue-Specific Regulatory Networks**; Josue Baeza[1]; Barbara Coon[2]; William Peranteau[2]; Ben Garcia[1]; *[1]University of Pennsylvania School of Medicine, Philadelphia, PA; [2]Childrens Hospital of Pennsylvania, Philadelphia, PA*

10:55-11:10 am   **The Initiating Events in Direct Cardiomyocyte Reprogramming Involve Temporally Distinct Gene Expression and Proteome Abundance Signatures**; Todd Greco[1]; Kimberly Sauls[2]; Li Wang[2]; Meng Zou[2]; Michelle Villasmil[2]; Li Qian[2]; Frank Conlon[2]; Ileana Cristea[1]; *[1]Princeton University, Princeton, NJ; [2]Univ of North Carolina McAllister Heart Institute, Chapel Hill, NC*

9:50 – 11:10 AM: PARALLEL SESSION
INTACT PROTEIN STRUCTURAL ANALYSIS, *Ballroom 1*
Session Chair: Lingjun Li (University of Wisconsin)

9:50-10:15 am   **Analysis of Intact Proteins and Protein Complexes by Ultraviolet Photodissociation Mass Spectrometry**; Jennifer Brodbelt; *University of Texas at Austin, Austin, TX*

10:15-10:40 am   **Native MS in Structural Biology: Surface Collisions of Protein Complexes**; Vicki Wysocki; *The Ohio State University, Columbus, OH*

10:40-10:55 am   **Linear and Differential Ion Mobility Separations of Middle-Down Proteoforms**; Alyssa Garabedian[1]; Matthew Baird[2]; Jacob Porter[1]; Kevin Jeanne Dit Fouque[1]; Pavel Shliaha[3]; Ole Jensen[3]; Todd Williams[4]; Francisco Fernandez-Lima[1]; Alexandre Shvartsburg[2]; *[1]Florida International University, Miami, FL; [2]Wichita State University, Wichita, KS; [3]University of Southern Denmark, Odense, Denmark; [4]University of Kansas, Lawrence, KS*

10:55-11:10 am   **Characterizing Transmembrane Domains of Membrane Proteins with Top-Down Ultra-Violet Photodissociation (UVPD) High-Resolution Mass Spectrometry**; Julian Whitelegge[1]; Romain Huguet[2]; Chris Mullen[2]; Vlad Zabrouskov[2]; *[1]UCLA, Los Angeles, ; [2]Thermo Fisher Scientific, San Jose, CA*

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

Presentation Order

Tues 01   **Spritz: An RNA-Seq Analysis Engine that Enables Proteogenomics on Windows;** <u>Anthony Cesnik</u>; Michael Shortreed; Brian Frey; Lloyd Smith; *UW-Madison, Madison, WI.* ***See Poster 17.***

Tues 02   **Connecting the Dots Between Metabolic Reprogramming and Protein Function in Cancer;** <u>Emily Cannon</u>**;** Monique Pare Speirs; Max Jones; John Price; *Brigham Young University, Provo, UT.* ***See Poster 03.***

Tues 03   **A Novel Approach for Isolating Exosomes From Clinical Sample;** <u>Blake Ebert</u>; Alex J Rai, PhD; *Columbia University Medical Center, New York, NY.* ***See Poster 06.***

Tues 04   **A Quantitative Evaluation of Algorithms for Isotopic Trace Extraction via Ion Chromatogram Clustering;** <u>Mathew Gutierrez</u>; Amber Yascavage; Rob Smith; *University of Montana, Missoula, MT.* ***See Poster 14.***

Tues 05   **Detection of Post-Translational Modifications Using Mass Spectrometry**; <u>Nathan Zuniga</u>; Marcus Hadfield; Lavender Lin; David Parkins; John Price; *Brigham Young University, Provo, UT.* ***See Poster 48.***

Tues 06   **Big Data: Ensuring Integrity of Differential Analyzes From Large Scale DIA-MS Experiments**; <u>Andrea Matlock</u>[1]; Erin Crowgey[2]; Vidya Venkatraman[1]; Victoria Dardov[1]; Jennifer Van Eyk[1]; *[1]Cedars-Sinai Medical Center, Los Angeles, CA; [2]Nemours Children's Health System, Wilmington, DE.* ***See Poster 52.***

Tues 07   **PTM Knowledge Networks and LINCS Multi-Omics Data for Kinase Inhibitor Drug-Analytics in Lung Cancer;** Xu Zhang[1]; <u>Karen Ross</u>[2]; Jake Jaffe[3]; Michele Forlin[4]; Cathy Wu[5]; Udayan Guha[1]; *[1]CCR, NCI, NIH, Bethesda, MD; [2]Georgetown University Med. Ctr., Washington DC, Washington DC; [3]Broad Institute of Harvard and MIT, Cambridge, MA; [4]University of Miami, Coral Gables, FL; [5]University of Delaware, Newark, DE.* ***See Poster 04.***

Tues 08   **Investigation of the Effect of Reduced Synthesis and Degradation on in Vivo Protein Stability**; <u>Lavender Hsien-Jung Lin</u>; Marcus Hadfield; Nathan Zuniga; John C Price; *Brigham Young University, Provo, UT.* ***See Poster 58.***

Tues 09   **Phosphoproteomic Investigation of the Mechanism of Calorie Restriction Attenuation by Dietary Protein Signaling;** <u>Joshua Mcphie</u>[1]; Marco Hadisurya[2]; Aaron Moss[3]; Richard Carson[1]; John Price[1]; *[1]Brigham Young University, Provo, UT; [2]Brigham Young University - Hawaii, Laie, HI; [3]Brigham Young University - Idaho, Rexburg, ID.* ***See Poster 60.***

Tues 10   **Integrated Proteogenomic Analyses Reveal Extensive Tumor Heterogeneity and Validate Expression of Somatic Mutations in Lung Adenocarcinoma;** <u>Xu Zhang</u>[1]; Paul Rudnick[2]; Shaojian Gao[1]; Shivangi Awasthi[1]; David Fenyo[3]; Udayan Guha[1]; *[1]CCR, NCI, NIH, Bethesda, MD; [2]Spectragen Informatics LLC, Bainbridge Island, WA; [3]NYU School of Medicine, New York, NY.* ***See Poster 75.***

Tues 11   **Large Datasets and You: Using Proteomics to Design a Biosensor;** <u>John B</u>[1]; Minervo Perez[2]; Laurie L. Parker[3]; *[1]UMN, Minneapolis, MN; [2]University of Minnesota, Minneapolis, MN; [3]University of Minnesota Twin Cities, Minneapolis, MN.* ***See Poster 63.***

Tues 12   **Development of Hydrogen-Deuterium Exchange Methodology Coupled to Top- and Middle-Down Mass spectrometry Enables High-Resolution Analysis of Histone Dynamics;** <u>Kelly Karch</u>; Mariel Coradin; ZhongYuan Kan; Ben Black; Benjamin Garcia; *University of Pennsylvania School of Medicine, Philadelphia, PA.* ***See Poster 67.***

Tues 13   **Antibody-Antigen Interaction Characterization Using Stopped Flow Assisted Hydrogen Deuterium Exchange Mass Spectrometry**; <u>Zhe Wang</u>[1]; Kellye Sutton[1]; Michael Ashby[1]; Kenneth Smith[2]; Si Wu[1]; *[1]University of Oklahoma, Norman, OK; [2]Oklahoma Medical Research Foundation, Oklahoma City, OK.* ***See Poster 68.***

Tues 14   **Development of A High-Performance Sheathless CZE-MS Interface for Top-Down Proteomics**; <u>Lushuang Huang</u>; Zhe Wang; Si Wu; *University of Oklahoma, Norman, OK.* ***See Poster 70.***

Tues 15   **MetaMorpheus for Enhanced Global PTM Discovery (G-PTM-D) and Quantitative Proteomics;** <u>Stefan Solntsev</u>; Michael Shortreed; Brian Frey; Lloyd Smith; *University of Wisconsin, Madison, WI.* ***See Poster 51.***

Tues 16   **Improved Protein Sequence Analysis by 21 Tesla FT-ICR MS/MS and Advanced Mass Spectral Interpretation**; <u>Lissa Anderson</u>[1]; Jeffrey Shabanowitz[2]; Chad Weisbrod[1]; Greg Blakney[1]; Donald Smith[1]; Donald Hunt[2]; Christopher Hendrickson[1]; *[1]NHMFL, Tallahassee, FL; [2]Dept. of Chemistry University of Virginia, Charlottesville, VA.* ***See Poster 72.***

Tues 17   **Examination of the Specific Binding of Cytochrome c on MPA-Coated Gold Nanoparticles via Protein Footprinting;** Emily Tollefson[1]; Xi Zhang[2]; Nikita Rozanov[3]; Caley Allen[3]; Rigoberto Hernandez[3]; Catherine Murphy[2]; Erin Carlson[1]; *[1]University of Minnesota, Minneapolis, MN; [2]University of Illnios Urbana Champaign, Urbana, IL; [3]Johns Hopkins University, Baltimore, MD.* **See Poster 73.**

Tues 18   **Active Kinase Characterization Using an Isobaric Labeling Activity-Correlated Protein Profiling Platform (TMT-ACPP);** Hongyan Ma; Paul Sims; Si Wu; *University of Oklahoma, Norman, OK.* **See Poster 77.**

Tues 19   **A High-throughput Omics Pipeline for Comprehensive Analysis of Challenged Human Cells;** Danielle Gutierrez[1]; Carrie Romer[1]; Jamie Allen[1]; Yuan-Wei Nei[2]; Melissa Farrow[3]; Randi Gant-Branum[1]; Stacy Sherrod[1]; Nicole Muszynski[1]; Eric Spivey[1]; Salisha Hill[1]; Kristie Rose[1]; John Wikswo[1]; John McLean[1]; Eric Skaar[3]; D. Borden Lacy[3]; Jeremy Norris[1]; Richard Caprioli[1]; *[1]Vanderbilt University, Nashville, TN; [2]Quest Diagnostics, Chantilly, VA; [3]Vanderbilt University Medical Center, Nashville, TN.* **See Poster 79.**

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**, *Deer-Elk Lake*

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

In this seminar, the use of TMT based multiplexing quantitative proteomics for profiling subcutaneously implanted breast cancer patient-derived xenografts (PDXs) will be discussed. PDXs are the best model of primary human tumors and enable cancer researchers to study drug response and tumor biology. The stroma plays an important role in breast cancer progression. In PDX models, the tumor-associated stroma is mouse-derived and can be differentiated from the tumor (human-derived) by analysis of species-unique peptides. Precise and accurate TMT-based multiplexed quantitative proteomics enabled us to discover, in a cohort of 21 breast PDX tumors, that the education of the stroma was highly individualized but biologically coordinated. In particular, proteins involved in immune signaling varied in a subtype- and stage-specific manner. These findings may have future implications for treatment stratification and provide a platform from which to understand tumor-stroma interactions on a large-scale protein level.

Multiplexed Mass Spectrometry Solutions for Cancer Proteomics
Translational proteomics workflows place much greater emphasis on biological replicate analysis of large, well-defined cohorts instead of fewer samples and greater numbers of technical replicates. These also need to be quantitative, with changes across cohorts of samples measured to be precise and accurate. Multiplexed tandem mass tag (TMT) solutions offer greater parallelization potential in quantitative mass spectrometry experiments resulting in greater throughput.

Jason Held, *Ph.D., Assistant Professor, Washington University School of Medicine, Departments of Medicine, Oncology Division and Department of Anesthesiology*

12:15 – 1:30 PM: **SCIEX**, *Birch-Pine Lake*

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

Many challenges exist in realizing the potential of Precision Medicine, especially in producing robust, reproducible quantitative proteomics measurements. This workshop will address many of the challenges that currently exist in making these measurements in a high-throughput, industrialized fashion:

- Microflow LC for enhanced workflow robustness and sample throughput
- Cloud-based data processing to address the challenges of large data file and large sample numbers
- Integration with other 'omics data to provide more complete biological insight

Accelerating Quantitative Proteomics – microflow SWATH® Acquisition
Speaker: Arianna Jones & Guest Speaker

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

3:00 – 4:20 PM: PARALLEL SESSION
METABOLISM AND DISEASE, *Ballroom 2*
Session Chair: Ben Garcia (University of Pennsylvania)

3:00-3:25 pm **Defining Mitochondrial Protein Function through Systems Biochemistry**; David Pagliarini; *Morgridge Institute for Research, Madison, WI*

3:25-3:50 pm **Chemical-Proteomic Strategies to Investigate Reactive Cysteines**; Eranthii Weerapana; *Boston College, Boston, MA*

3:50-4:05 pm **Mapping the Changes to the Serum and Urinary Proteomes when the Pancreas is Removed**; Tue Bjerg Bennike[1, 2]; Melena Bellin[4]; Saima Ahmed[1, 2]; Zobeida Cruz-Monserrate[3]; Darwin Conwell[3]; Hanno Steen[1, 2]; *[1]Boston Children's Hospital, Boston, MA; [2]Harvard Medical School, Boston, MA; [3]The Ohio State University Wexner Medical Center, Columbus, OH; [4]University of Minnesota Medical Center, Minneapolis, MN*

4:05-4:20 pm **Sirtuin-Regulated Lipoylation is an Evolutionarily Conserved Mediator of Metabolic Health and Disease**; Elizabeth Rowland; Todd Greco; Caroline Snowden; Cora Betsinger; Ileana Cristea; *Princeton University, Princeton, NJ*

3:00 – 4:20 PM: PARALLEL SESSION
IMMUNOPEPTIDOMICS, *Ballroom 1*
Session Chair: Melanie Patterson (AbbVie)

3:00-3:25 pm **Immunopeptidomics: Accelerating the Development of Personalized Cancer Immunotherapy**; Michal Bassani-Sternberg; *CHUV, Lausanne, Switzerland*

3:25-3:50 pm **Chemical Biology to Investigate the Immunopeptidome**; Stephanie Jensen; Greg Potts; Melanie Patterson; *AbbVie, Chicago, IL*

3:50-4:05 pm **Characterizing the Intracellular Peptidome to Define Protein Areas Efficiently Processed and Presented across Multiple HLAs**; Julie Boucau[1]; Carl Kadie[2]; David Heckerman[2]; Sylvie Le Gall[1]; *[1]Ragon Institute of MGH, MIT and Harvard, Cambridge, MA; [2]Microsoft Research, Redmond, WA*

4:05-4:20 pm **Improved Discovery of Post-Translationally Spliced Peptides Using Separate b- and y-Ion Databases**; Zach Rolfs; Stefan Solntsev; Michael Shortreed; Brian Frey; Mark Scalf; Alan Attie; Lloyd Smith; *University of Wisconsin, Madison, Wisconsin*

TUESDAY, MARCH 13

4:30 – 5:50 PM: PARALLEL SESSION
PTMs: NEW APPROACHES AND APPLICATIONS, *Ballroom 2*
Session Chair: Chris Barnes (NovoNordisk)

4:30-4:55 pm   **Peptide Biosensors for Measuring Kinase Activity in Cells;** Laurie Parker; *University of Minnesota, Minneapolis, MN*

4:55-5:20 pm   **Judit Villen**; *University of Washington, Seattle, WA*

5:20-5:35 pm   **Deciphering the Human Sirtuin 3 Antiviral Functions and the Temporal Mitochondrial Acetylome during Herpesvirus Infection**; Xinlei Sheng; Laura Murray; Morgan Sly; Ileana Cristea; *Princeton University, Princeton, NJ*

5:35-5:50 pm   **Human Succinyl-CoA Ligase Deficiency Causes Widespread Protein Hyper-Succinylation in Patient-Derived Fibroblasts and Myotubes**; Philipp Gut[1, 2]; Jesse G. Meyer[3]; Sanna Matilainen[4]; Chris Carrico[3]; Pieti Pällijeff[4]; Birgit Schilling[3]; Anu Suomalainen[4]; Eric Verdin[3]; *[1]Gladstone Institutes, UCSF, San Francisco, CA; [2]Nestle Institute of Health Sciences, Ecublens, Switzerland; [3]Buck Institute, Novato, CA; [4]University of Helsinki, Helsinki, Finland*

4:30 – 5:50 PM: PARALLEL SESSION
GLYCOMICS FOR CLINICAL APPLICATIONS, *Ballroom 1*
Session Chair: Hui Zhang (Johns Hopkins University)

4:30-4:55 pm   **Integrated Workflows for Intact Glycopeptide Analysis**; Sharon J. Pitteri; *Stanford University, Palo Alto, CA*

4:55-5:20 pm   **Assignment of Site-Specific Glycosylation in Complex Proteoglycan Samples**; Joshua Klein; Le Meng; Joseph Zaia; *Boston University, Boston, MA*

5:20-5:35 pm   **Immunoglobulin G Fc Region N-linked Glycosylation as a Clinical Biomarker for Insulin Resistance**; Andrew Lipchik[1, 2]; Michael Snyder[1, 2]; *[1]Stanford University, Stanford, CA; [2]Stanford University, Stanford, CA*

5:35-5:50 pm   **N-Glycopeptide Feature Identification by Revealing Trends Between Analyte Composition and Compensation Field Through FAIMS-Coupled MS Platform**; Daniel Delafield[1]; Zhe Wang[1]; Matthew Baird[2]; Alexandre Shvartsburg[2]; Si Wu[1]; *[1]University of Oklahoma, Norman, OK; [2]Wichita State University, Wichita, KS*

6:00 –7:30 PM: **CAREER DEVELOPMENT EVENT**
Deer-Elk Lake

This special event is sponsored by Bio-Techne

A special program coordinated by a committee of young researchers (Kyle Delaney, *University of Chicago*; Joey Huang, *University of Chicago;* Kevin Janssen, *University of Pennsylvania*; Matthew Perez-Neut, *University of Chicago*) with Laurie Parker. All are invited, but the target participants are graduate students, post-docs, and other early career researchers. The program will feature a panelist poster session followed by a reflection exercise and discussion.

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!

8:00 – 8:30 AM: **EARLY MORNING COFFEE & PASTRIES**, *Foyer*

8:30 – 9:20 AM: PLENARY SESSION   **ANNOUNCEMENT OF BEST STUDENT AND POST-DOC POSTER AWARD WINNERS**   **TIPS & TRICKS (TECHNOLOGY FOCUS) LIGHTNING SESSION**   *Ballroom 2*   *Five-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   **High Pressure Denaturation Improves Protein Digestion by Trypsin;** <u>Vera Gross</u>; Nicole Cutri; Gary Smejkal; Alexander Lazarev; *Pressure BioSciences, Inc., South Easton, MA.* **See Poster 45.**

Wed 02   **Abundant Protein Depletion and Multiplexed Protein Quantitation of Human Plasma Samples – A Reproducibility and Scaling Study;** Sergei Snovida; Katherine Herting; Ramesh Ganapathy; Ryan Bomgarden; Barbara Kaboord; Chris Etienne; <u>Monica O'Hara</u>; John Rogers; *Thermo Fisher Scientific, Rockford, IL.* **See Poster 01.**

Wed 03   **High-Throughput Untargeted Data-Independent Analysis of Misoprostol Challenged HL60 Cells as a Model for Chemical Threat Assessment**; <u>Roy Martin</u>[1]; Brad Williams[1]; Danielle Gutierrez[2]; Jeremy Norris[2]; [1]*Waters, Beverly, MA;* [2]*Mass Spectrometry Research Center, Vanderbilt University, Nashville, TN.* **See Poster 90.**

Wed 04   **Optimizing Collision Energy in Collision-Induced Dissociation for Peptide Sequencing;** Nandhini Sokkalingam; Luke Schneider; William Wright; Siamak Ashrafi; Adam Tenderholt; <u>Jeffrey Peterson</u>; *Veritomyx Inc., Palo Alto, CA.* **See Poster 19.**

Wed 05   **Development of a Novel LC Concept for Clinical Proteomics**; <u>Nicolai Bache</u>[1]; Philipp Geyer[2]; Ole Hoerning[1]; Lasse Falkenby[1]; Peter Treit[2]; Sophia Doll[2]; Igor Paron[2]; Florian Meier[2]; Ole Vorm[1]; Matthias Mann[2]; [1]*Evosep, Odense, Denmark;* [2]*Max-Planck Inst. for Biochemistry, Martinsried, Germany.* **See Poster 40.**

Wed 06   **timsTOF Pro Powered by PASEF for High Speed and Sensitive Shotgun Proteomics**; <u>Gary Kruppa</u>[1]; Juergen Cox[2]; Heiner Koch[3]; Scarlet Koch[3]; Baozhen Shan[4]; [1]*Bruker Daltonics Inc., Billerica, MA;* [2]*Max Planck Inst. of Biochem., Martinsried, Germany;* [3]*Bruker Daltonik, GmbH, Bremen, Germany;* [4]*Bioinformatics Solutions Inc, Waterloo, ON, Canada.* **See Poster 42.**

Wed 07   **Stroma Liquid Biopsy – Pan-Cancer Dysregulation of the Serum Proteome;** <u>Matthew Kuruc</u>[1]; Haiyan Zheng[2]; Amenah Soherwardy[2]; Swapan Roy[1]; [1]*Biotech Support Group LLC, Monmouth Junction, NJ;* [2]*Rutgers Center for Proteomics, Piscataway , NJ.* **See Poster 05.**

Wed 08   **Liquid Extraction Surface Analysis (LESA) and LESA Plus Chromatography as Novel Surface Analysis Tools for Mass Spectrometry**; Daniel Eikel; *Advion, Ithaca, NY.* **See Poster 44.**

9:20 - 9:50 AM: **COFFEE BREAK**, *Foyer*   Tips & Tricks posters will be featured in the foyer.

9:50 – 11:10 AM: PARALLEL SESSION
NEXTGEN CLINICAL MS-BASED PROTEOMICS, *Ballroom 2*
Session Chairs: Victoria Zhang (University of Rochester Medical Center) and Henry Rodriguez (NIH, NCI)

9:50-10:15 am **Adapting Proteomics for Patient Care**; Mari DeMarco[1, 2]; [1]*University of British Columbia, Vancouver, Canada;* [2]*Providence Health Care, Vancouver, Canada*

10:15-10:40 am **Mass Spectrometry Imaging for Surgical Neuropathology and Neurooncology;** Nathalie Agar; *Brigham and Women's Hospital, Harvard Medical School, Boston, MA*

10:40-10:55 am **Selecting Patients with Colorectal Cancer for 5-fluorouracil-based Adjuvant Chemotherapy Using Quantitative Proteomic Analysis**; Yuan Tian[1]; Dongyao Yan[1]; Ji Hyung Hong[2]; Hee Yeon Lee[2]; Jae Ho Byun[2]; Fabiola Cecchi[1]; Sarit Schwartz[1]; Wei-li Liao[1]; Eunkyung An[1]; Todd A. Hembrough[1]; [1]*Nantomics, Rockville, MD;* [2]*Incheon St. Mary's Hospital, Incheon, Incheon, South Korea*

10:55-11:10 am **Optimization of Sample Preparation Methods for Using Residual Pap Test Fixatives for Mass Spectrometry-Based Proteomic Identification of Ovarian Cancer Biomarkers**; Anna Rogers[1]; Kristin Boylan[1]; Melissa Geller[1]; Peter Argenta[1]; Samantha Hoffman[1]; Timothy Griffin[1]; Nasrin Perskvist[2]; Amy Skubitz[1]; [1]*University of Minnesota, Minneapolis, MN;* [2]*Karolinska Institute, Stockholm, Sweden*

9:50 – 11:10 AM: PARALLEL SESSION
INTEGRATIVE 'OMICS: MULTI-OMICS, *Ballroom 1*
Session Chair: David Fenyo (New York University)

9:50-10:15 am **Identifying Therapeutic Targets in Cancer using Proteogenomics**; Kelly Ruggles; *New York University, New York, NY*

10:15-10:40 am **From Raw Data to New Discoveries: Towards a Complete Proteogenomic Informatics Solution**; Praveen Kumar, James E. Johnson, Thomas McGowan, Matthew C. Chambers, Subina Mehta, Caleb Easterly, Ray Sajulga, Shane Hubler, Candace R. Guerrero, Pratik D. Jagtap and Timothy J. Griffin; *University of Minnesota, Minneapolis, MN*

10:40-10:55 am **Identification of Protein Isoforms Resulting from Alternative pre-mRNA Splicing by the Integration of Mass Spectrometry and RASL-Seq** ; Laura Agosto[1, 2]; Simone Sidoli[1, 2]; Amber K. Weiner[1, 2]; Kristen W. Lynch[1]; Benjamin A. Garcia[1, 2]; [1]*University of Pennsylvania School of Medicine, Philadelphia, PA;* [2]*Epigenetics Institute, Philadelphia, PA*

10:55-11:10 am **Multi-Omic Molecular Profiling of Lung Cancer Risk in Chronic Obstructive Pulmonary Diseas**; Brian Sandri[1]; Adam Kaplan[1]; Shane Hodgson[2]; Mark Peterson[1]; Svetlana Avdulov[1]; LeeAnn Higgins[1]; Todd Markowski[1]; Ping Yang[3]; Andrew Limper[3]; Tim Griffin[1]; Peter Bitterman[1]; Eric Lock[1]; Chris Wendt[2]; [1]*University of Minnesota, Minneapolis, MN;* [2]*Veterans Affairs Medical Center, Minneapolis, MN;* [3]*Mayo Clinic, Rochester, MN*

11:10 AM – 12:00 PM: **PLENARY LECTURE + CLOSING SESSION**, *Ballroom 2*
Session Chair: Josh Coon (University of Wisconsin)

11:10 -11:55 am **Spatiotemporal Proteome Organization of the Human Cell**; Emma Lundberg, *KTH Royal Institute of Technology*

11:55 am-12:00 pm Closing Remarks

Mon Talk 9:50-10:15 am: Metabolic Activity within the Gut Microbiome and Its Impact on Human Health

Kelsey Veldkamp; Kimberleigh Romano; Federico Rey; Daniel Amador-Noguez
University of Wisconsin, Madison, WI

The human gut microbiome is a dense ecosystem that impacts our development, nutrition and physiology. The gut microbiota performs a myriad of chemical transformations that modulate human physiology including degradation of complex dietary substrates into short-chain fatty acids, synthesis of vitamins, modification of bile acids and xenobiotics and regulation of the immune system and host metabolism. Despite the importance of the gut microbiome as a mediator of chemical transformations, little is known about the scope of molecules that this community metabolizes, the specific bacterial species involved, or the physiological effects of the molecules produced. During this talk, I will discuss the use of mass spectrometry-based metabolomic approaches to systematically investigate chemical transformations by the gut microbiome and their impact on human health, presenting bile acids and choline transformation as two relevant examples.

Mon Talk 10:40-10:55 am: Reproducible, Robust Quantitative Functional Analysis of Metaproteomes Using the Galaxy Platform

Caleb Easterly[1]; Joel Rudney[1]; James Johnson[1]; Carolin Kolmeder[2]; Andrea Argentini[3]; Thomas Mcgowan[1]; Bjoern Gruening[4]; Praveen Kumar[1]; Subina Mehta[1]; Lennart Martens[3]; Tim Griffin[1]; Pratik Jagtap[1]
[1]University of Minnesota, Minneapolis, MN; [2]Max Planck Institute for Developmental Biology, Tuebingen, Germany; [3]Ghent University, Ghent, Belgium; [4]University of Freiburg, Freiburg, Germany

Characterization of expressed microbial proteins in a given microbiome helps determine the microbiome's functional state and its effects on the host organism or environment. Aligning peptides identified by mass spectrometry (MS) to known proteins or protein groups can be used to infer the functional state. However, the protein inference problem is further compounded in metaproteomic experiments since an identified peptide might be shared across multiple organisms. In addition, drawing meaningful biological conclusions from large lists of proteins or functions is difficult without a method to separate interesting from uninteresting assignments. We present a workflow in the open source bioinformatics software Galaxy to address these difficulties. First, we align peptides to orthologs in the eggNOG database to mitigate protein inference difficulties, using the published tool eggNOG-mapper (doi:10.1093/molbev/msx148). Second, we use precursor intensities (obtained through moFF (doi:10.1038/nmeth.4075)) to derive ortholog abundance fold changes between paired samples, then rank the orthologs by fold change and determine the functions associated with the most variable orthologs, focusing on Gene Ontology (GO) term assignments. As an illustrative example, we analyse MS data from a published study on oral microcosm biofilm models of dental caries exposed to sucrose (doi:10.1186/s40168-015-0136-z). The resulting Galaxy workflow is automated, self-contained, and reproducible, and is being made available on the Galaxy-P metaproteomics instance (z.umn.edu/metaproteomicsgateway). In the illustrative example, preliminary results show 105,864 peptide identifications, 43,296 quantified peptides, and 35,642 peptides mapped to an ortholog by eggNOG-mapper, of which 59.1% were assigned GO terms. Enriched proteins in the sucrose-exposed sample were associated with enolase activity, 30S ribosomal protein S1, chaperone proteins and thioredoxin. The workflow presented here allows for reproducible, quantitative functional analysis of complex microbiomes, and is adaptable to many study designs.

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]; Zuo-Fei Yuan[1]; Kevin Janssen[1]; Yemin Lan[1]; Nathaniel Snyder[2]; Gary Wu[1]; Benjamin Garcia[1]
[1]University of Pennsylvania, Philadelphia, PA; [2]AJ Drexel Autism Institute, Drexel University, Philadelphia, PA

The gut microbiota is a complex microbial community that inhabits the human intestinal tract and accomplishes functions related to host defense and digestion. Defining the molecular interactions that take place between the microbiota and the host is necessary for understanding how the microbiota affects host physiology, which is important given the association of numerous diseases with an altered microbiota. Small molecule metabolites represent one such avenue of interaction. For instance, microbial fermentation of dietary polysaccharides generates the short-chain fatty acid butyrate, which inhibits histone deacetylases (HDACs) and thus, has the potential to modulate host epigenetics and gene expression. To assess how the microbiota influences histone modifications in the gut, we analyzed hundreds of uniquely modified histone peptides from the colonic epithelial cells of conventional and germ-free mice by quantitative mass spectrometry. As expected, germ-free mice, which lack microbiota-derived butyrate, displayed lower levels of histone acetylation, primarily on histone H4. While the reduced acetylation in germ-free mice may stem from more HDAC activity due to less inhibition by butyrate, an alternative explanation is that germ-free mice have decreased histone acetyltransferase (HAT) activity due to less oxidation of butyrate to acetyl-CoA, the necessary cofactor for HATs. Consistent with butyrate acting as a carbon source for acetylation, treatment of a cell line with 13C-labeled butyrate shifted the isotopic distributions of acetyl-CoA and acetylated histone peptides. Furthermore, we recapitulated these isotope tracing results in a more physiological model, in which we treated mice with 13C-labeled inulin, a plant polysaccharide that undergoes fermentation by the microbiota. Our current findings and ongoing work, which focuses on identifying the genetic loci that harbor less acetylation in germ-free mice via butyrate-dependent and -independent mechanisms, will aid in understanding how the microbiota affects epigenetic modifications and gene expression, which may be relevant to diseases featuring an altered microbiota.

Mon Talk 10:40-10:55 am: Integrating virology and proteomics to define mechanisms of cellular innate immune signaling during herpesvirus infection

Krystal Lum; Benjamin Diner; Catherina Pan; Timothy Howard; Ileana Cristea
Princeton University, Princeton, New Jersey

At the heart of mammalian intrinsic and innate immunity against DNA viruses is the recognition of intracellular foreign DNA. Our lab was at the forefront of uncovering the ability of human cells to distinguish viral DNA from human DNA in the nuclei of infected cells. This discovery has provided an important new avenue of research for understanding cellular defense against nuclear-replicating DNA viruses. The principal finding involved characterizing the interferon-inducible protein IFI16 as a nuclear sensor of viral DNA, and crucial immunosuppressive effector against herpes simplex virus-1 (HSV-1) and human cytomegalovirus. We previously established that the IFI16 pyrin domain is critical for localization to the nuclear periphery where IFI16 binds viral DNA to further suppress viral transcription and protein levels and induce cytokine levels. Yet, how the pyrin domain facilitates these antiviral functions remains unknown.

Here, we integrate proteomics with virology, live-cell microscopy, CRISPR technology, and biochemistry to define contributions of the pyrin domain in IFI16-mediated cellular immunity. By immunoaffinity purification (IP) and mass spectrometry (MS), we explore protein interactions specifically mediated by the IFI16 pyrin domain during active immune signaling. Our IP-MS study discovered pyrin domain interactions with nuclear pore proteins, antiviral PML nuclear bodies, and another DNA sensor cGAS. Via CRISPR technology, we establish that PML enrichment to incoming HSV-1 genomes is dependent on IFI16. Additionally, we show IFI16 and cGAS to act independently in recognizing HSV-1 DNA. Lastly, through mutagenesis assays, we demonstrate that IFI16 pyrin oligomerization is required for IFI16 association with viral DNA at the nuclear periphery, and resulting suppression of viral replication. We further discover the charged, solvent-exposed residues that facilitate IFI16 oligomerization. We also demonstrate the conservation of pyrin domain function and oligomerization within several antiviral proteins. Altogether, we

demonstrate the utility of this integrative approach in mechanistically probing antiviral immune signaling.

Mon Talk 10:55-11:10 am: Proteomic analysis reveals novel hydroxyproline-dependent cellular pathways in cancer cells

Luke Erber; Tong Zhou; Yue Chen
University of Minnesota, Minneapolis, MN

Proline hydroxylation (Hyp) is a critical cellular mechanism regulating oxygen-response pathways in tumor initiation and progression. We developed a proteomics strategy based on immunoaffinity enrichment to identify novel hydroxyproline-modified substrates. By integrating antibody based hydroxyproline enrichment with exhaustive HPLC-mass spectrometry analysis, we identified 562 proline hydroxylation sites on 272 protein substrates in HeLa cells. These identifications include many well-known proline hydroxylation targets including collagen, actin and FKBP10 proteins, and many previously unreported proline hydroxylation substrates. Our pathway enrichment analysis results showed that proline hydroxylation substrates are highly enriched in the biological processes including multi-organism processes, macromolecular assembly, RNA splicing, and regulation of response to stress.

In this study, we identified and validated Bromodomain-containing protein 4 (Brd4) as a novel proline hydroxylation substrate in leukemia cells. Brd4 transcription activity is crucial in cancer and metabolic diseases. Understanding the mechanisms that regulate Brd4 transcriptional activity has broad impact in characterizing Brd4-mediated transcriptional networks in cellular processes. PHD inhibition led to significantly decreased prolyl hydroxylation abundance on Brd4 relative to its unmodified peptide (Hyp stoichiometry from 59% to 24% upon DMon treatment). Co-immunoprecipitation experiments revealed BRD4 interaction with the prolyl hydroxylase domain enzyme, PHD2. To confirm enzymatic regulation of Brd4 hydroxylation, overexpression of PHD2 led to a significantly increased BRD4 Hyp stoichiometry (2-fold increase). Functional experiments revealed prolyl hydroxylation reduced Brd4-mediated transcriptional activity. Inhibition of prolyl hydroxylase enzymatic activities significantly diminished the transcription of several of known Brd4 transcriptional targets, c-Myc, Ran and Rad21. DMon treatment significantly reduced Brd4 binding to the c-Myc promoter and significantly inhibited cell proliferation in AML leukemia cells.

MONDAY 3:00 – 4:20 PM
INFORMATICS: EMERGING & NEW APPROACHES, *Ballroom 2*

Mon Talk 3:50-4:05 pm: Improved Protein Inference for Multiple Protease MS Data Using a Single Database Search

Rachel Miller[1]; Connor Hoffmann[1]; Gloria Sheynkman[2, 2]; Robert Millikin[1]; Stefan Solntsev[1]; Anthony Cesnik[1]; Michael Shortreed[1]; Lloyd Smith[1, 3]
[1]University Wisconsin-Madison, Madison, Wisconsin; [2]Center for Cancer Systems Biology (CCSB), Boston, MA; [3]Genome Center of Wisconsin, Madison, WI

Peptides that are detected by tandem mass spectrometry (MS/MS) in bottom-up proteomics serve as a proxy for the proteins expressed in a certain cell or tissue type. In recent years, the use of multiple proteases for protein digestion has grown because it greatly improves sequence coverage. Following MS analysis, peptide identification and protein inference for multiple protease MS data is currently performed separately for each protease, and the results of such analyses are manually aggregated; no existing software allows for peptide identifications from multiple proteases to be used simultaneously for protein inference. We have developed a new software tool that performs peptide identification and protein inference on data from multiple proteases in a single search task. This method improves the overall accuracy of protein inference by eliminating a significant number of protein groups that are erroneously identified by the traditional method. Peptide identifications from these false positive protein groups were reassigned to other groups based on additional evidence for those proteins based on data from alternative proteases. This increased accuracy of protein inference may improve downstream targeted quantitative proteomics and reduce wasted effort in pursuit of erroneously identified proteins.

Mon Talk 4:05-4:20 pm: A sampling of perceptions in the field of mass spectrometry data processing software

Rob Smith
University of Montana, Missoula, MT

Mass spectrometry is an important tool used by many scientists throughout the world. Nonetheless, feedback on the strengths and limitations of current software is often restricted to anecdote rather than formal inquiry. Over the course of interviews with 100 mass spectrometry professionals, surprising patterns coalesced on several software-based topics: perception of the frontier, perception of software quality, and differences between commercial and non-profit environments. Scientists' anonymized responses are presented and summarized into their suggestions for improving the state of the art.

MONDAY 3:00 – 4:20 PM
PROTEOMICS OF CANCER & DISEASE, *Ballroom 1*

Mon Talk 3:00-3:25 pm: Proteomic Analysis of Stalled Replication Forks

Anja Bielinsky[1]; Ya-Chu Chang[1]; Rebecca Rivard[2]; Yee Mon Thu[1]; Susan Kaye Van Riper[1]; LeeAnn Higgins[2]; Todd Markowski[1]; Katarzyna Kulej[2]; Jack Hedberg[1]; Luke Erber[1]; Yue Chen[1]; Eric Brown[2]
[1]University of Minnesota, Minneapolis, MN; [2]University of Pennsylvania, Philadelphia, PA

Ataxia telangiectasia and Rad3-related protein kinase (ATR) is the central player in response to replication stress. Replication stress is caused by conditions that challenge the progression of replication forks and lead to fork stalling. ATR deficiencies in humans are associated with Seckel syndrome, a disease that is characterized by growth retardation and severe microcephaly. In mouse models, mutations in *ATR* result in premature aging phenotypes that have been linked to the replicative exhaustion of stem cells. To better understand the underlying mechanisms of these observations, we sought to characterize ATR deficiency in the context of replication fork stalling. The isolation of proteins on nascent DNA (iPOND) combined with quantitative proteomics allowed us to study the proteomes associated with active and stalled replication forks. Following iPOND, we applied a novel algorithm (RIPPER), coupled with directed tandem mass spectrometry (DRIPPER for *directed* RIPPER) to establish a robust and powerful tool to reveal molecular consequences of ATR deficiency under conditions of replication stress.

Upon challenging cells with the replication inhibitor aphidicolin, stalled forks remained initially stable but relocated to nuclear pores, a known site of DNA repair. After prolonged fork stalling, core replisome components dissociated from chromatin, indicative of fork collapse. Interestingly, this dissociation was dependent on p97 segregase, suggesting that these factors are removed via ubiquitin-dependent proteasomal degradation. In ATR-deficient cells, association of repair factors with stalled forks was delayed, and forks were unable to relocate to the nuclear pore in a timely manner. In summary, these results show that stalled forks are localized to nuclear pores before the replication machinery gets disassembled to allow for the repair of damaged DNA and that ATR is critical for this process. Supported by NIH R01GM074917 and CA189743.

Mon Talk 3:25-3:50 pm: Hunting Circulating Mediators of Systemic Vascular Dysfunction after Carbon Nanotube Exposure

Andrew Ottens
Virginia Commonwealth University, Richmond, VA

Engineered carbon nanomaterials are growing in diversity and popularity for manufacturing strong, lightweight components. Yet, health risk assessments on these materials have only recently begun. Concern over their small size and ease with which they disperse and become trapped within the lung raises questions over their pulmonary as well as extrapulmonary health effects. Recent studies have demonstrated functional consequences outside the lung, and in particular on the vasculature. Blood from exposed animals can cause inflammation and dysfunction in naïve cells and tissues, yet the carbon nanotube material itself is restricted principally to the lung. Studies are ongoing to assess factors introduced into circulation that contribute to systemic health consequences, such as disruption of the blood-brain barrier and induced neuroinflammation. Male C57BL/6 mice were exposed to two doses of multi-walled carbon nanotubes (Matsui-7, 49

nm mean diameter) or vehicle (n=6/group). Samples were size fractionated and assessed using label-free data independent analysis. Mass spectrometry revealed over 1,700 circulating factors significantly altered by nanotube exposure that correlated with levels present within bronchial lavage fluid. Lung tissue showed significant 2-fold or greater increases in 12 matrix proteases supporting a pulmonary origin of peptide proteolytic products released into circulation. Peptide analysis identified products from known matrix protease substrates and secretory proteins, which offered functional relevance to immuno-inflammatory responses, extracellular matrix remodeling, and receptor interactions. The tested fraction was further evaluated and found to induce inflammatory marker release in naïve endothelial cells as well as vasodilatory dysfunction in naïve blood vessels. Of particular interest was a 58-mer thrombospondin fragment that contains antiangiogenic CD36 binding characteristics as needed to induce the observed vascular dysfunction. Results from these studies underlie an indirect mechanism by which pulmonary insults, such as to nanomaterials, can translate into systemic health consequences.

Mon Talk 3:50-4:05 pm: Polycomb loss enhances oncogenesis but leads to therapeutic vulnerabilities in malignant peripheral nerve sheath tumors

John Wojcik[1, 2]; Dylan Marchione[1, 2]; Simone Sidoli[1, 2]; Ben Garcia[1, 2]
[1]University of Pennsylvania, Philadelphia, PA; [2]University of Pennsylvania School of Medicine, Philadelphia, PA

Malignant peripheral nerve sheath tumors (MPNST) are aggressive sarcomas in which loss of function mutations in the polycomb repressive complex 2 (PRC2) promote tumor progression. To better understand how PRC2 loss contributes to pathogenesis, we conducted parallel proteomic and epigenomic analysis of human MPNSTs with and without PRC2 loss ($MPNST_{LOSS}$ vs. $MPNST_{RET}$).

$MPNST_{LOSS}$ showed decreased H3K27me3 and increased of H3K27 acetylation. This was accompanied by hyperacetylation of H4, a marker of open chromatin, and increased H3K36me2 and DNA methylation. At the level of the proteome, $MPNST_{LOSS}$ had an increased abundance of chromatin remodelers and markers of cell growth and division and decreased interferon signaling and antigen presentation.

To assess whether PRC2 function was directly linked to the proteome changes seen in human tumors, we restored PRC2 function in $MPNST_{LOSS}$ cell lines and measured the proteome response. PRC2 reconstitution downregulated pathways that were upregulated in $MPNST_{LOSS}$ and increased interferon pathway expression and MHC presentation. The same effects were observed in $MPNST_{LOSS}$ cell lines with knockdown of NSD2, the methyltransferase responsible for H3K36me2, suggesting functional antagonism between H3K36me2 and H3K27me3.

NSD2 knockdown also decreased proliferation and DNA methylation and activated transcription of endogenous retroviral elements (ERV), thereby linking H3K36me2 loss with interferon activation, which occurs as a consequence of ERV expression. $MPNST_{LOSS}$ were found to be highly sensitive to both DNA methyltransferase inhibitors (DNMTi) and histone deacetylase inhibitors (HDACi), both of which similarly activate interferons through induction of ERVs.

Together, these results suggest that PRC2 loss promotes global increases in open chromatin that enhance oncogenic pathway expression but promote genomic instability, and render MPNST heavily reliant on DNA methylation to prevent spurious transcription initiation, including of ERV. Consequently, MPNST are highly sensitive to therapeutics promoting further destabilization through increased acetylation (HDACi) or decreased DNA methylation (DNMTi).

Mon Talk 4:05-4:20 pm: A proteomics approach to understand the role of autophagy in colorectal cancer and enhance chemosensitivity

Emily Cannon; Monique Speirs; John Price
Brigham Young University, Provo, Utah

Colorectal cancer (CRC) is the third leading cause of cancer deaths, and CRC recurrence and chemoresistance limit the 5-year survival rate to 8%. Understanding how metabolic adaptations promote the transition from normal to malignant colon cells is necessary to improve treatments, and identify chemosensitizing drug targets. Autophagy is a conserved protein degradation pathway by which cytosolic components are engulfed, degraded by lysosomes, and recycled. Cancer cells respond to microenvironmental and treatment-induced stress by increasing autophagy-driven protein turnover. This increase is linked to stress tolerance and chemoresistance by unknown mechanisms. Identifying whether autophagy is selective for specific proteins and/or organelles is crucial to understand how autophagy provides a metabolic advantage in CRC. We are using kinetic proteomics techniques to measure autophagy and its effects on the turnover of specific protein types in human colon tumor (HCT116) cells. Our data suggest that autophagy is selective in CRC and plays key roles in stress tolerance, growth, and resistance to conventional CRC chemotherapeutics. Distinguishing between definitive autophagy substrates at the proteome level will help us identify specific anti-autophagy targets that may be used to sensitize resistant CRC cells to chemotherapy.

MONDAY 4:30 – 5:50 PM
QUANTITATIVE PROTEOMICS I, *Ballroom 2*

Mon Talk 4:30-4:55 pm: Drug Target Identification by Label-Free Differential Mass Spectrometry

Nathan Yates
University of Pittsburgh, Pittsburgh, PA

Few analytical techniques offer as many possibilities for the identification of novel drug targets as proteomics. Here we describe the development of label-free differential mass spectrometry (dMS) as a sensitive and selective method for the unbiased identification of proteins that bind to small molecule drugs. This lecture will highlight the application of our dMS target identification platform to different classes of approved drugs (e.g. NSAIDs, metformin, and carbonic anhydrase inhibitors) as well as novel cancer therapeutics (e.g. mdivi-1). Using the approach, we have identified known and novel targets that have been confirmed by quantitative western blot and functional assays. We have found the approach to be quite efficient and versatile, allowing for up to five compounds to be screened in less than a week on a single mass spectrometer. A strength of the label-free dMS approach is the precise quantification of all features that are detected in full-scan high-resolution mass spectra, not just peptides that have been identified via MS/MS experiments. Results are ranked by statistical significance and statistical power can be easily adjusted by increasing sample size. Overall, our results support the utility of proteomics for target identification and demonstrate a straightforward method that can be readily carried out as part of an efficient phenotypic screening drug development strategy.

Mon Talk 5:20-5:35 pm: FlashLFQ: Ultrafast Label-Free Quantification of Peptides in Proteomics

Robert J. Millikin; Stefan K. Solntsev; Michael R. Shortreed; Lloyd M. Smith
University of Wisconsin, Madison, WI

The rapid and accurate quantification of peptides is a critical element of modern proteomics that has become more challenging as proteomic data sets increase in size and complexity. FlashLFQ is a computer program for high-speed label-free quantification of peptides following a search of bottom-up mass spectrometry data. It is approximately an order of magnitude faster than established label-free quantification methods, making it practical to base quantification upon all charge states for a given peptide rather than solely upon the charge state that was selected for MS2 fragmentation. This increases the number of quantified peptides, improves replicate-to-replicate reproducibility, and increases quantitative accuracy. FlashLFQ is integrated into the MetaMorpheus search software GUI, allowing it to work together with the global post-translational modification discovery (G-PTM-D) engine to accurately quantify modified peptides. It is also available as a NuGet package, facilitating its integration into other software, and as a

standalone command line software program for the quantification of search results from other programs (e.g., MaxQuant).

Mon Talk 5:35-5:50 pm: Optimization of Experimental Parameters in Data-Independent Mass Spectrometry Significantly Increases Depth and Reproducibility of Results
Florian Marty; Roland Bruderer; Lukas Reiter
Biognosys AG, Schlieren, Switzerland
Comprehensive, reproducible and precise analysis of large sample cohorts is one of the key objectives of quantitative proteomics. Here, we present an implementation of DIA that surpasses the limitation of serial MS2 acquisition of DDA. Using deep single shot DIA, we identified and quantified over 7,100 proteins in human cell lines. In mouse tissues 8,121 proteins were identified and quantified. Missing values for proteins were within 0.3 to 2.1% and median coefficients of variation of 4.7 to 6.2% among technical triplicates. In very complex mixtures, we could quantify 12,192 proteins in total showing that DIA is not limited to low complexity samples.

MONDAY 4:30 – 5:50 PM
PROTEOMICS OF AGING & AGE-RELATED DISEASE, *Ballroom 1*

Mon Talk 4:30-4:55 pm: How Protein PTMs Inspired Pharmacological Chaperones and Crosslinkers That Minimize Dead-End Modifications.
Jeff Agar
Northeastern University, Boston, MA
We will address three topics. 1: Protein PTMs that cause or contribute to neurodegenerative disease. 2: Protein PTMs that ameliorate neurodegenerative disease. 3: A new click-type reaction that can protect proteins from disease-associated modification-induced unfolding, and be used to create dead-end modification-free crosslinkers. These topics will be addressed within the confines of ALS-associated mutations of SOD1 and Parkinson-associated mutations of DJ-1, using a combination of mass spectrometry and neurotoxicology.

Mon Talk 5:20-5:35 pm: Proteome-wide modulation of degradation dynamics in response to growth arrest
Tian Zhang[1,2]; Kevin Welle[2]; Jennifer Hryhorenko[2]; Sina Ghaemmaghami[2]
[1]*Harvard Medical School, Boston, MA;* [2]*University of Rochester, Rochester, NY*
In dividing cells, cytoplasmic dilution is the dominant route of clearance for long-lived proteins whose inherent degradation is slower than the cellular growth rate. Thus, as cells transition from a dividing to a non-dividing state, there is a propensity for long-lived proteins to become stabilized relative to short-lived proteins, leading to alterations in the abundance distribution of the proteome. However, it is not known if cells mount a compensatory response to counter this potentially deleterious proteostatic disruption. We used a proteomic approach to demonstrate that fibroblasts selectively increase degradation rates of long-lived proteins as they transition from a proliferating to a quiescent state. The selective degradation of long-lived proteins occurs by the concurrent activation of lysosomal biogenesis and upregulation of macroautophagy. Through this mechanism, quiescent cells avoid the accumulation of aged long-lived proteins that would otherwise result from the absence of cytoplasmic dilution by cell division.

Mon Talk 5:35-5:50 pm: Identifying ApoE isoform dependent changes for protein turnover in the brain
Joseph Creery; Joshua Chamberlain; Russell Denton; John Price
Brigham Young University, Provo, UT
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. For AD, being an age-related disease, this is critical information. Comparing transgenic mice (C57BL/6), expressing one of the three isoforms of human ApoE, we can observe differences in relative concentrations of a variety of proteins. Taking a step further, we have labeled our transgenic mice with deuterated water and utilize newly developed mass spectrometry programs to monitor proteome wide analysis of the effects APOE has on protein turnover within the brain tissue of the mice. This allows us to identify even more protein interactions, on a proteome wide level, than ever before. We are progressing toward an overall proteopathy picture, where protein turnover and homeostasis is affected in the presence of ApoE isoform differences. This data, compared to lipidomics data, with a similar approach to receive cell wide lipidomics, enhances our understanding of various protein turnover steps taken in disease progression. We hypothesize that more proteomic and lipidomic relations can be identified, with new and upcoming technology, in a push to create an overall picture, tissue wide, for the identification of crucial relationships in the progression of proteopathy.

TUESDAY 9:50 – 11:10 AM
QUANTITATIVE PROTEOMICS II, *Ballroom 2*

Tues Talk 9:50-10:15 am: Quantitative multidimensional characterization of protein-protein interactions in cancer
Danielle Swaney[1]; Minkyu Kim[1]; Margaret Soucheray[1]; Kyumin Kim[1]; Fan Zheng[2]; Jennifer Grandis[1]; Trey Ideker[2]; Nevan Krogan[1]
[1]*University of California, San Francisco, San Francisco, CA;* [2]*University of California, San Diego, San Diego, CA*
DNA sequencing of cancer genomes has produced a wealth of information regarding the mutational landscape of cancer, however, translating these alterations into functional and clinical outcomes is complicated by the significant heterogeneity among cancers of the same type, and even within a single tumor. Previous work has demonstrated that placing these alterations in the context of protein interaction networks can transform heterogeneous genetic mutations into stratified classes of cancer subtypes with distinct survival rates. Here we have used a quantitative affinity purification mass spectrometry (APMS) approach to characterize the dynamic nature of protein-protein interactions for genes altered in two cancer types (breast, head and neck), across different cell lines representing different clinical subtypes, and across different protein mutational states. Our analysis has revealed novel physical connections between genes altered in these cancer types, connections that could serve as therapeutic targets. Furthermore, this work has revealed novel protein interaction pairs altered genes and existing drug target proteins. These results demonstrate the highly dynamic and context dependent nature of protein-protein interactions and illustrate the utility of multidimensional experiments across cell types, mutational status, drug perturbation, etc. to reveal novel PPIs that can enhance our understanding of disease biology and serve as candidates for potential therapeutic intervention.

Tues Talk 10:40-10:55 am: Quantifying protein synthesis rates during fetal development reveals temporal and tissue-specific regulatory networks
Josue Baeza[1]; Barbara Coon[2]; William Peranteau[2]; Ben Garcia[1]
[1]*University of Pennsylvania School of Medicine, Philadelphia, PA;* [2]*Childrens Hospital of Pennsylvania, Philadelphia, PA*
During development, complex genomes generate different cell types in a highly ordered and reproducible manner. Precise spatial and temporal control of the gene expression program is critical for mammalian 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 newly synthesized proteins at specific stages of mouse fetal development and relate these changes to regulatory networks of various tissues. To determine developmental stage specific protein synthesis, fetal mice were administered a single pulse of isotopic amino acids via the vitelline vein. This non-invasive procedure bypasses the need to administer labeled amino acids in the diet of the pregnant mouse and allows for the precise control 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 find that the developing fetus reprograms the proteome in a tissue and stage specific manner.

Tues Talk 10:55-11:10 am: The Initiating Events in Direct Cardiomyocyte Reprogramming Involve Temporally Distinct Gene Expression and Proteome Abundance Signatures

Todd Greco[1]; Kimberly Sauls[2]; Li Wang[2]; Meng Zou[2]; Michelle Villasmil[2]; Li Qian[2]; Frank Conlon[2]; Ileana Cristea[1]
[1]*Princeton University, Princeton, NJ; [2]Univ of North Carolina McAllister Heart Institute, Chapel Hill, NC*

Cardiovascular diseases are a leading cause of human mortality. Underlying these diseases is the improper development or loss of cardiomyocytes, the contractile cells of the heart muscle, which occurs in congenital or adult-onset heart diseases, respectively. These diseases are characterized by an abundant population of scar-forming fibroblasts. Therefore, therapies based on direct reprogramming of fibroblasts into cardiomyocyte-like cells (iCM) hold great potential for restoration of cardiac function and may lead to therapeutic applications in human patients. One approach to achieve direct reprogramming is by retroviral transduction of three cardiac lineage-specific transcription factors, Mef2C, Gata4, and Tbx5 (MGT). Yet, the application of this technique is limited by our lack of understanding of the molecular mechanisms that drive the initial stages of reprogramming.

Here, we performed gene expression (~500 targets) and global proteome abundance profiling during the initial phases of reprogramming in MGT-transduced mouse embryonic fibroblasts. Analysis of cardiomyocyte markers by qRT-PCR showed no changes at 24 hours, but an increase at 48 and 72 hours, concomitant with a decrease in fibroblast markers. At the proteome level, the relative abundances of 3,463 proteins were measured using TMT-based proteomics at 48 and 72 hour timepoints in triplicate. The proteins of the extracellular matrix were found upregulated at both timepoints using Gene Set Enrichment Analysis, while interestingly, those in translation and chromatin regulation were temporally distinct, being down-regulated at 24 and 48 hours, respectively. Potential key regulators of direct iCM reprogramming were identified using hierarchical clustering and assembly of functional protein networks. One of the most up-regulated proteins was agrin, an ECM protein recently shown to promote cardiac proliferation and repair. Overall, these findings demonstrate the temporal coordination of specific gene and protein abundance signatures during the initiating events of reprogramming, providing a resource for targeted studies of downstream gene and protein signaling networks.

**TUESDAY 9:50 – 11:10 AM
INTACT PROTEIN STRUCTURAL ANALYSIS**, *Ballroom 1*

Tues Talk 9:50-10:15 am: Analysis of Intact Proteins and Protein Complexes by Ultraviolet Photodissociation Mass Spectrometry

Jennifer Brodbelt
University of Texas at Austin, Austin, TX

Developments in mass spectrometry instrumentation and experimental design have motivated new applications in the field of proteomics and structural biology. Ultraviolet photodissociation (UVPD) results in broad sequence coverage of intact proteins via more extensive backbone fragmentation than can be obtained using other MS/MS methods, and ion activation/dissociation can be accomplished using a single 5 ns laser pulse. This translates to a compelling MS/MS technology for characterization of intact proteins, including mapping post-translational modifications and ligand binding sites. There has been growing interest in employing top-down approaches to characterize proteins and to examine native-like protein structures by using MS/MS to disassemble the complexes and sequence the constituent proteins. UVPD provides high levels of sequence coverage for native-like protein complexes, and the relative abundances of fragment ions correlate with variations in the intramolecular and intermolecular interactions that stabilize particular regions of the proteins. Products retaining non-covalently bound ligands reflect the fast, high energy activation of UVPD.

Tues Talk 10:15-10:40 am: Native MS in structural biology: surface collisions of protein complexes

Vicki Wysocki, *The Ohio State University*

Characterization of the overall topology and inter-subunit contacts of protein complexes, and their assembly/disassembly and unfolding

pathways, is critical because protein complexes regulate key biological processes, including processes important in understanding and controlling disease. Conventional structural biology methods such as X-ray crystallography, cryoEM, and nuclear magnetic resonance provide high-resolution information on the structures of protein complexes. However, other biophysical methods that provide structural data (e.g. stoichiometry and subunit connectivity) on protein complexes are also important. Native mass spectrometry is an approach that provides critical structural information with higher throughput on low sample amounts. The power of native MS increases when coupled to ion mobility (IM-MS), a technique that measures rotationally averaged collisional cross sections and thus direct information on conformational changes, or to high resolution mass spectrometry (HRMS). This presentation illustrates surface-induced dissociation/ion mobility SID/IM MS and SID HRMS for characterization of topology, intersubunit connectivity, and other structural features of multimeric protein complexes. Data for a number of protein-partner complexes are under investigation, where the partner can be small molecule ligand, protein, DNA, or RNA.

Tues Talk 10:40-10:55 am: Linear and Differential Ion Mobility Separations of Middle-Down Proteoforms

Alyssa Garabedian[1]; Matthew Baird[2]; Jacob Porter[1]; Kevin Jeanne Dit Fouque[1]; Pavel Shliaha[3]; Ole Jensen[3]; Todd Williams[4]; Francisco Fernandez-Lima[1]; Alexandre Shvartsburg[2]
[1]*Florida International University, Miami, FL; [2]Wichita State University, Wichita, KS; [3]University of Southern Denmark, Odense, Denmark; [4]University of Kansas, Lawrence, KS*

Comprehensive characterization of proteomes comprising same proteins with distinct post-translational modifications (PTMs) is a staggering challenge. Many such proteoforms are isomers (localization variants) with same PTMs in different positions. As such variants often coexist in cell and have disparate biological activity, we need to detect and quantify them individually. While tandem mass spectrometry (MS), especially using electron transfer dissociation (ETD), can distinguish pure variants, common mixtures of more than two cannot be disentangled for lack of unique fragments. This is most pertinent to histones that combine great biomedical significance with diversity of proteoforms comprising numerous variants. Hence variants must be resolved prior to the MS step, but chromatography could do that only for small peptides. However, proteolysis to the bottom-up level obliterates the crucial PTM connectivity information, and properly mapping proteoforms requires top-down or middle-down approaches.

The variants for middle-down peptides were recently resolved by differential ion mobility spectrometry (FAIMS) relying on the mobility increment at high electric fields and identified by ETD, but not previously by linear IMS based on absolute mobility. We now use complete H3.1 histone tails (~50 residues) with diverse PTMs (methylation, trimethylation, acetylation, phosphorylation) on alternate biological sites to demonstrate that high-resolution linear IMS, here trapped IMS (TIMS) with resolving power about 200, broadly separates the variants fully or into binary mixtures quantifiable using tandem MS. This largely ensues from orthogonal separations across charge states, which multiplies peak capacity by their number produced by electrospray ionization (ESI). Separations employing traveling-wave IMS are similar (with much lower resolution), despite ~100x shorter residence times and different source conditions. This reproducibility across IMS implementations, timescales, and ESI source parameters shows transferability of results over linear IMS instruments. The linear IMS and FAIMS dimensions are ~50% orthogonal, suggesting FAIMS/IMS/MS as the ultimate platform for proteoform analyses.

Tues Talk 10:55-11:10 am: Characterizing transmembrane domains of membrane proteins with top-down ultra-violet photodissociation (UVPD) high-resolution mass spectrometry

Julian Whitelegge[1]; Romain Huguet[2]; Chris Mullen[2]; Vlad Zabrouskov[2]
[1]*UCLA, Los Angeles, ; [2]Thermo Fisher Scientific, San Jose, CA*

The most common structural element of integral membrane proteins is the lipid bilayer spanning transmembrane helix. These hydrophobic domains typically lack charged side chains and backbone amide/carbonyl polarity is minimized by the regular hydrogen bonding of the alpha-helix secondary motif. Peptides from transmembrane domains are poorly recovered in bottom-up proteomics experiments so the top-down approach presents an attractive alternative to access

transmembrane regions when structural information in this domain is desired. While collisionally activated dissociation can be highly effective for some transmembrane domains this is rarely widespread when a protein has more than one. Previously we showed that ion activation with infra-red laser excitation dramatically enhanced product-ion yield in electron-capture dissociation, presumably due to breakage of transmembrane alpha-helix hydrogen bonds that otherwise remain stable in the gas phase. With widening availability of instrumentation for ultra-violet photodissociation (UVPD) we set out to test the comparative efficacy of this technique applied to the two transmembrane helix c-subunit of the ATP-synthase of the thylakoid membrane (AtpH) under non-native conditions using an Orbitrap instrument equipped with a solid state UV laser (213 nm). The 7997.36 Da (monoisotopic) protein isolated from S. oleracea retains its initiating formyl-Met residue and yields ions with either 4 or 5 charges (m/z 2001 and 1334 respectively) and both charge states were analyzed by static nano-electrospray to yield a precursor intensity of 1e6. These ions were subjected to CAD, HCD, ETD, EThcD and UVPD and product ions assigned using Prosight PC 4.0 software. While EThcD pushed coverage above 50% compared with CAD, HCD and ETD, UVPD gave 70 - 80% coverage effectively covering both transmembrane domains of the protein. UVPD on the 5-charge precursor gave better coverage compared to the 4-charge precursor. UVPD appears to be especially effective for detailed structural characterization of the transmembrane domains of integral membrane proteins.

TUESDAY 3:00 – 4:20 PM
METABOLISM AND DISEASE, *Ballroom 2*

Tues Talk 3:00-3:25 pm: Defining mitochondrial protein function through systems biochemistry
David Pagliarini
Morgridge Institute for Research, Madison, WI

Mitochondrial dysfunction is associated with many human diseases, including cancer and neurodegeneration, that are often linked to proteins and pathways that are not well-characterized. To help define the functions of such poorly characterized proteins and determine how their expression is regulated, we are performing deep mass spectrometry profiling to map the proteomes, lipidomes, and metabolomes of yeast strains and human cell lines, each lacking a single gene related to mitochondrial biology. To explore the resulting large-scale data, we devised a multi-omic data analysis and visualization tool that we use to find covariance networks that can predict molecular functions, identify correlations between profiles of related gene deletions, spotlight gene-specific perturbations that reflect protein functions, and elucidate a global respiration deficiency response that results from mitochondrial dysfunction. We used this approach to elucidate uncharacterized features of mitochondrial coenzyme Q (CoQ) biosynthesis—an essential pathway disrupted in many human diseases. In particular, our analyses defined previously unrecognized roles for Hfd1p in producing the CoQ precursor, 4-hydroxybenzoate. We further leveraged this strategy to reveal that the RNA-binding protein, Puf3p, regulates expression of a select set of proteins involved in mitochondrial protein import, translation, complex assembly, and coenzyme Q (CoQ) biosynthesis—pathways essential to prime the full mitochondrial biogenesis program. Collectively, our results provide molecular insight into various aspects of mitochondrial biology and, more broadly, establish a high-throughput, multi-omic approach for quantifying diagnostic phenotypes and defining protein functions.

Tues Talk 3:50-4:05 pm: Mapping the changes to the serum and urinary proteomes when the pancreas is removed
Tue Bjerg Bennike[1, 2]; Melena Bellin[4]; Saima Ahmed[1, 2]; Zobeida Cruz-Monserrate[3]; Darwin Conwell[3]; Hanno Steen[1, 2]
[1]Boston Children's Hospital, Boston, MA; [2]Harvard Medical School, Boston, MA; [3]The Ohio State University Wexner Medical Center, Columbus, OH; [4]University of Minnesota Medical Center, Minneapolis, MN

The ultimate proteinaceous biomarker is a protein that has a clear functional role in the pathogenesis of the disease of interest. It is more likely to identify such an 'ideal' protein marker in proximal body fluids. However, proximal fluids are not always available or readily accessible thus peripheral systemic fluids are used for biomarker studies although little is known about the relationship between protein abundances in various body fluids.

We are interested in identifying biomarkers for pancreatic diseases. Although pancreatic fluid as proximal fluid is endoscopically collectable, such procedure is lengthy, expensive and requires hour-long sedation which hampers the collection of pancreatic fluid from positive/negative control patients for whom such collection is not medically indicated. Thus, we are interested in identifying markers in peripheral body fluids including blood and urine.

As a starting point for this pancreatic disease biomarker project, we studied the effect of the pancreas on the blood and urinary proteomes. We used a unique set of paired urine and serum samples from patients before and after complete removal of the pancreas (Total Pancreatectomy with Islet AutoTransplantation; TPIAT) in response to intractable pancreatitis with severe pain.

Expanding our recently developed high-throughput body fluid proteomics pipeline to serum and plasma, we used MStern blotting and DIA to process and analyze 44 urine and 96 serum samples. The subsequent analysis uncovered robust pancreas-dependent changes in the urinary and serum proteomes such as a downregulation of a desmosome subcomplex, changes in the rapidly (vs. slowly) clearing apolipoproteins probably due to altered lipid uptake, and changes in several cathepsins which have previously been associated with pancreatitis and pancreatic adenocarcinoma. In addition, this analysis provided insights into which proteins show dis-/concordant behavior in blood and urine, therefore advancing our knowledge about urine as a proxy for blood as a source of biomarkers.

Tues Talk 4:05-4:20 pm: Sirtuin-regulated lipoylation is an evolutionarily conserved mediator of metabolic health and disease
Elizabeth Rowland; Todd Greco; Caroline Snowden; Cora Betsinger; Ileana Cristea
Princeton University, Princeton, NJ

Lipoylation is an essential metabolic cofactor added as a posttranslational modification to a select few multimeric enzyme complexes. Evolutionary conservation is a prominent feature of lipoylation. Considering how rare this modification is, it is remarkable that lipoylated metabolic complexes are conserved from bacteria to mammals. These complexes are core regulators of cellular metabolism, and include the pyruvate dehydrogenase (PDH) and the alpha-ketoglutarate dehydrogenase (KDH) complexes, both critical for incorporating carbon into the TCA cycle.

Here, we demonstrate that regulation of lipoylation is also evolutionary conserved. Expanding upon our initial discovery of mitochondrial sirtuin 4 (SIRT4) as the first known cellular lipoamidase in mammals, we now demonstrate that this sirtuin enzymatic activity is conserved in bacterial sirtuins, which share close sequence homology to SIRT4. For this work, we used a multidisciplinary approach, integrating in vitro and in vivo assays, including biochemical, mass spectrometry-based proteomic, functional, and nutrient restriction assays. We show the ability of gram-negative and gram-positive bacterial sirtuin homologs to remove lipoylation from PDH and KDH. We establish the biological significance of this sirtuin activity, demonstrating that removal of lipoylation inhibits PDH and KDH, and we further show that lipoamidase activity responds to nutrient availability.

The striking conservation of lipoylation and sirtuins as lipoamidases highlight the critical role of lipoylation in healthy and disease states. Aberrant function of lipoylated complexes, like PDH, have been implicated in metabolic disorders, cancer, and Alzheimer's disease. Additionally, we previously discovered that human SIRT4 acts against DNA virus infections, while a bacterial sirtuin acts against bacteriophage infections. Using human cytomegalovirus (HCMV), we now further examine the antiviral role of SIRT4, and the mechanism through which HCMV inhibits SIRT4 functions. We show that understanding sirtuin-regulated lipoylation provides a unique and specific target to manipulate the activities of these metabolic complexes when studying disease or developing therapies.

ORAL ABSTRACTS

Tues Talk 3:00-3:25 pm: Immunopeptidomics: Accelerating the development of personalized cancer immunotherapy

Michal Bassani-Sternberg

CHUV, Lausanne, Switzerland

The remarkable clinical efficacy of the immune checkpoint blockade therapies has motivated researchers to discover immunogenic epitopes and exploit them for personalized vaccines. Mutated human leukocyte antigen binding peptides (HLAp) are currently the leading targets for T-cell recognition of cancer cells. Most studies attempt to identify neoantigens based on predicted affinity to HLA molecules. We have shown that the direct identification of tissue derived neoantigens by mass spectrometry is becoming feasible; however, methodologies for purification of HLAp for mass-spectrometry analysis have been a major limitation. We have recently designed a novel high-throughput, reproducible and sensitive method for sequential immuno-affinity purification of HLA-I and -II peptides from up to 96 samples in a plate format, suitable for both cell lines and tissues and for drug-screening assays.

 The massive amount of HLAp data we acquire while hunting down the neoantigens is highly valuable. We have compiled a large immunopeptidomics database across dozens of cell types and HLA allotypes. First, we have shown that by taking advantage of co-occurring HLA-I alleles across dozens of immunopeptidomics datasets we can rapidly and accurately identify HLA-I binding motifs. Consequently, training HLA-I ligand predictors on refined motifs significantly improves the identification of neoantigens. Second, our database captures the global nature of the *in vivo* peptidome averaged over many HLA alleles, and therefore, reflects the propensity of peptides to be presented, which is complementary to the existing neoantigen prediction features. We have shown as a proof of concept that our immunopeptidomics MS-based features improved neoantigen prioritization by up to 50%. Overall, immunopeptidomics facilitates direct identification of neoantigens and it can also improve the prediction of clinically relevant neoantigens for personalized anti-cancer vaccines.

Tues Talk 3:25-3:50 pm: Chemical Biology to Investigate the Immunopeptidome

Stephanie Jensen; Greg Potts; Melanie Patterson

AbbVie, Chicago, IL

Peptides presented to the immune system by the major histocompatibility complex (MHC) are important biomarkers that reflect the health of a cell. These peptides are significant immunotherapy targets for disease, including both infection and cancer. MHC-I peptides are largely generated from the intracellular proteolytic degradation of source proteins. Proteolysis targeting chimera (PROTAC) technology has provided a significant enhancement in the means to target and degrade cellular proteins selectively. We utilized mass spectrometry to investigate the capability of PROTAC compounds to degrade endogenous cellular target proteins, inducing the presentation of corresponding MHC class I peptides. Our results highlight the capability of PROTAC compounds to create new targets for immunotherapy, effectively broadening the scope of targetable peptides on the cell surface.

Tues Talk 3:50-4:05 pm: Characterizing the intracellular peptidome to define protein areas efficiently processed and presented across multiple HLAs

Julie Boucau[1]; Carl Kadie[2]; David Heckerman[2]; Sylvie Le Gall[1]

[1]Ragon Institute of MGH, MIT and Harvard, Cambridge, MA;
[2]Microsoft Research, Redmond, WA

While it is possible to identify thousands of peptides displayed by MHC-I at the cell surface, they only correspond to a fraction of the proteome. The sequence signatures predicting areas of self or viral proteins that are efficiently processed and presented across HLAs are lacking despite their critical role for immune monitoring. To avoid HLA bias and assess the role of degradation patterns in peptide generation, we identified the intracellular peptidome of 10 cell lines and 20 primary human cell samples by mass spectrometry. The 7107 intracellular peptides ranged from 5-46aa in length and originated from over 1500 proteins. These source proteins had varied cellular abundance, represented all subcellular compartments and showed a preference for small acidic proteins. Protein sampling was uneven ranging from 1-268 intracellular peptides/protein. Degradation peptides comprised overlapping sets with an overrepresentation of the 10% N- and C-termini of proteins. One third of the peptides could be assigned to a specific secondary structure within their source proteins, with a majority coming from alpha helices. All aa were represented at the N-terminus with 4 aa significantly enriched and 4 others underrepresented. C-termini included all aa but were enriched in frequent proteasomal cleavage sites. The analysis of 7107 intracellular peptides and 300,000 MHC-peptides reported in the literature showed that 61% of intracellular peptides correspond to exact or N/C-extended surface peptides. Fragment size distributions and computationally identified motifs other than HLA anchors distinguished intracellular peptides unable to yield surface peptides from the epitope precursors. These preliminary results revealed degradation motifs for the generation of intracellular peptides from their source protein and how HLA ligands are selected among the degradation products. Sequence signatures of protein areas efficiently generating intracellular peptides will be useful to identify areas of pathogens presentable across HLAs and relevant for immune targeting.

Tues Talk 4:05-4:20 pm: Improved Discovery of Post-Translationally Spliced Peptides Using Separate b- and y-Ion Databases

Zach Rolfs; Stefan Solntsev; Michael Shortreed; Brian Frey; Mark Scalf; Alan Attie; Lloyd Smith

University of Wisconsin, Madison, Wisconsin

Recent studies report the discovery of post-translationally spliced peptides (PTSPs), which has major implications for autoimmunity and cancer immunotherapy among others. Cis-PTSPs are the result of joining two peptides from the same protein whereas trans-PTSPs results from the joining of two peptides from two separate proteins. Current efforts to identify PTSPs are computationally demanding and constrained to cis-PTSPs. We developed Neo, a new software package to detect both cis- and trans-PTSPs in complex samples. Neo uses separate b- and y-ion databases to identify the N-terminal and C-terminal sequences of PTSPs. These two sequences are then fused in silico, and the resultant candidate is evaluated and retained if its mass is within a specified tolerance of the experimental precursor mass. All candidate PTSPs are then searched against the original species-specific database and removed if they better match existing sequences. Neo is thus able to rapidly identify cis- and trans-PTSPs in complex samples such as MHC-I and -II pulldowns as well as lysate. To benchmark Neo, a canonical database was altered to contain 5591 simulated PTSPs—actual peptides (not PTSPs) found to exist in an MHC-I pulldown dataset at a 1% FDR. Neo was able to identify 60% of these simulated PTSPs at a 1% FDR, indicating a reasonable false-negative rate of 40%. When searching for real PTSPs in MHC-I pulldowns from lymphoblastic cell lines, approximately 2% of identified antigens were found to be PTSPs. Prior studies of similar samples reported as high as one-third of antigens were cis-PTSPs. However, re-analysis of that data by examination of the observed and calculated retention times suggest that many of those reported results were false positives. The actual number of fusion peptides present in those samples is thus estimated to be much smaller.

Tues Talk 5:20-5:35 pm: Deciphering the human sirtuin 3 antiviral functions and the temporal mitochondrial acetylome during herpesvirus infection

Xinlei Sheng; Laura Murray; Morgan Sly; Ileana Cristea

Princeton University, Princeton, NJ

Viruses, such as the widely-spread herpesvirus human cytomegalovirus (HCMV), rely on cellular metabolic networks to provide energy and materials for their replication and spread. HCMV infection is known to induce mitochondrial fragmentation and striking alterations in cellular metabolism. In addition to these virus-induced modulations of mitochondria functions, our group recently found that mitochondria are also sites of host defense. We discovered the mitochondrial sirtuin 3 (SIRT3) as an antiviral factor during HCMV infection. SIRT3 is an NAD+-dependent deacetylase known to be a

29

critical regulator of metabolism. However, the mechanisms through which SIRT3 exerts its defense functions remain unknown. Here, we explore SIRT3 functions, its interactions with substrates, and the dynamic regulation of mitochondrial acetylation during HCMV infection. In agreement with the antiviral function of SIRT3, we observe that primary human fibroblasts rapidly elevate SIRT3 levels in response to infection. We confirm that SIRT3 knockdown results in increased virus titers, while SIRT3 overexpression decreases virus titers and viral protein levels. To characterize SIRT3 antiviral functions, we performed mitochondrial fractionation and isolated SIRT3 by immunoaffinity purification to identify its interaction partners during infection. Our mass spectrometry (MS) analyses revealed striking infection-induced changes in SIRT3 association with deacetylation substrates. We further used siRNA knockdowns to demonstrate the contribution of these SIRT3-substrate interactions to antiviral response. To place these changes into the broader context of mitochondrial regulation, we next characterized the temporal mitochondrial proteome, membrane potential and the pH during the replication cycle of HCMV. Finally, given the known deacetylase function of SIRT3, we hypothesized that mitochondrial acetylation is dynamically modulated during infection. We analyzed the mitochondrial acetylome using affinity enrichment of acetylated peptide and quantitative MS. Altogether, this study provides important insights into SIRT3 antiviral functions and the first characterization of the temporal mitochondrial protein composition and acetylation during HCMV infection.

Tues Talk 5:35-5:50 pm: Human Succinyl-CoA Ligase Deficiency Causes Widespread Protein Hyper-Succinylation in Patient-Derived Fibroblasts and Myotubes

Philipp Gut[1, 2]; Jesse G. Meyer[3]; Sanna Matilainen[4]; Chris Carrico[3]; Pieti Pällijeff[4]; Birgit Schilling[3]; Anu Suomalainen[4]; Eric Verdin[3]
[1]Gladstone Institutes, UCSF, San Francisco, CA; [2]Nestle Institute of Health Sciences, Ecublens, Switzerland; [3]Buck Institute, Novato, CA; [4]University of Helsinki, Helsinki, Finland

Reactive Acyl-CoA Species (RAS) are required metabolic intermediates produced by catabolism of sugars, fats, and amino acids. RAS are known to post-translationally modify (PTM) proteins, especially in the mitochondria, an environment where elevated RAS and pH facilitate non-enzymatic acylation of protein lysine residues. Cells have evolved mitochondria-specific, NAD-dependent deacylases, e.g. Sirt3 and Sirt5, which remove acetylation and succinylation, respectively.

Here, we investigated an incurable human disease caused by SUCLA2 mutation, resulting in succinyl-CoA ligase deficiency (SCL-d), which is characterized by progressive encephalomyopathy, and lethality by early adulthood. Because SUCLA2 protein is required for degradation of succinyl-CoA in the TCA cycle, we hypothesized that SCL-d might result in excess succinyl-CoA, and therefore, pathological protein hyper-succinylation, which we measured here.

Quantification of protein succinylation using data-independent acquisition (DIA) offers greater accuracy than precursor-only quantification. However, DIA-based quantification requires spectral libraries, which are usually generated by extensive sample fractionation and numerous data-dependent acquisitions. For studies of PTMs, new libraries are needed for every experiment, often consuming significant instrument time and precious sample. We recently described a new workflow and software tool for PTM Identification and Quantification from Exclusively Data-Independent Acquisition (PIQED, Meyer et al. Nature Methods, 2017), which connects several tools to automate label-free PTM analysis.

We applied PIQED to quantify site-level protein succinylation in fibroblasts and myotubes from patients with SCL-d. Protein-level quantification was automatically used by PIQED to correct site-level succinylation. We quantified 933 unique succinylation sites on 366 proteins from fibroblasts, and 194 unique succinylation sites on 91 proteins from myotubes. Over 96% of these modification sites were increased relative to controls (FDR < 0.05, fold-change>2), with median fold changes of 10- and 18-fold from fibroblasts and myotubes, respectively. The most-heavily succinylated sites in SUCLA2-/- samples are Sirt5-regulated, offering a potential therapeutic route for this incurable disease.

Tues Talk 4:30-4:55 pm: Integrated Workflows for Intact Glycopeptide Analysis

Sharon J. Pitteri
Stanford University, Palo Alto, CA

Glycosylation is a biologically important post-translational modification that plays critical roles in cellular processes and disease. However, glycosylation is a relatively poorly understood and difficult modification to study, particularly in complex mixtures. Recent developments in mass spectrometry have allowed the analysis of intact glycopeptides, thereby providing simultaneous information about the glycan and modified protein to be retained. The analysis of intact glycopeptides provides insight into glycan structure and composition, as well as the site of glycan modification on the peptide backbone. However, many analytical challenges remain in the analysis of intact glycopeptides including, for example, low ionization and overall abundance compared to non-glycopeptides and the heterogeneity of glycan occupancy at a particular glycosylation site. To help overcome these challenges, we have been developing analytical workflows to enable systematic studies of glycopeptides in complex mixtures. We have explored various enrichment strategies for glycosylated species at both the protein and peptide level including lectin affinity, strong anion exchange with electrostatic repulsion hydrophilic interaction chromatography (SAX-ERLIC), and hydrophlic interaction chromatography (HILIC). We have also employed metabolic incorporation strategies to label and enrich glycopeptides in cell culture systems. The development and application of these integrated workflows to biological and clinical samples including blood plasma, tissue, and cells will be presented. This work demonstrates the identification and characterization of thousands of intact glycopeptides in complex samples and the analysis and biological interpretation of the information.

Tues Talk 4:55-5:20 pm: Assignment of site-specific glycosylation in complex proteoglycan samples

Joshua Klein; Le Meng; Joseph Zaia
Boston University, Boston, MA

Proteoglycans (PGs) are distributed widely in all animals and play critical, multifaceted, physiological roles. Expressed in a spatially and temporally regulated manner, these molecules regulate growth factor availability to cell surface receptors and myriad protein-carbohydrate interactions in extracellular matrices. Much of the information on PG structures derives from classic biochemical studies on connective tissue. Most of the information on their abundances comes from western blot and immunohistochemical studies. Due to the high degree of glycosylation by glycosaminoglycans (GAGs) and other glycan classes, the peptide sequence coverage of complex PGs is poorly revealed by standard mass spectrometry-based proteomics methods. As a result, there is little information concerning how PG site specific glycosylation changes during normal- and patho-physiology. We analyzed the small leucine-rich proteoglycan decorin and the hyalectan proteoglycans neuorocan, brevican, and aggrecan, respectively. For aggrecan, the Swiss-Prot and PeptideAtlas databases contain peptides present in the globular domains (G1, G2 and G3) but lack peptide coverage in the CS-1 and CS-2 domains modified with GAGs. Here, we developed and utilized a novel workflow to improve sequence coverage and identification of peptides with GAG chains in proteoglycans. We detected more than 150 peptides in the CS-1 and CS-2 domains of aggrecan that have not been reported previously. Most importantly, we succeeded to detect more than 100 peptide sequences that bear chondroitin sulfate chains, which have not been reported before. Interestingly, these peptides showed high micro-heterogeneity in their GAG tetrasaccharide linkers.

Tues Talk 5:20-5:35 pm: Immunoglobulin G Fc Region N-linked Glycosylation as a Clinical Biomarker for Insulin Resistance

Andrew Lipchik[1, 2]; Michael Snyder[1, 2]
[1]Stanford University, Stanford, CA; [2]Stanford University, Stanford, CA

Type 2 diabetes mellitus (T2D) is a metabolic disease that is strongly tied to obesity and often preceded by insulin resistance and chronic inflammation in adipose, muscle and liver tissues. Recently, cells of the innate and adaptive immune system have been demonstrated to be important regulators of insulin sensitivity. B cells have been shown to drive obesity-associated insulin resistance through numerous

mechanisms including activation of T cells, secretion of inflammatory cytokines and production of pathogenic autoreactive immunoglobulin G antibodies. However, the link between pathogenic antibodies and onset of insulin resistance remains poorly understood.

Previously, we have demonstrated that treatment with purified IgG is sufficient to illicit an insulin resistant phenotype in vitro cell culture models; however, fragmentation of IgG revealed that the Fc region was the effector portion. We used a mass spectrometry-based approach to map the complete glycosylation profiles of purified IgG and compared profiles between insulin sensitivity and resistant individuals. This comparison has allowed us to identify differential glycosylation of the Fc region. The effector function of Fc N-linked glycosylation to cause an insulin resistant phenotype was validated in vitro measuring glucose uptake and mitochondrial respiration. Our findings suggest the pathogenicity of IgG in insulin resistance is mediated through alternative glycosylation of the Fc region rather than F(ab')2 mediated antigen recognition. Together these results demonstrate a direct link between IgG and insulin resistance as well as offers a novel biomarker for determining insulin resistance.

Tues Talk 5:35-5:50 pm: N-Glycopeptide Feature Identification by Revealing Trends Between Analyte Composition and Compensation Field Through FAIMS-Coupled MS Platform

Daniel Delafield[1]; Zhe Wang[1]; Matthew Baird[2]; Alexandre Shvartsburg[2]; Si Wu[1]
[1]University of Oklahoma, Norman, OK; [2]Wichita State University, Wichita, KS

With unmatched biological diversity and function, glycosylation as a post translational modification remains an area of great significance across chemistry and biology. However, though there has been much emphasis placed on further understanding of glycobiology and the characterization of such modified proteins, means of feature identification that are easily-implemented to an existing laboratory setup remain on the horizon. Traditional LC-MS methods are typically quite limited in deciphering the microheterogeneity inherent within glycoproteins, leaving most successful characterizations heavily dependent on labor-intensive, de-novo sequencing and fragmentation methods. Differential Ion Mobility offers further orthogonality to LC-MS due to its ability to separate analytes based on electrophoretic character, offering a remedy to the issue of co-eluted glycopeptides with similar features. Utilizing this sense of orthogonality to MS, our use of a LC-MS platform coupled with FAIMS filtering has yielded success in revealing correlations between high-mannose glycopeptides of various features and compensation field. Considering mass-to-charge ratio, charge state, and compensation field, we have demonstrated that tryptic glycopeptides from standard proteins (Ribonuclease B, Ovalbumin) exhibit unique trendlines based on constant backbones with varying glycan length (GlcNAc2Man5-9) and static glycan composition with varying peptide backbone when compared to other, non-glycosylated peptides. It is therefore suggested that unknown modified peptides can be rapidly characterized through FAIMS by comparison to these built trendlines. While such a method may be novel in its approach, the benefits of applying FAIMS to glycopeptide characterization increase when considering FAIMS can be implemented in front of an existing MS setup, allowing for greater overall orthogonality in glycopeptide identification without great expense. Understanding this demonstrated use of FAIMS, a platform such as this could be further applied to whole and digested proteins containing an array of modifications for more robust, orthogonal means of analytes recognition.

WEDNESDAY 9:50 – 11:10 AM
NEXTGEN CLINICAL MS-BASED PROTEOMICS, *Ballroom 2*

Wed Talk 9:50-10:15 am: Adapting Proteomics for Patient Care

Mari DeMarco[1, 2]
[1]University of British Columbia, Vancouver, Canada; [2]Providence Health Care, Vancouver, Canada

Although the translation of biomarker discovery efforts into tangible products for health care has been fraught with challenges, a success story for the field has been the adoption of analytical techniques developed for proteomics into health care. Quantitative (isotope dilution) mass spectrometry is now considered the gold standard (i.e. reference) method in laboratory medicine for a growing number of peptide and protein analytes. However, translation of these specialized reference methods into the routine testing paradigms of medical laboratories has proved challenging. Although the sample preparation steps and multi-day workflows common to proteomics experiments are neither complicated nor onerous for research laboratories, in medical laboratories they can be prohibitive (in time, cost and resources) to implementation. For example, trypsin digestion protocols have typically relied on lengthy digestion times to ensure effective proteolysis. For clinical laboratories, lengthy digestion times result in (1) challenges with staff scheduling, (2) increased potential for errors (different staff may be working on the same assay over multiple days), (3) throughput limitations (e.g. batches performed per week), and (4) an inability to report patient results in a timely manner. This example highlights the demand by medical laboratories for simple, rapid and cost-effective workflows for protein mass spectrometry assays. Herein, we will explore how we have adapted and re-envisioned proteomics techniques in the design of diagnostic tools for use in patient care.

Wed Talk 10:40-10:55 am: Selecting patients with colorectal cancer for 5-fluorouracil-based adjuvant chemotherapy using quantitative proteomic analysis

Yuan Tian[1]; Dongyao Yan[1]; Ji Hyung Hong[2]; Hee Yeon Lee[2]; Jae Ho Byun[2]; Fabiola Cecchi[1]; Sarit Schwartz[1]; Wei-li Liao[1]; Eunkyung An[1]; Todd A. Hembrough[1]
[1]Nantomics, Rockville, MD; [2]Incheon St. Mary's Hospital, Incheon, Incheon, South Korea

Background: 5-fluorouracil (5-FU) is a standard chemotherapeutic treatment for stage III and high-risk stage II colorectal cancer (CRC). However, about 20% of patients relapse within 48 months of treatment with 5-FU, even when combined with oxaliplatin (a common adjuvant treatment). We hypothesized that tumor expression of proteins involved in 5-FU activation or metabolism would identify responders within a group patients who had been treated with 5-FU. We used multiplexed selected reaction monitoring (SRM) to quantitate uridine-cytidine kinase 2 (UCK2) and other relevant biomarkers in archived tumor samples of patients with stage II/III CRC.

Methods: We developed and validated SRM assays for UCK2 in formalin-fixed, paraffin-embedded (FFPE) tumor tissues from 128 patients with stage II/III CRC who received adjuvant 5-FU, folinic acid, and oxaliplatin during 2000-2014. Tumor sections were microdissected and solubilized, and 67 candidate biomarkers were quantified using multiplexed SRM. Patient survival was assessed using the Kaplan-Meier method and log-rank test.

Results: By concentration curves, the limit of detection and the lower limit of quantitation of UCK2 SRM assays was 150 amol/µg and 200 amol/µg, respectively. Precision was assessed in 10 FFPE tumor samples across a range of spiked peptide concentrations from 125-10,000 amol/ug (n=3). The average inter-assay precision (different instruments, different days, and different operators) was 6% coefficient of variation (CV).

Of 128 patients, 37 had disease recurrence and 91 patients did not. UCK2 protein expression ranged from 192.6 amol/µg to 1605.44 amol/µg. Patients with UCK2 expression above 319 amol/µg (n = 104) had significantly longer relapse-free survival (hazard ratio: 0.45; p = 0.0134) and overall survival (hazard ratio: 0.33; p = 0.0033) than patients with lower UCK2 expression (n = 24).

Conclusions: UCK2 has potential for use as a predictive marker for selecting patients for 5-FU-based chemotherapy, which warrants validation in other patient cohorts.

Wed Talk 10:55-11:10 am: Optimization of sample preparation methods for using residual Pap test fixatives for mass spectrometry-based proteomic identification of ovarian cancer biomarkers

Anna Rogers[1]; Kristin Boylan[1]; Melissa Geller[1]; Peter Argenta[1]; Samantha Hoffman[1]; Timothy Griffin[1]; Nasrin Perskvist[2]; Amy Skubitz[1]
[1]University of Minnesota, Minneapolis, MN; [2]Karolinska Institute, Stockholm, Sweden

Current screening methods to detect ovarian cancer are not adequately sensitive or specific to detect early disease. In contrast, cervical cancer screening with Pap tests has been routinely performed for decades.

The liquid-based Pap test involves collecting cervical cells and placing them into an alcohol-based fixative for later identification of premalignant and malignant cells. We hypothesize that proteins shed by ovarian cancer cells are detectable in residual Pap test fixatives by mass spectrometry (MS)-based proteomic techniques. This biospecimen source is ideal for biomarker discovery since the samples are routinely collected, derived from a site near the tumor, and may not contain high abundance proteins that mask potential biomarkers. At the University of Minnesota, we optimized a protocol for obtaining "mock Pap tests" from patients with ovarian cancer and patients with benign or normal conditions. We have also received Pap test samples from the Clinical Cytology Biobank at the Karolinska Institute, which contains clinically annotated Pap test samples from thousands of Swedish patients. We determined the protein concentration in samples from each cohort by BCA assay. The average protein concentration in Minnesota "mock Pap tests" was 102 μg/mL. The average protein concentration from Swedish Pap tests was 44 μg/mL. The protein pattern appeared similar on silver stained SDS-PAGE gels, indicating that samples can be pooled for MS analysis. We observed acceptably low levels of albumin and immunoglobulin in these samples, suggesting little blood contamination. Three workflows were tested to determine optimal methods of protein concentration. We found acetone precipitation and Amicon filtration with filter-aided sample preparation (FASP) are promising methods of preparing Pap test fixative-derived protein samples for MS analysis, as these methods resulted in the identification of dozens of unique proteins. These results demonstrate that liquid-based Pap tests have the potential to identify ovarian cancer protein biomarkers using proteomic methods.

WEDNESDAY 9:50 – 11:10 AM
INTEGRATIVE 'OMICS: MULTI-OMICS, *Ballroom 1*

Wed Talk 10:15-10:40 am: From raw data to new discoveries: Towards a complete proteogenomic informatics solution
Praveen Kumar, James E. Johnson, Thomas McGowan, Matthew C. Chambers, Subina Mehta, Caleb Easterly, Ray Sajulga, Shane Hubler, Candace R. Guerrero, Pratik D. Jagtap; Timothy J. Griffin
University of Minnesota, Minneapolis, MN

Proteogenomics integrates large-scale genomic and mass spectrometry-based proteomics data. This approach provides a means to identify expressed protein sequence variants associated with human disease, as well as generate proteome-level evidence to improve the annotation of genome structure. Proteogenomics is enabled by informatics, and presents a number of challenges to wide-spread implementation by the research community. First, proteogenomics requires a pipeline employing a diverse suite of domain-specific software (e.g. RNA-Seq assembly tools, MS-based proteomics sequence database searching tools etc.). Second, customized software tools are necessary to ensure quality and confidence of putative protein variant sequences identified from these workflows. Third, tools for interpretation of results and hypothesis generation are also a necessity. Finally, to empower more wide-spread adoption by bench researchers, these tools must be made accessible in a platform that is usable, even by those who are not software developers or bioinformaticians. As a solution, we have been extending the Galaxy for proteomics (Galaxy-P) platform to provide a resource for complete proteogenomic informatics. Built on the highly flexible, extensible and scalable Galaxy bioinformatics platform, Galaxy-P provides an environment well-suited for multi-omic informatics, such as proteogenomics. Here, we will present an update on this ongoing work. We'll highlight a number of modules available in Galaxy-P, including: workflows for generating protein sequence databases from RNA-Seq data containing a diverse array of sequence variants; tools for matching tandem mass spectrometry (MS/MS) spectra to variant peptides, along with functionalities ensuring quality of these results; and downstream tools for visualizing and interpreting results. These collective tools, integrated within the accessible and usable Galaxy-P platform, are providing the foundation for complete proteogenomics informatics, from raw data input to results interpretation. Ongoing studies in human disease and cancer utilizing this platform will also be highlighted to demonstrate its utility.

Wed Talk 10:40-10:55 am: Identification of protein isoforms resulting from alternative pre-mRNA splicing by the integration of mass spectrometry and RASL-Seq
Laura Agosto[1,2]; Simone Sidoli[1,2]; Amber K. Weiner[1,2]; Kristen W. Lynch[1]; Benjamin A. Garcia[1,2]
[1]University of Pennsylvania School of Medicine, Philadelphia, PA; [2]Epigenetics Institute, Philadelphia, PA

Eukaryotic systems have some of the most diverse proteomes. This diversity arises via multiple mechanisms, including alternative pre-messenger RNA (pre-mRNA) splicing. Alternative splicing affects the formation of mature mRNAs, including stability, processing, reading frame, and translation. The extent and which differentially spliced mRNA isoforms are successfully translated into proteins is still an unanswered question in the RNA field. Here, we applied mass spectrometry (MS) based proteomics to identify proteins in a Jurkat T-cell line that result from alternative splicing events. Briefly, T-cells were stimulated with phorbol 12-myristate 13-acetate (PMA) for 48 hours prior to cell harvest. Total RNA was extracted from unstimulated and stimulated cells to quantitatively identify changes in splicing upon stimulation by RASL-Seq. In parallel, cells were fractionated into cytosolic and nuclear fractions to analyze the proteins by RPLC-MS/MS. Proteomic data was searched using two databases on Proteome Discoverer, a full human proteome database (UniProt) and a custom database using *in silico* translated RNA sequences from the RASL-Seq results. To confidently discriminate between protein isoforms it is necessary to identify peptides translated across exon junctions and/or alternatively included exons. We programmed a bioinformatics workflow to identify MS/MS spectra matching with protein sequences from both databases. In a systematic comparison of unstimulated vs stimulated T-cells, we identified 35,578 peptides across all samples, of which 89 were exclusively identified with the RASL-Seq custom database. Moreover, 102 of the peptides IDs with the custom database correspond to exon junction peptides. Together, we present a novel integration of high throughput sequencing with mass spectrometry applications in the interest of identifying scarcely characterized mechanisms like successful translation of alternatively spliced mRNAs.

Wed Talk 10:55-11:10 am: Multi-Omic Molecular Profiling of Lung Cancer Risk in Chronic Obstructive Pulmonary Diseas
Brian Sandri[1]; Adam Kaplan[1]; Shane Hodgson[2]; Mark Peterson[1]; Svetlana Avdulov[1]; LeeAnn Higgins[1]; Todd Markowski[1]; Ping Yang[3]; Andrew Limper[3]; Tim Griffin[1]; Peter Bitterman[1]; Eric Lock[1]; Chris Wendt[2]
[1]University of Minnesota, Minneapolis, MN; [2]Veterans Affairs Medical Center, Minneapolis, MN; [3]Mayo Clinic, Rochester, MN

Chronic obstructive pulmonary disease (COPD) is a known risk factor for developing lung cancer suggesting that the COPD stroma contains factors supporting tumorigenesis. Since cancer initiation is complex we used a multi-omic approach to identify gene expression patterns that distinguish COPD stroma in patients with or without lung cancer.

We obtained lung tissue from patients with COPD and lung cancer (tumor and adjacent non-malignant tissue) and those with COPD without lung cancer for proteomic and mRNA (cytoplasmic and polyribosomal) profiling. We used the joint and individual variation explained (JIVE) method to integrate and jointly analyze these three datasets. Proteomic data was acquired across 16 iTRAQ runs with both reverse-phase and high-pH liquid chromatography on an Orbitrap Velos instrument.

JIVE identified eight latent patterns that robustly distinguished and separated the three groups of tissue samples. Predictive variables that associated with the tumor, compared to adjacent stroma, were mainly represented in the transcriptomic data, whereas, predictive variables associated with adjacent tissue compared to controls was represented at the translatomic level. Kyoto Encyclopedia of Genes and Genome (KEGG) pathway analysis revealed extracellular matrix (ECM) and PI3K-Akt signaling pathways as important signals in the pre-malignant stroma. Thus, COPD stroma adjacent to lung cancer is unique and differs from non-malignant COPD tissue and is distinguished by the extracellular matrix and PI3K-Akt signaling pathways.

POSTER LIST

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Each day: even-numbered posters present 1:30-2:15 pm; odd-numbered posters present 2:15-3:00 pm.

Poster 01
Abundant Protein Depletion and Multiplexed Protein Quantitation of Human Plasma Samples – A Reproducibility and Scaling Study; Sergei Snovida; Katherine Herting; Ramesh Ganapathy; Ryan Bomgarden; Barbara Kaboord; Chris Etienne; Monica O'Hara; John Rogers; *Thermo Fisher Scientific, Rockford, IL. Featured in Wednesday's plenary on Tips & Tricks.*

Poster 02
The Power of Multiplexing- Combining TMT Discovery and Targeted Label Free Quantitation for Biomarker Analysis; Xiaoyue Jiang[1]; Sergei Snovida[2]; Julian Saba[3]; David Horn[1]; Vic Spicer[4]; Oleg Krokhin[4]; Rosa Viner[1]; Andreas Huhmer[1]; *[1]Thermo Fisher Scientific, San Jose, CA; [2]Thermo Fisher Scientific, Rockford, IL; [3]Thermo Fisher Scientific, Mississauga, Canada; [4]University of Manitoba, Winnipeg, MB, Canada*

Poster 03
Connecting the Dots Between Metabolic Reprogramming and Protein Function in Cancer; Emily Canon; Monique Pare Speirs; Max Jones; John Price; *Brigham Young University, Provo, UT. Featured as a Lightning Talk on Mon or Tues.*

Poster 04
PTM Knowledge Networks and LINCS Multi-Omics Data for Kinase Inhibitor Drug-Analytics in Lung Cancer; Xu Zhang[1]; Karen Ross[2]; Jake Jaffe[3]; Michele Forlin[4]; Cathy Wu[5]; Udayan Guha[1]; *[1]CCR, NCI, NIH, Bethesda, MD; [2]Georgetown University Med. Ctr., Washington DC, Washington DC; [3]Broad Institute of Harvard and MIT, Cambridge, MA; [4]University of Miami, Coral Gables, FL; [5]University of Delaware, Newark, DE. Featured as a Lightning Talk on Mon or Tues.*

Poster 05
Stroma Liquid Biopsy – Pan-Cancer Dysregulation of the Serum Proteome; Matthew Kuruc[1]; Haiyan Zheng[2]; Amenah Soherwardy[2]; Swapan Roy[1]; *[1]Biotech Support Group LLC, Monmouth Junction, NJ; [2]Rutgers Center for Proteomics, Piscataway , NJ. Featured in Wednesday's plenary on Tips & Tricks.*

Poster 06
A Novel Approach for Isolating Exosomes From Clinical Sample; Blake Ebert, MS; Alex J Rai, PhD; *Columbia University Medical Center, New York, NY. Featured as a Lightning Talk on Mon or Tues.*

Poster 07
Applying Proteomics and Metabolomics to Understand the Role Sphingolipid Signaling in Pancreatic Cancer and Drug Sensitivity; Max Jones; Monique Speirs; Connor Holman; John Price; *Brigham Young University, Provo, UT. Featured as a Lightning Talk on Mon or Tues.*

Poster 08
Using Isotope-Labeled Peptides to Investigate the Impact of Kinase Inhibitors in CML; Monica Johnson; Tzu-Yi Yang; Candace Guerrero; Laurie L. Parker; *University of Minnesota, Minneapolis, MN*

Poster 09
New Protocols to Monitor Proteome Dynamics in the Developing Chordate Heart; Burcu Vitrinel; Lionel Christiaen; Christine Vogel; *New York University, New York, NY. Featured as a Lightning Talk on Mon or Tues.*

Poster 10
Inhibition of KDM4C Leads to Histone Tail Clipping in a Triple Negative Breast Cancer Context; Shawn Egri[1]; Guillermo Peluffo[2, 3]; Malvina Papanastasiou[1]; Kornelia Polyak[2, 3]; Jacob Jaffe[1]; *[1]The Broad Institute, Cambridge, Massachusetts; [2]Dana Farber Cancer Institue, Boston, MA; [3]Harvard Medical School, Boston, MA. Featured as a Lightning Talk on Mon or Tues.*

Poster 11
Proteogenomics Approach to Measuring Minimal Residual Disease in Multiple Myeloma; Bob Bergen; Angela Dispenzieri; David Murray; *Mayo Clinic, Rochester, MN*

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Poster 12
Mapping the Pathways of Progressive Pulmonary Sarcoidosis; Maneesh Bhargava[1]; Caleb Easterly[1]; Kevin Viken[1]; Anna Levin[1]; Pratik Jagtap[1]; Timothy Griffin[1]; Lisa Maier[2]; *[1]University of Minnesota, Minneapolis, MN; [2]National Jewish Health, Denver, CO*

Poster 13
Development of a Multi-Protein Classifier for Ovarian Cancer Detection by the Simultaneous Measurement of 92 Serum Proteins; Amy Skubitz[1]; Kristin Boylan[1]; Kate Geschwind[1]; Qing Cao[1]; Timothy Starr[1]; Melissa Geller[1]; Robert Bast[2]; Karen Lu[2]; Joseph Celestino[2]; Joseph Koopmeiners[1]; *[1]University of Minnesota, Minneapolis, MN; [2]University of Texas, Houston, TX*

Poster 14
A Quantitative Evaluation of Algorithms for Isotopic Trace Extraction via Ion Chromatogram Clustering; Mathew Gutierrez; Amber Yascavage; Rob Smith; *University of Montana, Missoula, MT. Featured as a Lightning Talk on Mon or Tues.*

Poster 15
The Human Proteome as of 2018, from the HUPO Human Proteome Project; Gilbert Omenn[1]; Lydie lane[2]; Emma Lundberg[3]; Christopher Overall[4]; Young-Ki Paik[5]; Jennifer van Eyk[6]; Tadashi Yamamoto[7]; Eric Deutsch[8]; *[1]University of Michigan, Ann Arbor, MI; [2]Swiss institute of Bioinformatics, Geneva, Switzerland; [3]Scilifelab, Karolinska, Stockholm, Sweden; [4]University of British Columbia, Vancouver, BC, Canada; [5]Yonsei Research Center, Seoul, Korea; [6]Cedar-Sinai Hospital Center, Los Angeles, US; [7]Niigata University, Niigata, Japan; [8]Institute for Systems Biology, Seattle, WA , USA*

Poster 16
Progress Towards Development of an Open Source Human Kinetic Proteomics Software Tool; Bradley Naylor; Marcus Hadfield; David Parkinson; Austin Hannemann; Paul Hafen; John Dallon; Rob Hyldahl; John Price; *Brigham Young University, Provo, UT*

Poster 17
Spritz: An RNA-Seq Analysis Engine that Enables Proteogenomics on Windows; Anthony Cesnik; Michael Shortreed; Brian Frey; Lloyd Smith; *UW-Madison, Madison, WI. Featured as a Lightning Talk on Mon or Tues.*

Poster 18
A Portable Pathway for Processing Peptide Array Data in KNIME with an Interest in Protein-Protein Interactions; Lee Parsons; *Univ of Minnesota, Plymouth, MN. Featured as a Lightning Talk on Mon or Tues.*

Poster 19
Optimizing Collision Energy in Collision-Induced Dissociation for Peptide Sequencing; Nandhini Sokkalingam; Luke Schneider; William Wright; Siamak Ashrafi; Adam Tenderholt; Jeffrey Peterson; *Veritomyx Inc., Palo Alto, CA. Featured in Wednesday's plenary on Tips & Tricks.*

Poster 20
Increased Accuracy of Peptide *de novo* Sequencing Using Complementary AI-ETD and HCD Fragmentation; Kevin Schauer[1]; Dan Maloney[2]; Nicholas Riley[1]; Michael Westphall[1]; Baozhen Shan[2]; Joshua Coon[1]; *[1]University of Wisconsin, Madison, WI; [2]Bioinformatics Solutions Inc, Waterloo, Canada*

Poster 21
timsTOF Pro PASEF Data Processing with an Evolution of PEAKS™ Software; Baozhen Shan[2]; Gary Kruppa[1]; Heiner Koch[3]; Markus Lubeck[3]; Scarlet Koch[3]; *[1]Bruker Daltonics Inc., Billerica, MA; [2]Bioinformatics Solutions Inc, Waterloo, Ontario; [3]Bruker Daltonik GmbH, Bremen, Germany*

Poster 22
iOmicsPASS: Integrative-Omics Approach for Predictive Analysis with Subnetwork Signatures in Breast and Colorectal Cancer Subtypes; Hiromi Wai Ling Koh[1]; Hyungwon Choi[1, 2]; *[1]National University of Singapore, Singapore; [2]Institute of Molecular Cell and Biology, A*STAR, Singapore. Featured as a Lightning Talk on Mon or Tues.*

Poster 23
PTMscape: an Open Source Tool to Predict Generic Post-Translational Modifications and Map Hotspots of Modification Crosstalk; Ginny Li[1]; Christine Vogel[2]; Hyungwon Choi[1]; *[1]National University of Singapore, Singapore; [2]New York University, New York, NY*

Poster 24
Predicting Cell-Line Specific Protein and Phospho-protein Abundances in Cancers; Hongyang Li; Gilbert Omenn; Yuanfang Guan; *University of Michigan, Ann Arbor, MI. Featured as a Lightning Talk on Mon or Tues.*

Poster 25
Evolution of SWATH® Acquisition Provides Large Gains in Quantified Proteins; Christie Hunter[1]; Nick Morrice[2]; Joerg Dojahn[3]; Arianna Jones[4]; *[1]SCIEX, Redwood City, CA; [2]SCIEX, Warrington, UK; [3]SCIEX, Darmstadt, Germany; [4]SCIEX, Framingham, MA*

Poster 26
Identification of Putative Fibrous Plaque Marker Proteins by Unsupervised Deconvolution of Heterogeneous Vascular Proteomes; Sarah Parker[1]; LuLu Chen[4]; Georgia Saylor[3]; Chunhong Mao[2]; Vidya Venkatraman[1]; Mitra Mastali[1]; Weston Spivia[1]; Rakhi Pandey[1]; Yue Wang[4]; David Herrington[3]; Jennifer Van Eyk[1]; *[1]Cedars Sinai Medical Center, Los Angeles, CA; [2]Virginia Tech, Biocomplexity Institute, Blacksburg, VA; [3]Wake Forest University, Wake Forest, NC; [4]Virginia Tech, Arlington, VA*

Poster 27
Glycopeptide Analysis to Examine the Role of Chlamydial Protease-Like Activity Factor. ; Julian Saba[1]; Christa Feasley[2]; Fred Zinnel[3]; Stuart McCorrister[4]; Garrett Westmacott[4]; Grant McClarty[4]; Chris

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Grant[4]; [1]*Thermo Fisher Scientific, Mississauga, Canada;* [2]*Thermo Fisher Scientific, West Palm Beach, FL;* [3]*Thermo Fisher Scientific, Somerset, NJ;* [4]*Public Health Agency of Canada, Winnipeg, Manitoba*

Poster 28	**High Pressure-Accelerated PNGase F Deglycosylation of Native Proteins**; Vera Gross; Nicole Cutri; Alexander Lazarev; *Pressure BioSciences, Inc., South Easton, MA*
Poster 29	**Develop of a Robust and Reproducible Global Plasma Proteome Profiling Workflow;** Scott M. Peterman[1]; David Sarracino[2]; Amol Prakash[3]; Kerry Hassell[4]; [1]*Thermo Fisher Scientific, Cambridge, MA;* [2]*ThermoFisher, Cambridge, MA;* [3]*Optys Tech Corporation, SHREWSBURY, MA;* [4]*Thermo Fisher Scientific, Somerset, NJ*
Poster 30	**Approach to the Confident Determination of Elemental Compositions in Mass Spectrometry Imaging Using IR-MALDESI**; Sitora Khodjaniyazova[1]; Milad Nazari[1]; Kenneth Garrard[1]; Mayara Matos[2]; Glen Jackson[2]; David Muddiman[1]; [1]*North Carolina State University, Raleigh, NC;* [2]*West Virginia University, Morgantown, WV.* **Featured as a Lightning Talk on Mon or Tues.**
Poster 31	**Development of a Novel Strategy for Measurement of the Neurotoxin Beta-N-Methylamino-L-Alanine in Environmental Samples;** Kaylie Kirkwood; Joshua Beri; Michael Bereman; David Muddiman; *North Carolina State University, Raleigh, NC.* **Featured as a Lightning Talk on Mon or Tues.**
Poster 32	**Identification of Natural Products as Inhibitors of Bacterial Histidine Kinases**; Joseph Mckillip; Erin Carlson; *Department of Chemistry, University of Minnesota, Minneapolis, MN*
Poster 33	**Developing New Tools to Enhance the Galaxy-Based Metabolomics Workbench;** Arthur Eschenlauer; Adrian Hegeman; Timothy Griffin; *University of Minnesota, Saint Paul, MN*
Poster 34	**Novel High-Throughput Metabolomic Techniques and Mainstream Tools for the Discovery of Drug Mechanism of Action**; Akos Vertes[1]; Albert-Baskar Arul[1]; Andrew R. Korte[1]; Hang Li[1]; Peter Avar[1]; Peter Nemes[1]; Lida Parvin[1]; Sylwia Stopka[1]; Sunil Hwang[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 Chem., The George Washington University, Washington, DC;* [2]*SRI International, Menlo Park, CA;* [3]*GE Global Research, Niskayuna, NY.* **Featured as a Lightning Talk on Mon or Tues.**
Poster 35	**vkmz: Visualizing Metabolomics on a Van Krevelen Diagram through Galaxy;** Mark Esler; *University of Minnesota, Minneapolis, MN*
Poster 36	**Traumatic Brain Injury Proteomics Guides Novel KCC2-Targeted Therapy**; Pavel N. Lizhnyak; Demisha D.L. Porter; John T. Povloshock; Andrew K. Ottens; *Anatomy & Neurobiology, Virginia Commonwealth Univ, Richmond, VA.* **Featured as a Lightning Talk on Mon or Tues.**
Poster 37	**Comparison of the Rat and Human Dorsal Root Ganglion Proteome;** Adam Schwaid[1]; Alicja Krasowka-Zoladek[2]; An Chi[1]; Ivan Cornella-Taracido[1]; [1]*Merck & Co., Inc., Boston, MA;* [2]*Merck & Co., Inc., West Point, PA*
Poster 38	**High pressure (>30,000 psi) Packing of Capillary Columns for Shotgun Proteomics**; Evgenia Shishkova[1]; Alexander Hebert[1]; Michael Westphall[1]; Joshua Coon[1, 2]; [1]*University of Wisconsin - Madison, Madison, WI;* [2]*Morgridge Institute for Research, Madison, WI*
Poster 39	**In Silico Design and In Vitro Characterization of Universal Tyrosine Kinase Peptide Substrates;** Laura Marholz; *University of Minnesota, Minneapolis, MN*
Poster 40	**Development of a Novel LC Concept for Clinical Proteomics**; Nicolai Bache[1]; Philipp Geyer[2]; Ole Hoerning[1]; Lasse Falkenby[1]; Peter Treit[2]; Sophia Doll[2]; Igor Paron[2]; Florian Meier[2]; Ole Vorm[1]; Matthias Mann[2]; [1]*Evosep, Odense, Denmark;* [2]*Max-Planck Inst. for Biochemistry, Martinsried, Germany.* **Featured in Wednesday's plenary on Tips & Tricks.**
Poster 41	**Rapid Trypsin Digestion of Complex Protein Mixtures for Proteomics Analysis;** Judy Boland; Zhiyun Cao; Nicolas Caffarelli; Amber Henry; Jeffrey Turner; Kevin Ray; *MilliporeSigma, St. Louis, Missouri*
Poster 42	**timsTOF Pro Powered by PASEF for High Speed and Sensitive Shotgun Proteomics**; Gary Kruppa[1]; Juergen Cox[2]; Heiner Koch[3]; Scarlet Koch[3]; Baozhen Shan[4]; [1]*Bruker Daltonics Inc., Billerica, MA;* [2]*Max Planck Inst. of Biochem., Martinsried, Germany;* [3]*Bruker Daltonik, GmbH, Bremen, Germany;* [4]*Bioinformatics Solutions Inc, Waterloo, ON, Canada.* **Featured in Wednesday's plenary on Tips & Tricks.**
Poster 43	**TimsTOF Pro Powered by PASEF for High Sensitivity Phosphoproteomics** ; Paul Savage[1]; Koch Heiner[2]; Scarlet Koch[2]; Markus Lubeck[2]; Florian Maier[3]; Oliver Raether[2]; Andreas Brunner[3]; Matthias Mann[4]; [1]*Bruker Daltonics Inc., Billerica, MA;* [2]*Bruker Daltonik, GmbH, Bremen, Germany;* [3]*Max Planck Institute of Biochemistry, Martinsried, Germany;* [4]*Max-Planck Inst. for Biochemistry, Martinsried, N/A*

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Poster 44 **Liquid Extraction Surface Analysis (LESA) and LESA Plus Chromatography as Novel Surface Analysis Tools for Mass Spectrometry**; Daniel Eikel; *Advion, Ithaca, NY.*

Poster 45 **High Pressure Denaturation Improves Protein Digestion by Trypsin;** Vera Gross; Nicole Cutri; Gary Smejkal; Alexander Lazarev; *Pressure BioSciences, Inc., South Easton, MA.* **Featured in Wednesday's plenary on Tips & Tricks.**

Poster 46 **Impact of Three Different Mutations in Ehrlichia Chaffeensis in Altering the Global Gene Expression Patterns**; Chandramouli Kondethimmanahalli; Roman Ganta; *Kansas State University, Manhattan, KS.* **Featured as a Lightning Talk on Mon or Tues.**

Poster 47 **Quantitative Analysis of Signaling Pathways Using 11plex TMT Reagents and Comprehensive SMOAC Phosphopeptide Enrichment Strategies;** Bhavin Patel; Leigh Foster; Jae Choi; Ryan Bomgarden; John Rogers; *Thermo Fisher Scientific, Rockford, IL*

Poster 48 **Detection of Post-Translational Modifications Using Mass Spectrometry**; Nathan Zuniga; Marcus Hadfield; Lavender Lin; David Parkins; John Price; *Brigham Young University, Provo, UT.* **Featured as a Lightning Talk on Mon or Tues.**

Poster 49 **Adaptation of KALIP for the Development and Prediction of Artificial Peptide Substrates to Monitor FMS-Like Tyrosine Kinase 3 (FLT3) Activity;** Minervo Perez[1]; W. Andy Tao[3]; Laurie L. Parker[2]; [1]*University of Minnesota, Minneapolis, MN;* [2]*University of Minnesota Twin Cities, Minneapolis, MN;* [3]*Purdue University, West Lafayette, IN.* **Featured as a Lightning Talk on Mon or Tues.**

Poster 50 **Finding Proteins Connected to Rheumatoid Arthritis**; Marcus Hadfield[1]; David Parkinson[1]; Stephen Ames[1]; Brad Naylor[1]; Lavender Lin[1]; Colette Quinn[2]; Lee Hansen[1]; John Price[1]; [1]*Brigham Young University, Provo, UT;* [2]*TA Instruments, Draper, UT.* **Featured as a Lightning Talk on Mon or Tues.**

Poster 51 **MetaMorpheus for Enhanced Global PTM Discovery (G-PTM-D) and Quantitative Proteomics;** Stefan Solntsev; Michael Shortreed; Brian Frey; Lloyd Smith; *University of Wisconsin, Madison, WI.* **Featured as a Lightning Talk on Mon or Tues.**

Poster 52 **Big Data: Ensuring Integrity of Differential Analyzes From Large Scale DIA-MS Experiments**; Andrea Matlock[1]; Erin Crowgey[2]; Vidya Venkatraman[1]; Victoria Dardov[1]; Jennifer Van Eyk[1]; [1]*Cedars-Sinai Medical Center, Los Angeles, CA;* [2]*Nemours Children's Health System, Wilmington, DE.* **Featured as a Lightning Talk on Mon or Tues.**

Poster 53 **Complement C3 in Individual Plasma Lipoproteome as a Potential Biomarker of Alzheimer's Disease;** Fangying Huang; Danni Li; *University of Minnesota, Minneapolis, MN*

Poster 54 **Changes in Glycan and Protein Expression in Aging Brain Nigrostriatal Pathway for Adeno-Associated Virus Mediated Gene Therapy in Parkinson's Disease**; Rekha Raghunathan[1]; Nicole Polinski[2]; Joshua Klein[1]; John D. Hogan[1]; Chun Shao[1]; Kshitij Khatri[1]; Deborah Leon[1]; Mark McComb[1]; Caryl Sortwell[2]; Joseph Zaia[1]; [1]*Boston University, Boston, MA;* [2]*Michigan State University, Grand Rapids, MI.* **Featured as a Lightning Talk on Mon or Tues.**

Poster 55 **A Multi-omics Analysis of Lifespan Extension in Mice Through Short-term Calorie Restriction Reveals Mechanisms of Post-Transcriptional Proteome Regulation;** Richard Carson; Bradley Naylor; John Price; *Brigham Young University, Provo, UT*

Poster 56 **Investigating Mechanisms Which Support Improved Proteostasis**; John Price; *Brigham Young University, Provo, UT*

Poster 57 **Quantification of the Proteins in Pathways Affected by Autophagy Inhibition in Rat Myoblasts;** Edgar Arriaga; Katherine Muratore; Omprakash Nacham; *University of Minnesota, Minneapolis, Minnesota*

Poster 58 **Investigation of the Effect of Reduced Synthesis and Degradation on in Vivo Protein Stability**; Lavender Hsien-Jung Lin; Marcus Hadfield; Nathan Zuniga; John C Price; *Brigham Young University, Provo, UT.* **Featured as a Lightning Talk on Mon or Tues.**

Poster 59 **The Effects of Apolipoprotein E Isoforms on the Metabolism of Lipid and Protein in Alzheimer's Disease Related Metabolic Pathways;** Russell Denton; Joseph Creery; Joshua Chamberlain; John Price; *BYU, Provo, UT*

Poster 60 **Phosphoproteomic Investigation of the Mechanism of Calorie Restriction Attenuation by Dietary Protein Signaling;** Joshua Mcphie[1]; Marco Hadisurya[2]; Aaron Moss[3]; Richard Carson[1]; John Price[1]; [1]*Brigham Young University, Provo, UT;* [2]*Brigham Young University - Hawaii, Laie, HI;* [3]*Brigham Young University - Idaho, Rexburg, ID.* **Featured as a Lightning Talk on Mon or Tues.**

Poster 61 **Dynamics of Proteomics Changes in Presence of ER Stress in ALS Relevant Mouse Neuronal Model;** Shuvadeep Maity[1]; Disi An[2]; Justin Rendleman[1]; Esteban Mazzoni[1]; Christine Vogel[1]; [1]*Center for Genomics*

POSTER LIST

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and System Biology, NYU, New York, NY; [2]Department of Biology, NYU, New York, NY. **Featured as a Lightning Talk on Mon or Tues.**

Poster 62   **Utilizing Multi-Omic Network Analysis to Rapidly Assess the Biological Mechanism of Action of Methotrexate;** Nicole Muszynski[1]; Melissa Farrow[2]; Danielle Gutierrez[1]; Stacy Sherrod[1]; Eric Spivey[1]; Tina Tsui[1]; James Pino[1]; Michael Ripperger[1]; Randi Gant-Branum[1]; Matthew Hensen[2]; Kaycei Moton-Melancon[2]; Jeremy Norris[1]; D. Borden Lacy[2]; John McLean[1]; Eric Skaar[2]; Carlos Lopez[1]; John Wikswo[1]; Richard Caprioli[1]; [1]Vanderbilt University, Nashville, TN; [2]Vanderbilt University Medical Center, Nashville, TN

Poster 63   **Large Datasets and You: Using Proteomics to Design a Biosensor;** John B[1]; Minervo Perez[2]; Laurie L. Parker[3]; [1]UMN, Minneapolis, MN; [2]University of Minnesota, Minneapolis, MN; [3]University of Minnesota Twin Cities, Minneapolis, MN. **Featured as a Lightning Talk on Mon or Tues.**

Poster 64   **Determining Peptide Substrates for Bruton's Tyrosine Kinase Biosensors via the KALIP Process;** Lindsay Breidenbach[1]; Minervo Perez[2]; John B[3]; Laurie L. Parker[4]; [1]University of Minnesota, Maple Grove, MN; [2]University of Minnesota, Minneapolis, MN; [3]UMN, Minneapolis, MN; [4]University of Minnesota Twin Cities, Minneapolis, MN. **Featured as a Lightning Talk on Mon or Tues.**

Poster 65   **Application for Nanofluidic Devices Towards Single-Cell Proteomics: A Study with Xenopus Laevis Embryos;** Anumita Saha-Shah[1]; Melody Esmaeili[1]; Peter Klein[1]; Benjamin A. Garcia[2]; [1]University of Pennsylvania, Philadelphia, Pennsylvania; [2]University of Pennsylvania School of Medicine, Philadelphia, PA. **Featured as a Lightning Talk on Mon or Tues.**

Poster 66   **Proteome-Wide Structure-Based Function Prediction Reveals Roles of Proteins Responsible for *E. coli* Fitness;** Chengxin Zhang; Mehdi Rahimpour; Peter Freddolino; Yang Zhang; University of Michigan, Ann Arbor, MI. **Featured as a Lightning Talk on Mon or Tues.**

Poster 67   **Development of Hydrogen-Deuterium Exchange Methodology Coupled to Top- and Middle-Down Mass spectrometry Enables High-Resolution Analysis of Histone Dynamics;** Kelly Karch; Mariel Coradin; ZhongYuan Kan; Ben Black; Benjamin Garcia; University of Pennsylvania School of Medicine, Philadelphia, PA. **Featured as a Lightning Talk on Mon or Tues.**

Poster 68   **Antibody-Antigen Interaction Characterization Using Stopped Flow Assisted Hydrogen Deuterium Exchange Mass Spectrometry;** Zhe Wang[1]; Kellye Sutton[1]; Michael Ashby[1]; Kenneth Smith[2]; Si Wu[1]; [1]University of Oklahoma, Norman, OK; [2]Oklahoma Medical Research Foundation, Oklahoma City, OK. **Featured as a Lightning Talk on Mon or Tues.**

Poster 69   **Expanding Proteoform Identifications and Constructing Proteoform families in Top-Down Proteomics Using Proteoform Suite;** Leah V. Schaffer; Michael R. Shortreed; Anthony J. Cesnik; Brian L. Frey; Stefan K. Solntsev; Mark Scalf; Lloyd M. Smith; University of Wisconsin, Madison, WI

Poster 70   **Development of A High-Performance Sheathless CZE-MS Interface for Top-Down Proteomics;** Lushuang Huang; Zhe Wang; Si Wu; University of Oklahoma, Norman, OK. **Featured as a Lightning Talk on Mon or Tues.**

Poster 71   **Finding a Needle in a Haystack: Novel Top-down Proteomics Tools for Serum Antibody Analysis;** Zhe Wang[1]; Ken Smith[2]; Si Wu[1]; [1]University of Oklahoma, Norman, OK; [2]OMRF, Oklahoma City, OK

Poster 72   **Improved Protein Sequence Analysis by 21 Tesla FT-ICR MS/MS and Advanced Mass Spectral Interpretation;** Lissa Anderson[1]; Jeffrey Shabanowitz[2]; Chad Weisbrod[1]; Greg Blakney[1]; Donald Smith[1]; Donald Hunt[2]; Christopher Hendrickson[1]; [1]NHMFL, Tallahassee, FL; [2]Dept. of Chemistry University of Virginia, Charlottesville, VA. **Featured as a Lightning Talk on Mon or Tues.**

Poster 73   **Examination of the Specific Binding of Cytochrome c on MPA-Coated Gold Nanoparticles via Protein Footprinting;** Emily Tollefson[1]; Xi Zhang[2]; Nikita Rozanov[3]; Caley Allen[3]; Rigoberto Hernandez[3]; Catherine Murphy[2]; Erin Carlson[1]; [1]University of Minnesota, Minneapolis, MN; [2]University of Illnios Urbana Champaign, Urbana, IL; [3]Johns Hopkins University, Baltimore, MD. **Featured as a Lightning Talk on Mon or Tues.**

Poster 74   **Tumor-Specific HLA-peptide Identification for Cancer Immunotherapy Using Accurate Epitope Prediction and Targeted Mass Spectrometry;** Jennifer Busby[1]; Michele Busby[1]; Tyler Murphy[1]; Brendan Bulik-Sullivan[1]; Matthew Davis[1]; Lauren Young[1]; Andrew Clark[1]; Fujiko Duke[1]; Firaz Mohideen[1]; Corinne Gustafson[2]; Assunta De Rienzo[2]; William G. Richards[2]; Nhien T. Dao[2]; Hyeong R. Kim[3]; Jamie E. Anderson[2]; Chang-Min Choi[3]; Vincent De Montpreville[4]; Se Jin Jang[3]; Olaf Mercier[4]; Raphael Bueno[2]; Elie Fadel[4]; Joshua Francis[1]; Roman Yelensky[1]; [1]Gritstone Oncology, Cambridge, MA; [2]Division of Thoracic Surgery and Lung Center, BWH, Boston, MA; [3]Asan Medical Center, Seoul, Korea; [4]Centre Chirurgical Marie Lannelongue, Le Plessis-Robinson, France. **Featured in Wednesday's plenary on Tips & Tricks.**

All posters will be displayed Monday and Tuesday.
Each day: even-numbered posters present 1:30-2:15 pm; odd-numbered posters present 2:15-3:00 pm.

Poster 75 — **Integrated Proteogenomic Analyses Reveal Extensive Tumor Heterogeneity and Validate Expression of Somatic Mutations in Lung Adenocarcinoma;** <u>Xu Zhang</u>[1]; Paul Rudnick[2]; Shaojian Gao[1]; Shivangi Awasthi[1]; David Fenyo[3]; Udayan Guha[1]; *[1]CCR, NCI, NIH, Bethesda, MD; [2]Spectragen Informatics LLC, Bainbridge Island, WA; [3]NYU School of Medicine, New York, NY.* **Featured as a Lightning Talk on Mon or Tues.**

Poster 76 — **Using HUPO Proteomics Standards With Software Inputs and Outputs for Galaxy-Based Multi-Omic Informatics**; <u>Thomas Mcgowan</u>; James Johnson; Subina Mehta; Praveen Kumar; Pratik Jagtap; Tim Griffin; *University of Minnesota, Minneapolis, MN*

Poster 77 — **Active Kinase Characterization Using an Isobaric Labeling Activity-Correlated Protein Profiling Platform (TMT-ACPP);** <u>Hongyan Ma</u>; Paul Sims; Si Wu; *University of Oklahoma, Norman, OK.* **Featured as a Lightning Talk on Mon or Tues.**

Poster 78 — **Galaxy-Based Multi-Stage Two-Step Database Searching Pipeline for Improved multi-omics analysis**; <u>Praveen Kumar</u>[1]; James Johnson[1]; Thomas McGowan[1]; Matthew Chambers[2]; Mohammad Heydarian[3]; Subina Mehta[1]; Caleb Easterly[1]; Joel Rudney[1]; Pratik Jagtap[1]; Timothy Griffin[1]; *[1]University of Minnesota, Minneapolis, MN; [2]Vanderbilt University, Nashville, TN; [3]Johns Hopkins University, Baltimore, MD.* **Featured as a Lightning Talk on Mon or Tues.**

Poster 79 — **A High-throughput Omics Pipeline for Comprehensive Analysis of Challenged Human Cells;** <u>Danielle Gutierrez</u>[1]; Carrie Romer[1]; Jamie Allen[1]; Yuan-Wei Nei[2]; Melissa Farrow[3]; Randi Gant-Branum[1]; Stacy Sherrod[1]; Nicole Muszynski[1]; Eric Spivey[1]; Salisha Hill[1]; Kristie Rose[1]; John Wikswo[1]; John McLean[1]; Eric Skaar[3]; D. Borden Lacy[3]; Jeremy Norris[1]; Richard Caprioli[1]; *[1]Vanderbilt University, Nashville, TN; [2]Quest Diagnostics, Chantilly, VA; [3]Vanderbilt University Medical Center, Nashville, TN.* **Featured as a Lightning Talk on Mon or Tues.**

Poster 80 — **Multi-Omics Analysis of Stem Cell Neural Differentiation - Processing SWATH® Acquisition Data in the Cloud with the OneOmics™ Project**; Christie Hunter[4]; Katherine Williams[2, 3]; Hao Chen[3]; Joshua Robinson[3]; <u>Arianna Jones</u>[1]; *[1]SCIEX, Framingham, MA; [2]Sandler Moore Mass Spec Core Facility, UCSF, San Francisco, CA; [3]UCSF, Dept of ObGyn & Reproductive Sciences, San Francisco, CA; [4]SCIEX, Redwood City, CA*

Poster 81 — **A Comparison of Quantitative Reproducibility Between DDA Precursor and DIA Fragment Quantification Techniques;** <u>Jacob Lippincott</u>[1]; Susan Weintraub[2]; Sammy Pardo[2]; Phillip Seitzer[1]; Susan Ludwigsen[1]; Brian Searle[1]; *[1]Proteome Software, Portland, OR; [2]Univ. of Texas HSC, San Antonio, TX*

Poster 82 — **Comprehensive Benchmarking for Label-Free Quantitative Proteomics**; Thevaa Chandereng[2]; Shengchao Liu[2]; John Denu[1]; Anthony Gitter[2]; <u>James Dowell</u>[1]; *[1]Wisconsin Institute for Discovery, Madison, WI; [2]Morgridge Institute for Research, Madison, WI*

Poster 83 — **Statistical Model for dDtecting dDfferentially Abundant Proteins in Isobaric Labeling-Based Protein Quantification Experiments;** <u>Ting Huang</u>[1]; Meena Choi[1]; Manuel Tzouros[2]; Nikhil Pandya[2]; Balazs Banfai[2]; Tom Dunkley[2]; Olga Vitek[1]; *[1]Northeastern University, Boston, MA; [2]Roche Innovation Center Basel, Basel, Switzerland.* **Featured as a Lightning Talk on Mon or Tues.**

Poster 84 — **Cross-Species Comparison of Proteome Turnover Kinetics**; <u>Kyle Swovick</u>[1]; Kevin Welle[2]; Jennifer Hryhorenko[2]; Andrei Seluanov[1]; Vera Gorbunova[1]; Sina Ghaeemaghami[1]; *[1]University of Rochester, Rochester, NY; [2]University of Rochester Mass Spectrometry Lab, Rochester, NY*

Poster 85 — **Label-Free Quantitative Proteomics of Active Replication Forks;** <u>Ya-Chu Chang</u>[1]; Rebecca Rivard[2]; Yee Mon Thu[1]; Susan Kaye Van Riper[1]; LeeAnn Higgins[1]; Todd William Markowski[1]; Katarzyna Kulej[2]; Jack Hedberg[1]; Luke Erber[1]; Yue Chen[1]; Eric Brown[2]; Anja Bielinsky[1]; *[1]University of Minnesota, Minneapolis, MN; [2]University of Pennsylvania, Philadelphia, PA*

Poster 86 — **Accurate, Sensitive, and Precise Multiplexed Proteomics Using the Complement Reporter Ion Cluster**; Matthew Sonnett; Eyan Yeung; <u>Martin Wühr</u>; *Princeton University, Princeton, NJ*

Poster 87 — **Bayesian Confidence Intervals for Multiplexed Proteomics Integrate Ion-Statistics with Peptide Quantification Concordance;** Leonid Peshkin[2]; Lillia Ryazanova[1]; <u>Martin Wühr</u>[1]; *[1]Princeton University, Princeton, NJ; [2]Harvard Medical School, Boston, MA*

Poster 88 — **Intact Protein Quantitation in Complex Samples Using Protein-Level TMT Labeling and Top-down Mass Spectrometry;** <u>Dahang Yu</u>; Zhe Wang; Hongyan Ma; Si Wu; *University of Oklahoma, Norman, OK*

Poster 89 — **Evaluation of Precursor-Based Peptide Quantification Software Tools Within the Galaxy Framework;** <u>Subina Mehta</u>[1]; Caleb Easterly[1]; James E Johnson[1]; Björn Grüning[2]; Andrea Argentini[3]; Robert J Millikin[1]; Michael R Shortreed[4]; Lee S Parsons[1]; Thomas McGowan[1]; Praveen Kumar[1]; Lennart Martens[3]; Lloyd M Smith[4]; Timothy J Griffin[1]; Pratik Jagtap[1]; *[1]University of Minnesota, Minneapolis, MN;*

POSTER LIST

All posters will be displayed Monday and Tuesday.
Each day: even-numbered posters present 1:30-2:15 pm; odd-numbered posters present 2:15-3:00 pm.

[2]*University of Freiburg, Freiburg, Germany;* [3]*Ghent University, Ghent, Belgium;* [4]*University of Wisconsin, Madison, Wisconsin*

Poster 90	**High-Throughput Untargeted Data-Independent Analysis of Misoprostol Challenged HL60 Cells as a Model for Chemical Threat Assessment**; <u>Roy Martin</u>[1]; Brad Williams[1]; Danielle Gutierrez[2]; Jeremy Norris[2]; [1]*Waters, Beverly, MA;* [2]*Mass Spectrometry Research Center, Vanderbilt University, Nashville, TN.* ***Featured in Wednesday's plenary on Tips & Tricks.***
Poster 91	**Protein Turnover in a Whole Mouse Model;** <u>Erin Weisenhorn</u>[1]; Gary Wilson[1]; Emily Wilkerson[2]; Chris Rose[3]; Joshua Coon[1]; [1]*University of Wisconsin-Madison, Madison, WI;* [2]*University of North Carolina, Chapel Hill, NC;* [3]*Genentech, San Francisco, CA*
Poster 92	**Plasma Proteomics Using Label-free Quantification and Tandem Mass Tags in Non-Depleted and Immune-Depleted Samples**; <u>Ling Li</u>; Belinda Willard; *Cleveland Clinic, Cleveland, OH*

POSTER ABSTRACTS

All posters will be displayed Monday and Tuesday.
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Poster 01: Abundant Protein Depletion and Multiplexed Protein Quantitation of Human Plasma Samples – A Reproducibility and Scaling Study

Sergei Snovida; Katherine Herting; Ramesh Ganapathy; Ryan Bomgarden; Barbara Kaboord; Chris Etienne; Monica O'Hara; John Rogers
Thermo Fisher Scientific, Rockford, IL

The large dynamic range in protein abundance of plasma samples is the main problem associated with plasma/serum-based biomarker discovery experiments, and depletion of abundant proteins is required in order to identify and measure changes in prognostic or diagnostic plasma proteins. We have optimized the production and immobilization of immunoaffinity ligands to develop new top2 and top12 abundant protein depletion resins. In addition, we comprehensively evaluated specificity, efficiency, and reproducibility of abundant protein depletion from human plasma samples. Finally, we have optimized this procedure for the downstream applications with the Tandem Mass Tag (TMT) reagent-based workflows.

Commercially obtained pooled human plasma samples were used to assess selectivity, efficiency, and binding capacity of the depletion resins by SDS gel, ELISA, and liquid chromatography mass spectrometry experiments (LC-MS). Replicate samples were used to assess these parameters for reproducibility. Both label-free approaches and Tandem Mass Tag (TMT) reagents were used for relative quantitation of human plasma proteins by LC-MS. All samples were analyzed on Thermo Scientific Orbitrap Fusion™ Tribrid™ mass spectrometer and processed using Thermo Scientific™ Proteome Discoverer™ 1.4 software.

In our preliminary work, we have optimized the conjugation chemistries and blending protocols to achieve >95-99% depletion efficiency of target high abundance proteins in 10-100uL human plasma samples in spin columns. We are now investigating the use of TMT-based workflows in conjunction with high pH reversed-phase fractionation to enable deep proteome discovery and quantitation of human plasma samples.

Poster 02: The Power of Multiplexing-Combining TMT Discovery and Targeted Label Free Quantitation for Biomarker Analysis

Xiaoyue Jiang[1]; Sergei Snovida[2]; Julian Saba[3]; David Horn[1]; Vic Spicer[4]; Oleg Krokhin[4]; Rosa Viner[1]; Andreas Huhmer[1]
[1]Thermo Fisher Scientific, San Jose, CA; [2]Thermo Fisher Scientific, Rockford, IL; [3]Thermo Fisher Scientific, Mississauga, Canada; [4]University of Manitoba, Winnipeg, MB, Canada

Recently, isobaric labeling techniques TMT have become popular for biomarker discovery due to higher throughput and better precision and accuracy. The next verification step (10-50 patients) is still challenging when balancing the target numbers and devoted instrument time. Here, we propose a workflow for plasma proteomics from multiplexed TMT biomarker discovery to rapid and robust verification using capillary flow LC on a novel Orbitrap platform with up to 40Hz scan speed.

Plasma from normal and diabetic patients were depleted, digested, labeled with TMT six-plex reagents, mixed at 1:1 ratio, and fractionated. The fractions were separated in a 120min gradient followed by analysis on an Orbitrap instrument. Data were analyzed by Proteome Discoverer™2.2 software. For targeted analysis, the same depleted, but unlabeled, samples were separated at a flowrate of 2-5ul/min and analyzed using parallel reaction monitoring (PRM). The data were processed by Skyline or Spectronaut software.

The multiplexing capability of TMT labeling significantly saved instrument time and provided possibilities to perform extensive fractionation. Fractionation, combined with the new depletion columns, made the detection of plasma proteins spanning to 5 orders of magnitude accessible.

Over 200 peptide targets, which showed significant difference (>2 fold change) between normal and disease states in the above discovery experiment, were selected for label free targeted quantitation using PRM. Retention time prediction of unlabeled peptides was performed using adjusted hydrophobicity index calculations. LC separation at the capillary flow rate provided high sensitivity and offered improved retention time reproducibility and robustness. The fast scan speed on the new Orbitrap platform greatly facilitated the detection of hundreds of targets in a single experiment. This rapid and robust quantitation method confirmed the biomarker candidates found in isobaric labeled discovery experiment. The workflows described here enable the biomarker discovery and validation in a highly multiplexed and rapid manner.

Poster 03: Connecting the Dots Between Metabolic Reprogramming and Protein Function in Cancer

Monique Pare Speirs; Max Jones; John Price
Brigham Young University, Provo, Utah

Deregulation of protein metabolism is emerging as a definitive hallmark of cancer. Shifts in protein expression and metabolism drive protective phenotypic transformations, enabling cancer cells to withstand nutrient deprivation, selective pressure from the tumor microenvironment, chemotherapy, and increased demands for metabolic precursors to fuel both energy production and macromolecular synthesis. We are using proteomics and metabolomics techniques to identify cancer-promoting signals and understand the metabolic processes underlying their production, degradation, and functions in human cancer cells. Our results suggest that while cancer cells generally orchestrate a global metabolic shift, specific metabolic routes are modified to support growth and drug resistance in different types of cancer cells relative to healthy cells. The balance between pro-survival verses pro-apoptotic protein expression and metabolism largely determines whether these cells are sensitive or resistant to chemotherapeutic drugs. A therapeutic strategy that simultaneously targets oncogenic metabolic pathways and tumor-promoting protein functions may be beneficial for patients with aggressive and/or chemoresistant tumors. An increased understanding of the interplay between metabolic reprogramming and adaptive phenotypes at the proteome level may provide insight to improve conventional treatments and develop novel chemosensitizing strategies.

Poster 04: PTM Knowledge Networks and LINCS Multi-Omics Data for Kinase Inhibitor Drug-Analytics in Lung Cancer

Xu Zhang[1]; Karen Ross[2]; Jake Jaffe[3]; Michele Forlin[4]; Cathy Wu[5]; Udayan Guha[1]
[1]CCR, NCI, NIH, Bethesda, MD; [2]Georgetown University Med. Ctr., Washington DC, Washington DC; [3]Broad Institute of Harvard and MIT, Cambridge, MA; [4]University of Miami, Coral Gables, FL; [5]University of Delaware, Newark, DE

Kinase domain mutations in the Epidermal growth factor receptor (EGFR) are common drivers of lung adenocarcinoma. 1st generation EGFR tyrosine kinase inhibitors (TKIs), gefitinib and erlotinib, 2nd generation EGFR TKI, afatinib and 3rd generation EGFR TKIs osimertinib and rociletinib inhibit mutant EGFRs. While all the EGFR TKIs are active against TKI-sensitizing EGFR mutants, L858R and Del EGFR, only the 3rd generation TKIs are effective against EGFR T790M, the most common acquired resistance mechanism to 1st and 2nd generation TKIs. Patients often have a good initial response to these drugs, but resistance inevitably develops, due to either additional EGFR mutations or to activation of parallel signaling pathways. To understand the mechanisms of resistance to the 3rd generation EGFR TKIs, we conducted a mass spectrometry-based phosphoproteomic analysis comparing rociletinib-resistant and rociletinib-sensitive lung cancer cells. Using iPTMnet, a PTM resource that integrates data from text mining of the scientific literature and other PTM databases, we found that AKT and PKA kinases targeted many of the sites whose phosphorylation was up-regulated in resistant cells; these kinases may be part of signaling pathways that are aberrantly activated in these cells. Next, we used kinase-inhibitor target data (KinomeScan) and phosphoproteomic data (P100) generated by the NIH LINCS Program (http://www.lincsproject.org/) to identify drugs that might overcome drug resistance.

Our study demonstrated that PTM knowledge networks can be used in conjunction with phosphoproteomic data to identify aberrantly regulated kinase signaling pathways in drug resistant cells, and that

POSTER ABSTRACTS

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LINCS data (KinomeScan and P100) can be used to identify candidate drugs to be used in combination therapy to overcome resistance. In our ongoing work, we are testing drugs identified by LINCS analysis in cell culture assays, extending the analysis to other TKIs, and automating our workflow for overlay of PTM knowledge maps, LINCS data, and cancer omics data.

Poster 05: Stroma Liquid Biopsy – Pan-Cancer Dysregulation of the Serum Proteome

Matthew Kuruc[1]; Haiyan Zheng[2]; Amenah Soherwardy[2]; Swapan Roy[1]
[1]Biotech Support Group LLC, Monmouth Junction, NJ; [2]Rutgers Center for Proteomics, Piscataway , NJ

Discovery of protein biomarkers that can detect cancer early and personalize a treatment process has become an important research area in the proteomics field. For this, many proteomic approaches are being implemented in cancer research. Most biomarker investigations focus on very low abundance (pg/ml range) proteins shed from cancer cells, at the very limits of quantitative LC-MS analysis. To discover, characterize and monitor these 'needle in the haystack' biomarkers remains an industry wide challenge. By contrast, our investigations focused on the serum proteome mid-abundance range often not considered in biomarker investigations. Yet, an important advantage of the use of biomarkers within this window is that they are all highly observable with serum concentrations in the [μg-mg]/ml range. Furthermore, this range has been previously determined to be quantitative by LC-MS/MS with precision comparable to current clinical immunoassays. We hypothesized that because of tumor vasculature, changes in the serum proteome might result from the host cell response within the tumor-associated stromal microenvironments, and can thus be monitored by blood tests. We now report on protein measurements contributing to a Stroma Liquid Biopsy™, a pan-cancer proteome profile of dysregulation and consider its differential utility from previous liquid biopsy approaches. The special significance of this profile is that serum proteome changes were categorical and primarily contained within three host systemic response pathways: acute-phase inflammation, coagulation, and the complement cascade. Furthermore, this study signifies the importance of these pathways intercommunicating in the vast circuitry of cascading proteolytic events, the predominant mechanism for controlling acute insults in the bloodstream. Because proteolysis is irreversible and therefore highly regulated, the pathways in our model cannot be viewed as separate independent cascades, but rather as one interdependent system with extensive cross-talk, mutually fine-tuning their functional status.

Poster 06: A Novel Approach for Isolating Exosomes from Clinical Sample

Blake Ebert, MS; Alex J Rai, PhD
Columbia University Medical Center, New York, NY

Exosomes are cell-secreted, membrane-bound, transport nanovesicles that are increasingly being recognized as a rich source of novel biomarkers for the diagnosis, prognosis, prediction, and monitoring of drug response for a variety of disease states. Cancer in particular is poised to benefit significantly from exosome biomarker exploration because of the individualized nature of disease progression as well as the severely negative outcomes associated with late diagnosis. Despite the growing relevance of exosomes as cancer biomarkers within the clinical community, their use has been limited largely due to a lack of efficient, straightforward isolation procedures that can be used in a clinical laboratory setting. We report here the development of a simple, quick, inexpensive procedure requiring no specialized equipment that we have optimized into a standard operating procedure (SOP). To summarize, we processed native urine through a series of three centrifugation steps. The first spin removes dead cells and cellular debris from the sample. A second higher speed spin is used to remove microvesicles, along with Tamm-Horsfall protein filaments. This pellet is subsequently treated to remove the Tamm-Horsfall protein, thereby freeing the entrapped exosomes, and spun a third time to recover the vesicles in a small volume. Successful isolation was determined by western blot analysis using five previously identified protein markers: ALIX, CD81, HSP90b1, HSP1a1, and ACTN4. Comparison between native and processed urine revealed a robust enrichment of exosomes in the processed sample. This optimized extracellular vesicle isolation

methodology lays the groundwork for future investigations of cancer cell-secreted exosomes from urine and other clinical samples, and can ultimately form the basis of a procedure to develop appropriate vesicle based biomarker candidates for a clinical test.

Poster 07: Applying Proteomics and Metabolomics to Understand the Role Sphingolipid Signaling in Pancreatic Cancer and Drug Sensitivity

Max Jones; Monique Speirs; Connor Holman; John Price
Brigham Young University, Provo, Utah

Cancer cells often alter metabolism in response to external stressors, such as anaerobic conditions, lack of nutrition, and chemotherapeutic drugs, in order to survive. Recent studies also find that cancer cells alter their proteome to increase cell proliferation and reduce cell death. Our studies have focused on cell fate sphingolipid signaling in pancreatic cancer, and the proteins involved. Our results indicate that pancreatic cancer cells upregulate proteins associated with proliferative signaling, relative to healthy phenotypes, to increase their viability and resistance to chemotherapy. We are using isotopic labeling, proteomics, and metabolomics, in order to understand how protein expression affects the metabolism of sphingolipid-mediated growth signals, and how protein expression changes as cells gain chemoresitance. By tracking isotopically labeled substrates, we also hope to better understand the activity of proteins involved in their proliferative pathways. The better we understand the targets of sphingolipid signaling, the more likely we will be able to use them as chemotherapeutic targets.

Poster 08: Using Isotope-Labeled Peptides to Investigate the Impact of Kinase Inhibitors in CML

Monica Johnson; Tzu-Yi Yang; Candace Guerrero; Laurie L. Parker
University of Minnesota, Minneapolis, MN

Chronic myeloid leukemia (CML) is a blood cancer associated with poor prognosis and high mortality. CML is caused by the dysregulated Abl kinase, which is involved in governing cell proliferation. Typical treatment for this aggressive disease include inhibitors to reduce aberrant kinase activity. Peptide were designed to act as substrates for a specific kinase, in this case Abl, to monitor kinase activity. These biosensors are then used to study the impact of a kinase inhibitors on cancer cells. Different variations of isotope-labeling on a peptide gives the ability to differentiate the phosphorylated- and unphosphorylated-peptide mass products from different cellular treatments in a single mass spectrometry analysis. In our experiment, we collected cell lysate from multiple treatments, DMSO, pervanadate, and the kinase inhibitors Dasatinib and Imatinib (Gleevec), each with a different isotope-labeled peptide. Samples from each were pooled together for simultaneous analysis. The relative abundance of phosphorylated- to unphosphorylated-peptide ratios were calculated for each treatment, and subsequent analysis determined the effect of these treatments on Abl kinase. Pervanadate-treated cells presented the most phosphorylation events, followed by DMSO-treated cells, and Dasatinib and Imatinib with minimal phosphorylation. Phosphorylation levels reflect relative expected values, though the total peptide within the samples were not consistent based on mass spectrometry results. Future experiments will be investigating this issue, repeating this experiment for additional biological replicates, and possibly the use of inhibitor-resistant CML cell lines. In conclusion, isotope-labeled peptides are a useful tool to study the impact of anti-cancer drugs on the activity of a kinase.

Poster 09: New Protocols to Monitor Proteome Dynamics in the Developing Chordate Heart

Burcu Vitrinel; Lionel Christiaen; Christine Vogel
New York University, New York, NY

During chordate development, pluripotent cells are specified to their terminal fates. Differentiation of pluripotent progenitors to tissue-specific cells requires highly dynamic regulation at both the transcriptional and post-transcriptional levels. This regulation occurs in a tissue-specific manner where each lineage giving rise to a single tissue has a unique set of players executing said regulation. Focusing on a single lineage therefore requires investigation of the regulatory mechanisms in just a subset of cells in the developing embryo. While

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our understanding of transcriptional regulation is rapidly growing, post-transcriptional regulation in development is often overlooked due to the challenging nature of proteomics research, in particular when only small samples are available, as is the case when focusing on a particular tissue. To investigate the proteomic role of post-transcriptional regulation in heart development in very small samples, we exploit the simple chordate tunicate Ciona as a model organism. As tissue-specific markers begin to be expressed hours after fertilization, we can distinguish and FACS-purify specific differentiating cells very early on. We are developing proteomics methods to process these samples with as few as ~5,000 cells which is fewer than has been used in existing small sample protocols. Our method includes new protein extraction techniques and new applications of tandem mass tag labeling. Protein concentrations are measured reproducibly within few-fold variation across replicates. We compare the resulting protein concentrations to RNA measurements derived from single-cell and bulk sequencing and identify candidates for post-transcriptional regulation during heart development. Our method is generic and can be used for any small proteomics sample.

Poster 10: Inhibition of KDM4C Leads to Histone Tail Clipping in a Triple Negative Breast Cancer Context

Shawn Egri[1]; Guillermo Peluffo[2,3]; Malvina Papanastasiou[1]; Kornelia Polyak[2,3]; Jacob Jaffe[1]
[1]The Broad Institute, Cambridge, Massachusetts; [2]Dana Farber Cancer Institue, Boston, MA; [3]Harvard Medical School, Boston, MA

Post-translational modifications (PTMs) of histone proteins have been shown to be involved with a range of biological functions such as transcriptional regulation, DNA damage response, and chromosomal organization. Histone proteins allow for a range of PTMs including acetylation, methylation, and others. Global chromatin profiling uses liquid chromatography mass spectrometry to provide a high-throughput, robust readout of combinatorial PTMs present on bulk chromatin. Briefly, histones are purified from nuclear extracts via acid extraction, derivatized, and undergo tryptic digest followed by a second derivatization. Heavy, isotopically-labeled peptide standards are then spiked into each sample to provide accurate quantitation. Finally, a targeted, parallel reaction monitoring acquisition method is used to quantify PTM abundance.

A vast class of histone modifying enzymes (writers, readers, erasers) are responsible for generating the histone code. One eraser, KDM4C, has been shown to function as an oncogene in a subset of breast cancers. Monitoring changes in histone PTMs following chemical inhibition and genetic knockdown of this enzyme has provided insight into its biological activity. A decrease in abundance of all combinatorial forms of histone peptides occurring on the N-terminus of histone H3 was suggestive of a histone tail clipping event. Verification of this observation was completed using an untargeted data-dependent acquisition approach to identify Ala21 as the truncation site. Duncan, et al. have previously shown that a serine protease, Cathepsin-L (CTSL), is capable of cleaving at this site in murine stem cells. Subsequent biochemical investigations implicate CTSL in this breast cancer context as well. The biological role of CTSL in chromatin homeostasis in cancer is not well understood. To investigate further, we are performing immunoprecipitation mass spectrometry experiments to identify functional partners that may mediate CTSL targeting.

Poster 11: Proteogenomics Approach to Measuring Minimal Residual Disease in Multiple Myeloma

Bob Bergen; Angela Dispenzieri; David Murray
Mayo Clinic, Rochester, MN

Multiple myeloma is a plasma cell cancer. Under normal conditions plasma cells produce infection fighting antibodies. Unchecked growth of a clonal plasma cell population defines multiple myeloma. This clonal population produces a monoclonal antibody or in 20% of cases only free light chain with no heavy chain while 3% of patients have no circulating clone-derived immunoglobulin. Disease is monitored currently by 1) serum protein electrophoresis (**SPEP**), 2) multiparametric flow cytometry (**MFC**) for cells labeled in the six-color version with CD19, CD38, CD138 CD45, kappa and lambda light chains or more recently 3) next-generation sequencing (**NGS**) to monitor the heavy chain CDR3 sequence corresponding to the clone derived immunoglobulin. Of these methods, MFC and NGS are the most sensitive but both require an invasive bone marrow aspiration. Because a sensitive serum based test has distinct advantages for monitoring disease we have sought to develop an assay to monitor disease progress by monitoring the level of the circulating immunoglobulin. Monitoring a tryptic peptide covering the Ig heavy chain complementarity determining region 3 (**CDR3**) would suffice as it has the most unique sequence because of recombination events. Additionally, the heavy chain CDR3 DNA/RNA sequence is currently used successfully to monitor disease by NGS. Initial attempts to define unique sequences by de novo sequencing purified clonal Ig indicated very few heavy chain CDR3 sequences were being identified due to the heavy chain CDR3 tryptic peptides being exceptionally large (Bergen et al., Clin Chem 62(1): 243-251, 2016). We have subsequently incorporated genomics sequencing to identify the heavy chain CDR3 sequence and the corresponding tryptic peptide belonging to the plasma cell clone. Our initial results indicate this serum based approach has sensitivities similar to NGS and avoids all but an initial bone marrow aspiration.

Poster 12: Mapping the Pathways of Progressive Pulmonary Sarcoidosis

Maneesh Bhargava[1]; Caleb Easterly[1]; Kevin Viken[1]; Anna Levin[1]; Pratik Jagtap[1]; Timothy Griffin[1]; Lisa Maier[2]
[1]University of Minnesota, Minneapolis, MN; [2]National Jewish Health, Denver, CO

Sarcoidosis is a multisystem granulomatous disease of unknown etiology. Although spontaneous remission occurs, progressive pulmonary disease results in significant morbidity and mortality. Our goal is to identify proteins and protein networks that differentiate sarcoidosis from healthy controls and progressive from non-progressive sarcoidosis. We examined the bronchoalveolar lavage fluid (BALF) from four healthy controls, three non-progressive, and four progressive pulmonary sarcoidosis cases. Trypsin-digested medium and low-abundance BALF proteins obtained after depletion of high-abundance proteins were analyzed by gel LC-MS/MS on an Orbitrap Fusion mass spectrometer (MS). The RAW MS files were processed via MaxQuant software for Label-free Quantification (LFQ). Controlling for false discovery rate (FDR) of ≤ 1%, we identified 1056 proteins in sarcoidosis, a substantially higher number than previous studies. The RAW MS files were also analyzed within the Galaxy-P framework, implementing a SearchGUI/PeptideShaker algorithm. Using this method, we obtained similar coverage to MaxQuant for protein identification. To identify differentially expressed (DE) proteins, we used LFQ intensity for 590 proteins identified in two or more subjects/group. In sarcoidosis cases vs. controls, the proteins with a trend for DE (p-value<0.1) map to canonical pathways involved in signaling of CCR3, CXCR4, IL-8, B-cell receptor, mTOR and IL-12 and ROS production and signaling in macrophages. The proteins with a trend for DE between progressive and non-progressive cases map to glucocorticoid receptors, leukocyte extravasation, PI3/AKT, ERK/MAPK, PPAR, and TREM. Several proteins with differences in abundance between progressive and non-progressive cases showed similarities in expression pattern between non-progressive cases and controls, suggesting that non-progressive pulmonary sarcoidosis cases are more like controls in regards to protein expression. In summary, while we examined a small number of subjects, our findings provide proof-of-concept for systematic characterization of BALF in pulmonary sarcoidosis to gain insights into the mechanisms and markers of progressive pulmonary sarcoidosis.

POSTER ABSTRACTS

All posters will be displayed Monday and Tuesday.
Each day: even-numbered posters present 1:30-2:15 pm; odd-numbered posters present 2:15-3:00 pm.

Poster 13: Development of a Multi-Protein Classifier for Ovarian Cancer Detection by the Simultaneous Measurement of 92 Serum Proteins

Amy Skubitz[1]; Kristin Boylan[1]; Kate Geschwind[1]; Qing Cao[1]; Timothy Starr[1]; Melissa Geller[1]; Robert Bast[2]; Karen Lu[2]; Joseph Celestino[2]; Joseph Koopmeiners[1]
[1]University of Minnesota, Minneapolis, MN; [2]University of Texas, Houston, TX

Currently, there are no FDA approved screening tools for detecting ovarian cancer in the general population. The two best known ovarian cancer biomarkers, CA125 and HE4, are neither adequately sensitive nor specific. We hypothesized that by using a combination of protein biomarkers for screening, we could increase the sensitivity and specificity over CA125 alone. In this study, we used Proseek Multiplex Oncology II plates to simultaneously measure the expression of 92 cancer-related proteins in serum using proximity extension assays. This technology combines the sensitivity of the polymerase chain reaction with the specificity of antibody-based detection methods, allowing multiplex biomarker detection and high throughput quantification. We analyzed one microliter of serum from each of 60 women with ovarian cancer and compared the values obtained to those from 88 age-matched healthy women. Principle component analysis and unsupervised hierarchical clustering separated patients into the two major groups of ovarian cancer and healthy, with minimal misclassification. Data from the Proseek plates for CA125 levels exhibited a strong correlation with previously measured clinical values for CA125 (correlation coefficient of 0.91). CA125 and HE4 were detected at low levels in healthy samples, while higher levels were found in the ovarian cancer cases. We identified 45 proteins that showed a significant difference (p < 0.001) between ovarian cancer and healthy samples; many of which could serve as novel serum biomarkers for ovarian cancer. In total, 40 proteins had an estimated area under the ROC curve of 0.70 or greater. CA125 alone achieved a sensitivity of 0.93 at a specificity of 0.98. In addition, we generated a multi-protein classifier that increased the sensitivity of CA125 alone. Our data demonstrate that the Proseek technology can replicate the results established by conventional clinical assays for known biomarkers, identify new candidate biomarkers, and improve the sensitivity and specificity of CA125 alone.

Poster 14: A Quantitative Evaluation of Algorithms for Isotopic Trace Extraction via Ion Chromatogram Clustering

Mathew Gutierrez; Amber Yascavage; Rob Smith
University of Montana, Missoula, MT

Mass spectrometry provides a popular means to ascertain the identity and quantity of proteins in biological samples. Although methods exist for both tasks that rely on tandem MS, also known as MS/MS, several methods rely at least in part on isolating the precursor or MS1 signals of molecules measured in a sample (isotopic envelopes). These include techniques for quantifying, narrowing MS/MS search spaces, and mapping molecules across multiple replicates. Many techniques attempt to discover isotopic envelopes by searching for known isotope signal patterns, but these are limited by their computational complexity. A more efficient approach is to iteratively extract isotopic envelopes by first extracting extracted ion chromatograms (XICs), then clustering XICs into isotopic envelopes. Using a new quantitative MS1 ground truth data set, we present the first quantitative evaluation of three popular methods for XIC clustering: OpenMS, msInspect, and MaxQuant.

Poster 15: The Human Proteome as of 2018, from the HUPO Human Proteome Project

Gilbert Omenn[1]; Lydie lane[2]; Emma Lundberg[3]; Christopher Overall[4]; Young-Ki Paik[5]; Jennifer van Eyk[6]; Tadashi Yamamoto[7]; Eric Deutsch[8]
[1]University of Michigan, Ann Arbor, MI; [2]Swiss institute of Bioinformatics, Geneva, Switzerland; [3]Scilifelab, Karolinska, Stockholm, Sweden; [4]University of British Columbia, Vancouver, BC, Canada; [5]Yonsei Research Center, Seoul, Korea; [6]Cedar-Sinai Hospital Center, Los Angeles, US; [7]Niigata University, Niigata, Japan; [8]Institute for Systems Biology, Seattle, WA , USA

The Human Proteome Organization Human Proteome Project (HPP) progressed on its two overall goals: (1) completing the protein parts list, with annual updates of the HUPO draft human proteome; (2) making proteomics an integrated complement to genomics and transcriptomics throughout life sciences research. neXtProt v2017-01-23 had 17008 confident protein identifications (Protein Existence [PE] level 1) compliant with the HPP Guidelines v2.1 (https://hupo.org/Guidelines), up from 13664 in 2012-12 and 16518 in 2016-04. Remaining to be found by mass spectrometry/other methods were 2579 "missing proteins" (PE2+3+4), down from 2949 in 2016. PeptideAtlas 2017-01 had 15173 canonical proteins, accounting for nearly all of the 15290 PE1 proteins based on MS data. These resources also have extensive data on PTMs, single amino acid variants, and splice isoforms; the Human Protein Atlas v16 has 10492 highly curated protein entries with tissue and subcellular spatial localization of proteins plus transcript expression. Organ-specific popular protein lists have been generated for broad use in quantitative targeted proteomics using SRM-MS or DIA-SWATH-MS studies of biology and disease.

The HPP 5th special issue of *JPR* (Dec 2017) includes 25 manuscripts. Initial results from the C-HPP Next50 Missing Proteins Challenge detected 73 missing proteins that meet the HPP guidelines, using several novel approaches, notably a pan-proteome "data mining" approach by El-Guoshy et al. [2] (Chr X team, Japan). They searched the MS-based PeptideAtlas and GPM databases for stranded non-nested proteotypic pairs of 9+ aa peptides mapping to protein-coding genes. They found 360 in GPM, 1 in PeptideAtlas, and 41 bridging the two. For confirmation of the reported spectra, they used reference spectra of synthetic peptides available in SRMAtlas to generate 402 MP candidates. They identified good matches for 41 missing proteins. Baselines for the 2018 cycle of HPP progress are PeptideAtlas 2018-01/neXtProt 2018-02. Manuscripts deadline for JPR 2018 Special Issue: 31 May.

Poster 16: Progress Towards Development of an Open Source Human Kinetic Proteomics Software Tool

Bradley Naylor; Marcus Hadfield; David Parkinson; Austin Hannemann; Paul Hafen; John Dallon; Rob Hyldahl; John Price
Brigham Young University, Provo, UT

Living cells are dynamic, so understanding the proteome of a living system requires measuring changes in the proteome over time. Of particular interest is the average rate proteins are destroyed and replaced with new copies, a process called protein turnover. Measuring protein turnover requires providing a heavy isotope label to an organism and tracking the incorporation of that label into individual proteins on a proteomic scale throughout time. This process, called kinetic proteomics, is computationally intensive, which can cause difficulty for researchers new to the method.

To aid researchers new to kinetic proteomics, several labs, including ours, have developed various software tools to perform kinetic proteomics calculations for cell culture or experimental animals. While these tools are effective, kinetic proteomics experiments in human subjects have more limited software options. Humans require experimental changes that increase computational complexity and prevent most current software tools from calculating protein turnover rates in human subjects.

There have been efforts to create software tools to perform kinetic proteomics, but so far options are limited. We are building upon published calculations from previously published human kinetic proteomics experiments and work we have done creating our previous software tool for kinetic proteomics in animal models, to create an open source programming tool that will enable any lab to perform human kinetic proteomic calculations.

POSTER ABSTRACTS

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Poster 17: Spritz: An RNA-Seq Analysis Engine that Enables Proteogenomics on Windows

Anthony Cesnik; Michael Shortreed; Brian Frey; Lloyd Smith
UW-Madison, Madison, WI

Each human individual has thousands of variations in their genome and transcriptome that lead to protein sequence variations. Some of these variations play important roles in human diseases by changing the interactions and functions of certain proteins. The rising tide of computational workflows that aim to detect such variations has collectively been termed proteogenomics. These workflows involve using a core set of computational genomics tools that perform tasks such as aligning sequencing reads to a reference genome and calling variations from those reads. Then, after writing a protein database containing the sequence changes, a set of proteomics tools is used to analyze mass spectrometry proteomics data to detect these variant proteins. A major problem in proteogenomics is the integration and steep learning curve for these tools. Tools for genomics have typically been written to run in Unix command-line environments, e.g. Linux and Macintosh consoles, whereas proteomics tools are typically written for Windows. Learning how to use a command-line interface takes time and practice, and installing another operating system is inconvenient. To facilitate the learning curve and streamline the integration of these tools, we have developed Spritz, a software tool written in C# that dynamically generates scripts to run complete genomics analysis workflows natively on Windows using the Windows Subsystem for Linux. This tool will run several genomics analysis workflows with the click of a button, including generating proteogenomic databases to more easily detect proteoforms containing amino acid sequence variations.

Poster 18: A Portable Pathway for Processing Peptide Array Data in KNIME with an Interest in Protein-Protein Interactions

Lee Parsons
Univ of Minnesota, Plymouth, MN

Peptide arrays have been applied previously to questions including protein-protein interactions, though the lack of standardized methods for interpreting their resulting data sets has impeded their development and acceptance. This method aims to assist in standardization and portability of processing peptide array data by introducing a KNIME workflow that takes in an array of intensities from peptide array experiments and through statistical analysis (including steps written in R) can produce graphs and heatmaps that describe the interactions observed. The data set from Parsons & Wilkens (PLoS ONE 2012) is used for the construction of this workflow, and the results from the analysis in that publication (which was analyzed in the MeV software) are compared to the results of this workflow.

This workflow requires three files for input – a numerated table of probes with sequences, a numerated table of targets (array spots) with sequences, and a numerated table of intensity values for the array experiments – and produces heat maps that cluster and characterize the interactions shown in the data. In these first statistical steps guided clustering is done based on the intensity values and the assigned identities of the probes. The clustered set is then tested with one-way Analysis of Variance (ANOVA), which is used to build a heatmap as was done when the same data was analyzed in MeV.

New to this workflow are additional characterization of probes and targets based on their primary sequence. For both classes, molecular weight, hydrophobicity, isoelectric point, charge, and proposed helical content are calculated. With these numbers the hits for each probe are categorized accordingly to evaluate the specificity of the hits for each probe or whether a probe has a tendency to hit a specific type of target.

Poster 19: Optimizing Collision Energy in Collision-Induced Dissociation for Peptide Sequencing

Nandhini Sokkalingam; Luke Schneider; William Wright; Siamak Ashrafi; Adam Tenderholt; Jeffrey Peterson
Veritomyx Inc., Palo Alto, CA

Although there have been advances in mass spectrometers, ionization techniques and hybrid data collection approaches, it remains a challenge to derive a peptide's sequence from its product ion spectrum. Bottom up proteomic proficiency studies still show low sequencing efficiency and high error rates on known peptides. At least part of the problem may be related to collision-induced dissociation (CID) conditions. Previous studies show that CID parameters have a pronounced effect on the fragmentation of a given peptide ion and determine the useful sequencing information found in the spectrum. The goal of this study is to examine how collision energy (CE) affects the information content of the resulting MS^2 spectrum, and how optimal CID conditions may change with peptide composition.

The singly-charged precursor ions of a set of synthetic peptides were fragmented over a wide range of CEs. Three separate versions of each polypeptide (peptide with lengths ranging from 4 to 18 residues of the same amino acid) were generated with a single proline included at the N-termini, C-termini or the middle of the peptide to discriminate internal ions and any sequence dependence in the resulting fragments. The information content at each CE was calculated as the product of the fraction of the total unique peaks and the fraction of the total ion current observed in the resulting fragments.

Optimal CE was determined more by peptide length than peptide sequence and amino acid composition. While a fixed collision energy is often used for high throughput proteomics, this study found no single CE that provided more than half of the possible MS^2 information content for any peptide. This data set facilitates *in silico* optimization of the CE profile to maximize fragment information gained from a peptide CID spectrum.

Poster 20: Increased Accuracy of Peptide *de novo* Sequencing using Complementary AI-ETD and HCD Fragmentation

Kevin Schauer[1]; Dan Maloney[2]; Nicholas Riley[1]; Michael Westphall[1]; Baozhen Shan[2]; Joshua Coon[1]
[1]University of Wisconsin, Madison, WI; [2]Bioinformatics Solutions Inc, Waterloo, Canada

In bottom-up proteomics, ETD has often been used to complement HCD to provide more accurate peptide *de novo* sequencing. By alternating fragmentation of the same precursor with HCD and ETD, complementary fragment ions can be collected from the same precursor, allowing for more confident peptide identifications. However, during standard ETD reactions, non-dissociative electron transfer (ETnoD) is common, where backbone cleavage occurs but non-covalent, gas-phase interactions prevent dissociation of the fragments. ETnoD is especially common when analyzing precursor ions with low charge density. AI-ETD reduces ETnoD by concurrently irradiating the ETD reaction with IR photons, resulting in slow heating of the precursor and increased dissociation of ETD-formed fragments. Here we demonstrate that replacing ETD with AI-ETD as the complementary fragmentation method to HCD provides more accurate *de novo* sequencing of larger, low charge density precursors. Data was collected on a Thermo Scientific, Orbitrap Fusion Lumos that has been equipped with a continuous wave CO_2 laser to allow for AI-ETD fragmentation. Peptide *de novo* sequencing was performed using PEAKS Studio, where fragment ion frequencies of different fragmentation types were calculated using machine learning. ADEPTS was used to de novo sequence complementary spectra. In an investigation of isolated bovine thyroglobulin using complementary AI-ETD and HCD spectra, the average confident *de novo* tag length increased from 6.3 to 7.2 for doubly-charged precursors, compared to HCD fragmentation alone. Even greater gains in *de novo* tag length were observed for +3 (7.5 to 9.3) and +4 (7.9 to 9.4) charged precursors by the addition of AI-ETD.

POSTER ABSTRACTS

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Poster 21: TimsTOF Pro PASEF Data Processing with an Evolution of PEAKS™ Software

Baozhen Shan[2]; Gary Kruppa[1]; Heiner Koch[3]; Markus Lubeck[3]; Scarlet Koch[3]
[1]Bruker Daltonics Inc., Billerica, MA; [2]Bioinformatics Solutions Inc, Waterloo, Ontario; [3]Bruker Daltonik GmbH, Bremen, Germany

We have previously introduced the "Parallel Accumulation Serial Fragmentation" method (PASEF, Meier et al., JPR 2015, PMID: 26538118) for trapped ion mobility spectrometry (TIMS) quadrupole time of flight (QTOF) instruments, which allows for the increase of sequencing speed and sensitivity of MS/MS scans at high resolution. The combination of an extremely high acquisition rate (> 100 Hz) with the ultra-high MS and MS/MS resolution (50 000 FWHM) and the increased sensitivity and selectivity gained from parallel accumulation and TIMS separation results in the acquisition of high-quality data within one LC-MS/MS run. Here we describe how such a dataset can be processed and evaluated using a modified version of the well-known PEAKS™ proteomics processing software suite.

PEAKS Studio 8.5 has been modified to enable direct reading and processing of raw PASEF datasets. Data refinement, Database search, Spider™ DeNovo Sequencing, PTM Screening and Label-Free Quant functionalities have been enabled for use with PASEF datasets.

High throughput DDA measurements acquired from 100 ng of a HeLa tryptic digest and separated using 30 – 90 min gradient length resulted in identifications of 25,000 – 45,000 unique peptides and 4000 to 5300 protein groups. Extremely high depth and excellent reproducibility (R^2 = 0.98) in the label-free intensities could be achieved. Using only 12 ng of the same digest and a 60 min gradient resulted in the identification of 13,000 unique peptides, corresponding to 3000 protein groups. Using data from IMAC-enriched phosphopeptide digests allowed the distinction of isoforms from isobaric phosphopeptides, which have been separated in the IMS dimension and isolated/fragmented.

The ability of a cutting-edge proteomics software to interrogate PASEF data is displayed within these results.

Poster 22: iOmicsPASS: Integrative-Omics Approach for Predictive Analysis with Subnetwork Signatures in Breast and Colorectal Cancer Subtypes

Hiromi Wai Ling Koh[1]; Hyungwon Choi[1, 2]
*[1]National University of Singapore, Singapore, Singapore; [2]Institute of Molecular Cell and Biology, A*STAR, Singapore, Singapore*

mRNA expression data are often used as a surrogate measure of protein levels. However, the two are not always correlated and analysis of each data type yields different results. Hence, orthogonal information provided by each -omics data should be integrated. Availability of proteomic and transcriptomic data in large-scale clinical studies such as The Cancer Genome Atlas (TCGA) creates opportunities to better characterize the molecular landscape in complex human diseases. Also, past literature has accumulated a vast amount of experimental data validating molecular interactions and regulatory connections. This cumulative knowledge can be used to infer the biological correlation between different molecular levels in a large number of tumor specimens of cancer patients, while achieving the primary goal of phenotype prediction.

We developed a bioinformatics workflow, called iOmicsPASS, which combines multiple –omics data to build predictive gene signatures in the space of biological networks for clinical outcome prediction. The predictive model leverages on the widely used "shrunken centroid" prediction method, where the patient group centroids are searched with a network-based shrinkage method. In the case of protein, mRNA, DNA copy number-based prediction of cancer subtypes, predictive multi-omics signatures of subtypes are constructed based on profiles consistent with the transcription factor regulatory networks for protein-mRNA targets and protein-protein interaction networks. Here we also proposed a novel method for pathway-level scoring of the predictive subnetworks identified, to rank important and predictive biological pathways involved within each of the cancer subtypes.

We illustrate iOmicsPASS using the invasive breast cancer and colorectal cancer cohort data in TCGA. Our results show the predictive subnetworks signatures identified across the subtypes by utilizing multi-omics data are not only biologically sensible, but also enables highly robust prediction of cancer subtypes, improving the current mRNA-based molecular subtyping.

Poster 23: PTMscape: An Open Source Tool to Predict Generic Post-Translational Modifications and Map Hotspots of Modification Crosstalk

Ginny Li[1]; Christine Vogel[2]; Hyungwon Choi[1]
[1]National University of Singapore, Singapore, Singapore; [2]New York University, New York, NY

While tandem mass spectrometry can now detect post-translational modifications (PTM) at the proteome scale, reported modification sites are often incomplete and include false positives. Computational approaches can complement these datasets by additional predictions, but most available tools are tailored for single modifications and each tool uses different features for prediction. We developed an R package called PTMscape which predicts modifications sites across the proteome based on a unified and comprehensive set of descriptors of the physico-chemical microenvironment of modified sites, with additional downstream analysis modules to test enrichment of individual or pairs of modifications in functional protein regions. PTMscape is generic in the ability to process any major modifications, such as phosphorylation and ubiquitination, while achieving the sensitivity and specificity comparable to single-PTM methods and outperforming other multi-PTM tools. Maintaining generalizability of the framework, we expanded proteome-wide coverage of five major modifications affecting different residues by prediction and performed combinatorial analysis for spatial co-occurrence of pairs of those modifications. This analysis revealed potential modification hotspots and crosstalk among multiple PTMs in key protein domains such as histone, protein kinase, and RNA recognition motifs, spanning various biological processes such as RNA processing, DNA damage response, signal transduction, and regulation of cell cycle. These results provide a proteome-scale analysis of crosstalk among major PTMs and can be easily extended to other modifications.

Poster 24: Predicting Cell-Line Specific Protein and Phospho-Protein Abundances in Cancers

Hongyang Li; Gilbert Omenn; Yuanfang Guan
University of Michigan, Ann Arbor, MI

Protein abundance and post-translational modification are driving forces of phenotypic variation and of numerous diseases. In response to the 2017 NCI-CPTAC Dialogue on Reverse Engineering Assessment and Method (DREAM) proteogenomics challenge, we investigated the correlation among mRNA, protein and phospho-protein abundances across 77 breast and 105 ovarian cancers. The baseline correlation between mRNA and protein abundances was 0.40. This baseline assumes that the expression of one gene is independent of other genes. By integrating the mRNA levels of multiple genes, we created random forest models to learn the non-linear inter-dependencies among genes, which improved the predictive correlation to 0.48. Interestingly, we found that our models of breast and ovarian cancers are transferable to each other; the ensemble of the two models increased the correlation to 0.52. We applied similar strategies to predicting phospho-protein abundance, in which the baseline correlation between protein and phospho-protein abundance was only 0.32. Our model achieved a high correlation of 0.66 in the breast cancers. Moreover, we found that multiple phosphorylation sites of the same protein are highly associated. Through weighting the predictions of multiple sites based on the ratio of missing observations, we further improved the correlation to 0.68. In sum, our state-of-the-art method ranked first in predicting protein and phospho-protein abundances in both breast and ovarian cancer sets in the NCI-CPTAC DREAM challenge.

POSTER ABSTRACTS

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Poster 25: Evolution of SWATH® Acquisition Provides Large Gains in Quantified Proteins

Christie Hunter[1]; Nick Morrice[2]; Joerg Dojahn[3]; Arianna Jones[4]
[1]SCIEX, Redwood City, CA; [2]SCIEX, Warrington, UK; [3]SCIEX, Darmstadt, Germany; [4]SCIEX, Framingham, MA

The data independent acquisition strategy termed SWATH® Acquisition was first described and commercialized in 2012. The acquisition strategy used was to step 25 Da Q1 windows across the peptide mass range, collecting high resolution MS/MS data in each step. Since the first description of the technique, there has been a lot of progress made by the SWATH user community in better understanding the best way to collect and process data for large scale protein quantitation experiments. Innovations in data acquisition, improvements in instrument hardware, advances in library generation, and large increases in scale of studies have all resulted in large improvements, resulting in the workflow used today.

Here we will summarize and quantify the improvements made due to the various method improvements. Holding much of the parameters constant (LC, instrument, sample load) and running the various acquisition methods back to back, a good measure of the impact of the techniques can be obtained. Comparing to the original implementation of the SWATH method (34fx x 25Da with a small 1D library), significant gains (>100% protein and 300% peptides quantified) are observed when comparing to results obtained by using a 100 variable window approach with a large ion library.

Poster 26: Identification of Putative Fibrous Plaque Marker Proteins by Unsupervised Deconvolution of Heterogeneous Vascular Proteomes

Sarah Parker[1]; LuLu Chen[4]; Georgia Saylor[3]; Chunhong Mao[2]; Vidya Venkatraman[1]; Mitra Mastali[1]; Weston Spivia[1]; Rakhi Pandey[1]; Yue Wang[4]; David Herrington[3]; Jennifer Van Eyk[1]
[1]Cedars Sinai Medical Center, Los Angeles, CA; [2]Virginia Tech, Biocomplexity Institute, Blacksburg, VA; [3]Wake Forest University, Wake Forest, NC; [4]Virginia Tech, Arlington, VA

Atherosclerosis is an indolent condition involving progressive remodeling of blood vessels, leading to severe complications including coronary artery disease (CAD), stroke, and peripheral limb disease, that collectively comprise leading causes of morbidity and mortality world-wide. Circulating biomarker panels indicative of early stage lesion status would aid in more accurate risk stratification of individuals for appropriate prioritization of therapeutic intervention, which remains a significant clinical challenge. To identify candidate biomarkers of early atherosclerosis, we performed DIA-MS-based proteomic analysis of left anterior descending coronary (LAD) and abdominal aorta (AA) artery tissue from a large cohort of young human donors exhibiting a range of pathology biased toward none/low-to-moderate atherosclerotic remodeling. To parse molecular and phenotypic heterogeneity, an unsupervised deconvolution algorithm termed Convex Analysis of Mixtures (CAM) was used, which identified four cellular or pathological subtypes within the homogenates along with marker protein sets for each identified subtype. One of these subtypes corresponded highly with pathologist estimated fibrous plaque burden in both LAD and AA specimens, yielding putative tissue-derived FP marker proteins. We validated these markers in 'pure' specimen of FP (N=4), FS (N=3), or Normal (N=3) status from separate human donors, demonstrating that 83% of the 58 putative FP-markers were statistically significantly different between pure normal and pure FP specimens. Finally, we used scheduled multiple reaction monitoring mass spectrometry (MRM) to 39of the putative FP-markers reliably detectable in plasma from women with established CAD (N=46) and aged matched, healthy controls (N=40). Adaptive elastic net regression modeling and k-fold leave-one-out validation identified a parsimonious set of ten proteins, training a model capable of predicting CAD status with an average ROC AUC of 0.997 (95% CI 0.979 – 0.999) and a 3.5% misclassification rate (N=10,000 iterations). With additional development this pipeline has promise for identification of informative and, potentially, population-stratified panels of atherosclerosis biomarkers.

Poster 27: Glycopeptide Analysis to Examine the Role of Chlamydial Protease-Like Activity Factor

Julian Saba[1]; Christa Feasley[2]; Fred Zinnel[3]; Stuart McCorrister[4]; Garrett Westmacott[4]; Grant McClarty[4]; Chris Grant[4]
[1]Thermo Fisher Scientific, Mississauga, Canada; [2]Thermo Fisher Scientific, West Palm Beach, FL; [3]Thermo Fisher Scientific, Somerset, NJ; [4]Public Health Agency of Canada, Winnipeg, Manitoba

Recently proteomics studies have been performed to examine the role of chlamydial protease-like activity factor (CPAF), a secreted virulence factor and its role in immune evasion. However, experiments thus far have ignored glycopeptides that are prevalent in the sample as large scale intact glycopeptide analysis remains challenging by mass spectrometry. Here, we perform large scale intact glycopeptide analysis to examine the role of CPAF targets and derive additional insights from glycoproteomics experiments.

Hela 229 cells were infected with Chlamydia trachomatis L2 RST5 CPAF-sufficient strain and RST17 CPAF-deficient strain. Proteins were extracted, trypsin digested and enriched for glycopeptides. Samples were analyzed on Orbitrap Fusion Lumos MS. Data analysis was performed using Proteome Discoverer with Byonic node.

In proteomics experiment ~40-50% of MS/MS spectra are identified. Thus, researchers have focused on developing software algorithms to sequence remainder of the spectra with the hypothesis that these are indeed identifiable spectra from conventional peptides. This assumption ignores the fact that the spectra are the result of PTMs. We have observed some of these unidentified spectra are from glycopeptides. Unfortunately, conventional fragmentations are not ideal for glycopeptide sequencing. Further exasperating the issue is that these glycopeptides are present in low abundance. In our proteomics experiments we observed that 10% of our MS/MS spectra were from glycopeptides. XICs of m/z 204.087 (HexNaAc oxonium ion) were performed on LC-MS/MS runs. As precursor ions containing the m/z 204.087 could not be sequenced, these were targeted in a glycoproteomics experiment. Overall we identified over 3000 unique intact glycopeptides in a single LC-MS/MS run and over 5000 in triplicate runs, translating into over 600 unique glycoproteins.

To our knowledge this is by far the largest number of intact glycopeptides reported in a single experiment..

Poster 28: High Pressure-Accelerated PNGase F Deglycosylation of Native Proteins

Vera Gross; Nicole Cutri; Alexander Lazarev
Pressure BioSciences, Inc., South Easton, MA

The positive effects of hydrostatic pressure on digestion with proteases such as trypsin, lys-C, and chymotrypsin, are well established. Pressure-induced denaturation of substrate proteins leads to better access of enzymes to previously inaccessible, or poorly accessible, target sites, resulting in improved and accelerated digestion. Here we report that deglycosylation of native proteins is also accelerated when the reactions are carried out under pressure. Unlike most other enzymes commonly used in proteomics workflows, PNGase F is not a protease. It is a glycosidase that cleaves N-linked high mannose, hybrid, and complex oligosaccharides from glycoproteins. We tested PNGase F activity under high pressure conditions using two model glycoproteins, human alpha1 acid glycoprotein (α1AGP), and bovine fetuin. Deglycosylation of fetuin results in a single, characteristic shift of the protein band on SDS-PAGE. Alpha1 acid glycoprotein contains five N-linked oligosaccharides; partial deglycosylation results in multiple bands on SDS-PAGE.

Deglycosylation of native, unreduced protein is a slow process that requires significantly more enzyme compared to deglycosylation of protein that is denatured and where disulfide bonds have been reduced. For this reason, most protocols call for reduction of disulfide bonds and heat denaturation of proteins prior to treatment with PNGase F. However, by incubating the reactions under high pressure, deglycosylation of native, unreduced protein can be accelerated significantly. Our results indicate that PNGase F enzyme is quite pressure-stable and shows enhanced activity even at pressures above

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50,000psi. Data generated with the two model proteins suggest that PNGase F can be greatly accelerated by performing the reactions in a Barocycler under high pressure. Reactions carried out at high pressure for an hour or less, resulted in significantly more complete deglycosylation compared to reaction incubated overnight at ambient pressure.

Poster 29: Develop of a Robust and Reproducible Global Plasma Proteome Profiling Workflow

Scott M. Peterman[1]; David Sarracino[2]; Amol Prakash[3]; Kerry Hassell[4]
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Plasma has re-emerged as the primary bio-fluid evaluated for biomarker identification but requires alternative strategies for large-scale, comprehensive analysis due to the number of proteins and dynamic protein expression ranges. The primary goal is to routinely quantify over 500 proteins in non-depleted plasma samples supporting large-scale studies. This requires experimental workflows to overcome the dynamic range problem associated with protein expression range, robustness of the experimental instrumentation, and confident, robust data processing. We have modified our separation methods by incorporated a trapping column packed with PS/DVB particles, a second divert valve, and a 50-cm analytical column to increase the loading amount that has resulted in significantly increasing plasma proteome coverage.

To evaluate the proposed workflow, we have prepared a series of plasma samples from a single donor. Samples were divided into two experiments, a loading study and ruggedness evaluation. The loading study increased plasma digest loaded on column from 7 to 220 µg per replicate. The ruggedness study evaluated 321 different samples and analyzed continuously for 22 days with 90 µg of digest injected per sample. Both experiments utilized identical instrumental methods and all data was analyzed using Pinnacle.

The number of proteins confidently identified and quantified from the load study ranged from 326 to 745 and 1233 to 1963 peptides using the 52-minute gradient. In addition, the average retention time difference between 7 and 220 µg loading was ca. 6 seconds and FWHM peak widths changed by an average of 0.05 minutes despite substantially increasing the loading amounts. The results from the ruggedness study showed an average of 596 proteins and 2296 peptides were profiled across the 32 different groups. The average retention time variance was 0.5% and peak width (FWHM) values were 0.05 minutes and the calculated variance for peptide area values were less than 25%.

Poster 30: Approach to the Confident Determination of Elemental Compositions in Mass Spectrometry Imaging Using IR-MALDESI

Sitora Khodjaniyazova[1]; Milad Nazari[1]; Kenneth Garrard[1]; Mayara Matos[2]; Glen Jackson[2]; David Muddiman[1]
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Infrared matrix-assisted laser desorption electrospray ionization (IR-MALDESI) source coupled to the Q Exactive Plus[TM] has been extensively used in untargeted mass spectrometry imaging (MSI) analyses of biological tissue sections. Although the Orbitrap is a high-resolution and accurate-mass (HRAM) mass analyzer, these attributes alone cannot be used for the reliable identification of unknown analytes observed in complex biological matrices. However, high mass measurement accuracy (MMA) combined with spectral accuracy (SA) can often lead to elucidation of a single elemental composition. SA is the ability of the mass spectrometer to accurately measure the isotopic distributions which, when used with high MMA, can facilitate the elucidation of a unique elemental composition. To investigate the effects of different ion populations on an Orbitrap's SA, a solution of caffeine, the tetrapeptide MRFA, and ultramark was analyzed using a Q Exactive Plus across eight distinct automatic gain control targets. The same compounds from the same lot numbers were also analyzed using isotope ratio mass spectrometry (IRMS) to accurately calculate the expected isotopic abundance of $^{13}C_1$, $^{15}N_1$, and $^{34}S_1$ peaks used for

carbon, nitrogen, and sulfur counting, respectively. These analyses allowed us to establish two experimentally determined optimal conditions for each of three arbitrarily-defined mass windows of small (100 < MW (Da) < 400), medium (400 < MW (Da) < 900), and large (1000< MW (Da) < 1500) compounds: 1) optimum absolute $^{13}C_1$, $^{15}N_1$, and $^{34}S_1$ ion abundances for accurate carbon, nitrogen, and sulfur counting, and 2) empirically determined thresholds for the absolute monoisotopic ion abundances required for good spectral accuracy. In the work presented here, we demonstrate that it is crucial to establish thresholds for absolute ion abundances when using the Orbitrap because changes in ion populations could influence the MMA and SA, and subsequently hinder the ability to confidently identify unknown analytes in MSI.

Poster 31: Development of a Novel Strategy for Measurement of the Neurotoxin Beta-N-Methylamino-L-Alanine in Environmental Samples

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We describe a new set of tools for the detection and quantification of the neurotoxin beta-N-methylamino-L-alanine (BMAA) including a novel stable isotope labeled BMAA standard and a chip-based capillary electrophoresis mass spectrometry platform. Baseline resolution of BMAA and its structural isomers, 2,4-DAB and AEG, was achieved using this method in less than one minute. Detection and linearity of response were demonstrated using parallel reaction monitoring (PRM), and the lower limit of detection and quantification for BMAA detection were experimentally determined. These tools were applied to detection of BMAA in environmental samples, including seafood purchased from Raleigh, NC with known harvesting locations.

Poster 32: Identification of Natural Products as Inhibitors of Bacterial Histidine Kinases

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The continued prevalence of antibiotic resistance in bacteria has increased the demand for new therapeutics against novel targets. Two-component systems (TCSs), composed of a histidine kinase sensor protein and a response regulator protein, are ubiquitous in bacterial signaling, having key functions such as defense, nutrient acquisition, and quorum sensing. These systems utilize an autophosphorylation event by the histidine kinase and subsequent transfer of the phosphate group to the response regulator to propagate a cellular response from an extracellular signal. As part of these crucial signaling pathways, the histidine kinase superfamily of proteins is a promising therapeutic target, with broad-spectrum antibiotic potential due to the conserved ATP-binding domain. Our goal is to discover natural products that bind to this domain, thus preventing the function of key signaling pathways and the ability of the organism to cause infection.

To this end, we used a fluorescence polarization-based high-throughput screen to assess over 25,000 natural product extracts from marine-derived strains of Streptomyces, a largely untapped source of therapeutic potential. From this screen, 16 extracts demonstrated does-dependent inhibition activity of histidine kinase, HK 853. Currently, we are focused on the isolation and structure elucidation of lead compounds from two strains. Due to the complexity of natural product extracts, extensive purification is required before studies to identify the compounds can begin. The isolation workflow involves resin-based extraction and reverse-phase chromatography purification. A fluorescence activity assay and comparative metabolomics is used to track the active compounds through each step. Structure elucidation is achieved through extensive mass spectrometry and NMR characterization. The compounds discovered from these studies will prove to be promising leads towards the development of a new class of antibiotics with a novel mechanism of action.

POSTER ABSTRACTS

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Poster 33: Developing New Tools to Enhance the Galaxy-Based Metabolomics Workbench

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Untargeted metabolomics contrasts metabolome-scale analyses of samples without first identifying individual metabolites. Workflow4Metabolomics (W4m) http://workflow4metabolomics.org/ provides a suite of Galaxy tools for untargeted metabolomics including univariate and multivariate statistical tools and tools for preprocessing LCMS, GCMS, and NMR spectra. We have created three additional tools to facilitate use of W4m in our lab: "Sample Subset", "OPLS-DA Contrasts", and "w4mkmeans".

Sample Subset facilitates downstream statistical analysis by providing several filters to choose a subset of features or samples, imputation of missing values, and elimination of features and samples having zero variance. **OPLS-DA Contrasts** facilitates selection and OPLS-DA analysis of pairs of sample-classes. **W4mkmeans** provides an alternative to multivariate analysis for discovering relationships among samples. Examples of Galaxy workflows using these tools will be presented.

Poster 34: Novel High-Throughput Metabolomic Techniques and Mainstream Tools for the Discovery of Drug Mechanism of Action

Akos Vertes[1]; Albert-Baskar Arul[1]; Andrew R. Korte[1]; Hang Li[1]; Peter Avar[1]; Peter Nemes[1]; Lida Parvin[1]; Sylwia Stopka[1]; Sunil Hwang[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]
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Multi-omics studies are essential for integrative systems biology that aims to interpret large-scale biological data across genome, proteome and metabolome. This field faces challenges in terms of throughput, molecular coverage and due to the diversity of data, efficient data integration. Genomic and proteomic components of the integrative studies generally have excellent and good molecular coverage, respectively, and optimized throughput. Untargeted metabolomics, however, is a bottleneck both in terms of coverage and throughput. To mitigate these obstacles, we explored the utility of three metabolomic techniques for identifying the mechanism of action (MoA) of five test compounds by combining the results from these novel methods with data from transcriptomics and proteomics. Conventional liquid chromatography mass spectrometry (LC-MS) provided deep metabolite coverage at the expense of relatively low throughput. Nanopost array laser desorption ionization (NAPA-LDI) MS and laser ablation electrospray ionization (LAESI) MS provided reduced but complementary molecular coverage with significant throughput advantage. All three techniques were evaluated in combination with quantitative proteomics (TMT 10plex analysis) and microarray-based transcriptomics. In blind studies, we identified the MoA of five individual compounds with known MoA (forskolin, nocodazole, bendamustine, Nexturastat A, and atorvastatin) acting on hepatocellular carcinoma cells (HepG2/C3A) by combining the results from two metabolomics techniques (either NAPA+LAESI or NAPA+LC-MS) with other omics data. LAESI-MS and NAPA-MS enabled 10 to 20 times shorter analysis time compared to LC-MS with a factor of ~5 reduction in molecular coverage. A trade-off between throughput and coverage of metabolites can be made depending on the nature of the molecular perturbation, e.g., metabolic modulators, vs. inducers of signaling pathways.

Poster 35: VKMZ: Visualizing Metabolomics on a van Krevelen Diagram through Galaxy

Mark Esler
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Metabolomics is a rapidly emerging field which studies the composition of small-molecules, metabolites, of a biological sample. Unlike biopolymers, such as proteins and DNA, metabolites vary greatly which makes identification and visualization difficult. vkmz is a metabolomics visualization tool for Galaxy which generates van Krevelen Diagrams from liquid chromatography mass spectrometery (LC-MS) data. A traditional van Krevelen Diagram plots the ratio of hydrogen-to-carbon against oxygen-to-carbon ratios. Chemicals from a class of metabolites share characteristic structures and relative ratios of elements. Thus, certain metabolite classes cluster in certain areas of a van Krevelen Diagram.

These diagrams convey the overall metabolomics profile of a LC-MS sample concisely.

The vkmz tool matches high resolution mass measurements of ions to those known for an extensive list of empirical formulas. Each ion is represented as a dot on the diagram. The color of the dot changes based on the ions retention time and the size optionally correspond to relative intensity. Diagrams can be 3D with nitrogen-to-hydrogen as the third axis. This tool produces interactive plots and tabular data.

vkmz can run standalone but is most effective as a Galaxy tool. Galaxy allows reproducible and shareable data-processing workflows. Key benefits of vkmz on the Galaxy platform are (1) using xcms for Galaxy to process LC-MS data as input and (2) ease of adding visualization to existing workflows.

Poster 36: Traumatic Brain Injury Proteomics Guides Novel KCC2-Targeted Therapy

Pavel N. Lizhnyak; Demisha D.L. Porter; John T. Povloshock; Andrew K. Ottens
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There remains a need for more effective therapeutics in the treatment of traumatic brain injury (TBI). This study tested whether post-translational effects of TBI revealed by temporal proteomics would serve as an effective guide for TBI interventions. Of interest were delayed-onset processes that could be managed after patient stabilization on the intensive care unit. Self-organizing map analysis was applied to the post-TBI temporal proteome, from which we focused on a cohort of proteins involved in ionic dysregulation that exhibited a decrease beginning one day following injury. From this map, we identified neuron-specific K+-Cl cotransporter 2 (KCC2) as an amiable target, an essential component for maintaining chloride homeostasis that is critical to inhibitory neurotransmission. The proteomic results further defined a potential therapeutic window of opportunity around phosphorylation events preceding the escalated functional loss of KCC2. Testing this window, we administered the KCC2-targeting compound CLP290 (50 mg/kg, p.o.) before, at, and after the identified KCC2 post-translational processing. The intervention was most effective at the predicted 1-day time point in preserving plasmalemmal KCC2 within perilesional somatosensory neocortex. Results from a pentylenetetrazole assay validated restored chloride homeostasis and inhibitory function with CLP290 therapy. Furthermore, treatment significantly improved functional recovery of the injured somatosensory cortex on rotarod and whisker adhesive removal task assessments. Study findings demonstrate how temporal proteomic profiling may guide therapeutic development through identifying novel targets and defining windows of intervention. Furthermore KCC2 represents a promising target in TBI and potentially other neurological insults known to involve chloride dysregulation such as epilepsy and stroke.

Poster 37: Comparison of the Rat and Human Dorsal Root Ganglion Proteome

Adam Schwaid[1]; Alicja Krasowka-Zoladek[2]; An Chi[1]; Ivan Cornella-Taracido[1]
[1]Merck & Co., Inc., Boston, MA; [2]Merck & Co., Inc., West Point, PA

Dorsal root ganglion (DRG) are a key tissue in the nervous system that have a role in neurological disease, particularly pain. Despite the importance of this tissue, the proteome of DRG is poorly understood, and it is unknown whether the proteome varies between organisms or different DRG along the spine. Therefore, we profiled the proteome of human and rat DRG. We identified 5303 proteins in human DRG and 5000 proteins in rat DRG. Across species the proteome is largely conserved with some notable differences. While the most abundant proteins in both rat and human DRG played a role in extracellular

POSTER ABSTRACTS

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functions and mylein sheeth, proteins detected only in humans mapped to roles in immune function and translation whereas those detected only in rat mapped to roles in localization and transport. The DRG proteome between human T11 and L2 vertebrae was nearly identical indicating DRG from different vertebrae are representative of one another. Finally, we asked if this data could be used to enhance translatability by identifying mechanisms that modulate cellular phenotypes representative of pain in different species. Based on our data we tested and discovered that MAP4K4 inhibitor treatment increased neurite outgrowth in rat DRG as in human SH-SY5Y cells.

Poster 38: High Pressure (>30,000 psi) Packing of Capillary Columns for Shotgun Proteomics

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Extreme sample complexity is an inherent challenge in shotgun proteomics. Modern mass spectrometers (MS) afford rapid scan rates and remarkable sensitivity to tackle this problem, but high quality of chromatographic separations is necessary to leverage these advancements. An analytical column is an essential component of any chromatographic setup; its physical characteristics, i.e., length and diameter, properties of packing material, and packing bed structure primarily dictate efficiency of engendered separations. However, extensive explorations beyond the current state-of-the-art practices in unidimensional peptide separations are hindered by the upper pressure limits of commercial liquid chromatography (LC) systems. Here we demonstrated that adoption of high pressure (HP) packing for capillary column fabrication facilitates formation of more structurally homogenous packing bed, enhancing chromatographic performance and therefore, depth of shotgun proteomics experiments. We manufactured three sets of three columns reaching different maximum packing pressure (11,000, 20,000, and 30,000 psi). First, we observed that HP columns exhibited lower back pressure (~2,000 psi) upon installation on an HPLC system. This decline in pressure indicated changes in the packing bed structure and offered diverse benefits for HPLC operation. We also measured ~20% reduction in the median base peak width of detected peptides that translated into a comparable increase in the median peak intensity. These changes produced a 10-35% gain in the number of peptides identified in various commonplace bottom-up proteomics samples. Moderate but systematic increases (10-12%) were detected in the analyses of complex digests of the whole yeast and human proteomes and pre-fractionated peptide mixtures. The number of identified acetylated and phosphorylated peptides also increased by 20-25%. The most pronounced improvements (16-35%) were observed when analyzing small peptide loads (10-100 ng) and while implementing longer (90- vs. 180-minute) gradients. Overall, our work corroborates advantage of HP column packing and universal prominence of peptide separations in modern LC-MS/MS.

Poster 39: In Silico Design and in vitro Characterization of Universal Tyrosine Kinase Peptide Substrates

Laura Marholz

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The majority of the 90 human protein tyrosine kinases (PTKs) are understudied 'orphan' enzymes with few or no known substrates. Designing experiments aimed at assaying the catalytic activity of these PTKs has been a long-running problem. In the past, researchers have used polypeptides with a randomized 4:1 molar ratio of glutamic acid to tyrosine as general PTK substrates. However, these substrates are inefficient and perform poorly for many applications. We applied the KINATEST-ID pipeline for artificial kinase substrate discovery to design a set of candidate "universal" PTK peptide substrate sequences. From this work, we identified two unique peptide sequences from this set that had robust activity with a panel of 15 PTKs tested in an initial screen. Kinetic characterization with seven receptor and non-receptor PTKs confirmed these peptides to be efficient and general PTK substrates. The broad scope of these artificial substrates demonstrates that they should be useful as tools to probe understudied PTK activity.

Poster 40: Development of a Novel LC Concept for Clinical Proteomics

Nicolai Bache[1]; Philipp Geyer[2]; Ole Hoerning[1]; Lasse Falkenby[1]; Peter Treit[2]; Sophia Doll[2]; Igor Paron[2]; Florian Meier[2]; Ole Vorm[1]; Matthias Mann[2]

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A conceptually novel low-flow chromatography system has been developed to delivers the robustness and throughput required for clinical applications while maintaining the sensitivity of current nano-flow LC instrumentation. The new system uses four low-pressure pumps in parallel to elute samples from a disposable trap column while simultaneously creating a final but offset chromatographic gradient with embedded analytes.

Initially, two low-pressure pumps form a primary gradient that flows through a disposable trap column thereby eluting analytes of interest while two additional low-pressure pumps positioned right after the trap column are modifying the eluent to create an "offset" that increases the retention of the now embedded analytes at the separation column. The offset gradient with the embedded analytes are moved into a long, narrow holding loop that subsequently is switched in-line with a single high-pressure pump and a separation column. Thus elution from the disposable trap column and gradient formation become de-coupled from the high-pressure separation.

We have characterized the performance of the new system regarding cross contaminations (<0.05%, total TIC), retention time shifts and peak widths (<8.5 sec) in over 1,500 HeLa runs. The extremely short overhead time of approximately 3 min/gradient allowed us to measure 60 samples per day (21 min gradient, 3 min overhead time) compared to 40 samples with a standard nano-LC systems (22 min gradient, 15 min overhead time). To benchmark reproducibility, we measured 100 human plasma samples in 40 hours and each resulted in several hundred quantified plasma proteins, including more than 50 FDA-approved biomarkers. Assured of a high reproducibility, we then aimed to find significant biological information in the human plasma proteome. For this purpose, we analyzed a longitudinal weight loss study that included a total of 319 plasma samples, where we found strongest effects on proteins of the lipid metabolism and inflammation system.

Poster 41: Rapid Trypsin Digestion of Complex Protein Mixtures for Proteomics Analysis

Judy Boland; Zhiyun Cao; Nicolas Caffarelli; Amber Henry; Jeffrey Turner; Kevin Ray

MilliporeSigma, St. Louis, Missouri

Reliable and reproducible results in mass spectrometry proteomics analyses are highly dependent on robust sample preparation. Sample preparation workflows are often cumbersome and generally involve chemical denaturation, reduction and alkylation, buffer exchange, and overnight protease digestion of the protein sample. These lengthy, complex protocols require 3-4 hours of lab work followed by overnight incubation, potentially introducing variability to results. Filter-assisted sample prep and other alternative protocols can speed up the workflow but with increased costs and limited applicability. In this study, we identified conditions allowing for rapid trypsin digest at elevated temperatures that yielded reliable, reproducible results in less than 2 hours on a wide variety of substrates. Compared to traditional protocols, this approach identified similar numbers of peptides and proteins in complex mixtures, without the need for denaturation or reduction and alkylation. Rapid digest of individual proteins yielded comparable sequence coverage, although proteins with high cysteine content may still require reduction and alkylation for efficient digestion. Overall, these results suggest that incorporation of rapid digest buffer and elevated temperature can reduce the labor requirements and turnaround time of sample preparation for proteomics analyses by mass spectrometry.

POSTER ABSTRACTS

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Poster 42: TimsTOF Pro Powered by PASEF for High Speed and Sensitive Shotgun Proteomics

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In data dependent acquisition (DDA) experiments around only 20% of eluting peptide features are targeted by current mass spectrometers, due to limitations in sequencing speed, sensitivity and resolution. The previously introduced "Parallel Accumulation Serial Fragmentation" method (PASEF, Meier et al., JPR 2015, PMID: 26538118) for trapped ion mobility spectrometry (TIMS) quadrupole time of flight (QTOF) instruments has been shown to increase the sequencing speed and sensitivity of MS/MS scans at high resolution. Here, we show the instrument performance on low sample amounts, offering unprecedented possibilities to investigate samples at high sensitivity and high throughput.

Low peptide sample amounts (12 – 100 ng) of HeLa protein digest were HPLC separated (nanoElute, Bruker Daltonics) on 250 mm pulled emitter columns (IonOpticks, Australia) and analyzed on a high-resolution timsTOF Pro instrument (Bruker Daltonics) using a gradient lengths of 30 – 90 min. Data were analysed using PEAKS (Bioinformatics solutions Inc.) and MaxQuant (Jürgen Cox, Max Planck Institute of Biochemistry).

High throughput DDA measurements acquired from 100 ng of a HeLa tryptic digest and separated using 30 – 90 min gradient length resulted in identifications of 25,000 – 45,000 unique peptides and 4000 to 5300 protein groups. Extremely high depth and excellent reproducibility (R^2 = 0.98) in the label-free intensities could be achieved using post-processing packages from PEAKS or MaxQuant. Using only 12 ng of the same digest and a 60 min gradient resulted in 13,000 unique peptides identified, corresponding to 3000 protein groups. Using a 90 min gradient, a sequencing speed of 126 Hz on average can be achieved without a lost in sensitivity at a resolution of 30,000 – 40,000.

Our results show that the timsTOF Pro instrument using the extremely high speed PASEF method can be used for high throughput and sensitive shotgun proteomics experiments.

Poster 43: TimsTOF Pro Powered by PASEF for High Sensitivity Phosphoproteomics

Paul Savage[1]; Koch Heiner[2]; Scarlet Koch[2]; Markus Lubeck[2]; Florian Maier[3]; Oliver Raether[2]; Andreas Brunner[3]; Matthias Mann[4]

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The global analysis of phosphopeptides enriched from complex protein digests is very challenging in terms of identification as well as providing confident site localization. Here, we evaluate the performance of the trapped ion mobility spectrometry quadrupole time of flight mass spectrometer (timsTOF Pro, Bruker Daltonics) powered by PASEF for single shot phosphoproteomics.

IMAC phosphopeptide enrichments from 200 µg proteolytic digests were HPLC separated (nanoElute, Bruker Daltonics) on 250 mm pulled emitter columns (IonOpticks, Australia) and analyzed on a high-resolution timsTOF Pro instrument (Bruker Daltonics) on a 90 min gradient. Post-processing analyses were performed in PEAKS (Bioinformatics Solutions Inc.) and MaxQuant (Jürgen Cox, Max Planck Institute of Biochemistry).

Applying the Parallel Accumulation Serial Fragmentation (PASEF) method on the timsTOF Pro instrument enables on average the selection of 12 precursors within 100 ms. Due to the extremely high speed and sensitivity of the PASEF method we could achieve more than 66,000 peptide spectrum matches (PSMs) resulting in around 25,000 unique peptides and 16,000 unique phosphopeptides that could be identified within a 90 min gradient run. Due to the TIMS separation

PASEF leads to cleaner MS/MS spectra making especially low abundant peptides visible and identifiable.

The combination of the extremely high speed and sensitivity provided by the PASEF method which is can be exclusively be done on the timsTOF Pro instrument enables to make a significant step towards comprehensive phosphoproteome analysis.

Poster 44: Liquid Extraction Surface Analysis (LESA) and LESA Plus Chromatography as Novel Surface Analysis Tools for Mass Spectrometry

Daniel Eikel

Advion, Ithaca, NY

Liquid Extraction Surface Analysis (LESA) utilizes a micro-extraction step in ca 1 µL of solvent, directly from a surface of interest, by way of a micro-liquid junction. Suitable surfaces can be fresh frozen tissue sections, but also others such as dried blood spots, bacteria grown on agar or protein chips. Following the extraction step, LESA can introduce the extracted analytes to the mass spectrometer (MS) directly as an infusion experiment for rapid analysis. LESAplus however introduces the samples onto an additional liquid chromatography column to separate the extracted analytes prior to electrospray ionization and analysis in the MS. The latter increases spatial resolution and can separate isobaric analytes, whereas the former is more suitable for a higher sample throughput.

Here, we will show examples for LESA and LESAplus analysis utilizing an Advion TriVersa-NanoMate automated ion source. We found the spatial resolution of LESA to be around 1 mm on target and a variety of samples were analyzed (pesticides from fruit surfaces, toxins from molded wood pieces, small molecule drugs from tissue sections of brain and whole body of the mouse).

We will further show that LESAplus can achieve an improved spatial resolution of 400 µm on target and separate isobaric compounds such as pesticides from a fruit surface or lipids from brain tissue. Examples from the literature demonstrate the ability to perform proteome wide analysis in a bottom-up fashion from tissue sections.

Poster 45: High Pressure Denaturation Improves Protein Digestion by Trypsin

Vera Gross; Nicole Cutri; Gary Smejkal; Alexander Lazarev

Pressure BioSciences, Inc., South Easton, MA

Traditional proteomics workflows often take advantage of the effect of chaotropes on protein unfolding. By adding urea (sometimes up to 6M) to trypsin digests, difficult-to-digest proteins can be digested. However, urea, especially at high concentrations, is known to interfere with trypsin activity and lead to undesired protein carbamylation. Therefore, there is an antagonistic relationship between improving digestion by unfolding the substrate, and hampering digestion by unfolding the enzyme. Similar antagonistic effects on substrate and enzyme may be observed with additives such as guanidine HCl or detergents.

Exposure to very high pressure levels, in the range of 70-90kpsi, leads to protein denaturation without the need for high concentrations of chaotropes or other additives. Once exposed to these pressure levels, proteins can be subsequently digested at a lower pressure, or even at atmosphere. Unlike denaturation with high urea or other reagents, which remain in the sample and must be removed or diluted before digestion, pressure-denatured samples can be treated in mild buffer alone, or with low concentrations of urea or organic solvent (e.g., N-propanol) which are directly compatible with enzyme activity. Here we show that pretreating IgG at 90kpsi for 20-30 mins at room temperature, significantly improves subsequent digestion by trypsin in both disulfide-intact and reduced IgG preparations. In addition, we demonstrate accelerated trypsin digestion of liver and spleen tissue lysates pre-treated with high pressure.

This approach, of using high pressure to pretreat/denature proteins prior to enzyme digestion, may be beneficial in applications were fragile enzymes require very mild digestion conditions, or where downstream analysis methods require sample preparation in mild, non-denaturing

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reagents. In addition, since brief exposure of tissue lysates to high pressure has been shown to reduce the activity of endogenous enzymes, high pressure pretreatment may be beneficial for proteomic profiling of samples with high levels of endogenous proteolytic activity.

Poster 46: Impact of Three Different Mutations in Ehrlichia Chaffeensis in Altering the Global Gene Expression Patterns

Chandramouli Kondethimmanahalli; Roman Ganta
Kansas State University, Manhattan, KS

Rickettsial pathogen Ehrlichia chaffeensis causes a tick-borne disease, human monocytic ehrlichiosis (HME). Mutations within certain genomic locations of the pathogen aid in understanding the pathogenesis and in developing attenuated vaccines. Our previous studies demonstrated that mutations in three different genomic sites in E. chaffeensis caused variable impacts on their growth and attenuation in vertebrate and tick hosts. Here, we assess the effect of three mutations on the global transcriptional changes using RNA deep-sequencing technology. RNA sequencing aided in detecting 80-96% of the transcripts of E. chaffeensis wildtype and mutant organisms. Mutation in an antiporter gene (ECH_0379) causing attenuation in vertebrate hosts resulted in down regulation of many transcribed genes. Similarly, mutation downstream to ECH_0490 gene, while having minimal impact on pathogen's in vivo growth, also caused up and down regulation of many genes. This mutation caused enhanced expression of genes involved in overcoming the host stress response. ECH_0660 gene mutation causing the pathogen's rapid clearance, which also aided vertebrate hosts in generating a protective response, had a minimal impact on the transcriptome. The transcriptomic data offer novel insights about the impact of mutations on the global gene expression and how they contribute to pathogen's resistance and/or clearance from the host.

Poster 47: Quantitative Analysis of Signaling Pathways using 11plex TMT Reagents and Comprehensive SMOAC Phosphopeptide Enrichment Strategies

Bhavin Patel; Leigh Foster; Jae Choi; Ryan Bomgarden; John Rogers
Thermo Fisher Scientific, Rockford, IL

There is broad interest in quantifying protein phosphorylation alterations in cellular signaling pathways under different conditions. The transient nature and low stoichiometry of phosphorylation and low abundance of many targets makes this challenging. Enrichment is necessary for better detection of the low abundant phosphorylated proteins, while multiplexed quantitation reagents parallelize analysis of several experimental conditions. We have combined SMOAC (Sequential enrichment by Metal Oxide Affinity Chromatography) method with 11 plex Tandem Mass Tag (TMT) isobaric labeling reagents to evaluate changes in phosphorylated proteins expressions under different stimulation conditions.

HeLa cells were grown with 10 different conditions of starvation and/or stimulations (Nocodazole/TPA/ hIGF-1/hEFG/hPDGF-bb/FBS/charcoal stripped FBS) before being subjected to in-solution digestion. Thermo Scientific TMT10plex tags plus a novel TMT11-131C reagent were used to label 0.45mg of HeLa digest for each condition. Five milligram of combined TMT11plex labeled peptides was subjected to Thermo Scientific Pierce Hi-Select^{TM} TiO$_2$ phosphopeptide enrichment kit (PN#A32993) and the TiO$_2$ eluent was saved. TiO$_2$ flow-through and wash fractions were pooled and enriched with the Thermo Scientific Pierce Hi-Select^{TM} Fe-NTA phophopeptide enrichment kit (PN#A32992). Both eluents were combined and fractionated using the Thermo Scientific Pierce™ High pH Reversed-Phase Peptide Fractionation Kit (PN#84868) before LC-MS analysis using Thermo Scientific Orbitrap Fusion instrument. Thermo Scientific Proteome Discoverer 2.1 software was used to localize the phosphorylation sites.

TMT11plex reagents with the SMOAC method allowed comprehensive identification and quantitation of >22,000 phosphopeptides across 11 different conditions. A peptide assay allowed for normalization of peptide amounts and high pH reversed phase fractionation method after phosphopeptide enrichment resulted in higher throughput and proteome depth for profiling changes in phosphopeptide expression. Excellent selectivity and specificity for phosphopeptides were achieved with this improved SMOAC workflow.

This comprehensive phosphopeptide analysis allowed quantitation of phosphorylation changes for thousands of signaling pathways proteins under different stimulation conditions.

Poster 48: Detection of Post-Translational Modifications using Mass Spectrometry

Nathan Zuniga; Marcus Hadfield; Lavender Lin; David Parkins; John Price
Brigham Young University, Provo, UT

Knowledge of disease pathogenesis has gone beyond the systematic mechanisms that cause illness. Advances in proteomics continually demonstrate that imbalances in proteostasis are at the core of pathogenesis. In addition, post-translational modifications also affect the physiological mechanisms in which proteins are involved. Thus, it is important to create diagnostic methods that can detect modified proteins in a patient's proteome. Recent proteomic studies performed on serum samples from Rheumatoid Arthritis patients indicate distinctions in protein melting curves, compared to the group of "healthy" individuals. These results potentially indicate protein modifications at different positions in the amino acid (AA) sequence, including AAs that are normally not available for modification. Using reporter assays developed to modify AA sites, alterations specific AAs, such as tyrosine, tryptophan, and methionine were explored. These modifications were introduced at different degrees of protein folding, and Mass Spectrometry (MS) was used as a potential method to identify protein modifications at the secondary and tertiary level. Combined with the protein denaturation curves, we are researching methods to used MS as a diagnostic method to detect detrimental protein modifications that lead to proteome-related disease.

Poster 49: Adaptation of KALIP for the Development and Prediction of Artificial Peptide Substrates to Monitor FMS-like Tyrosine Kinase 3 (FLT3) Activity

Minervo Perez[1]; W. Andy Tao[3]; Laurie L. Parker[2]
[1]University of Minnesota, Minneapolis, MN; [2]University of Minnesota Twin Cities, Minneapolis, MN; [3]Purdue University, West Lafayette, IN

Acute myeloid leukemia (AML) is an aggressive disease that is characterized by an abnormal level of immature myeloblasts in the blood and bone marrow. FLT3 is a receptor tyrosine kinase that plays an integral role in haematopoiesis, and alteration to this cohesive signaling machinery leads to haematopoietic malignancies including AML. One third of AML diagnoses have gain-of-function mutations in FLT3 that occur within the juxtamembrane and kinase domains. Computational modeling suggests that internal tandem duplication of the juxtamembrane domain or point mutation to aspartic acid 835 alters the protein structure leading to decreased FLT3 inhibitor potency. Currently, few FLT3 substrates and their phosphorylation sites are known, which limits our insight of how FLT3 interacts with signaling proteins under disease conditions. The incorporation and adaptation of the Kinase Assay Linked with Phosphoproteomics (KALIP) technique has allowed the high throughput identification of proteins and sites that are phosphorylated by the FLT3 variants. Incorporation of the identified substrate sequences into the KINATEST-ID pipeline has allowed for the identification of FLT3 and its variants' preferred peptide substrate motifs. Subsequently, a panel of eight candidate sequences were synthesized and assayed against the FLT3 kinase variants to validate them as artificial peptide substrates. Additionally, two candidate sequences were used in an inhibitor dose response ELISA-based assay with known FLT3 inhibitors to demonstrate how the artificial substrates can be used to monitor kinase activity. Ultimately, the generation of artificial peptide substrates, which can be used with high-throughput screening assays, will aid in the search for specific and selective FLT3 inhibitors for WT and mutant forms of the enzyme.

All posters will be displayed Monday and Tuesday.
Each day: even-numbered posters present 1:30-2:15 pm; odd-numbered posters present 2:15-3:00 pm.

Poster 50: Finding Proteins Connected to Rheumatoid Arthritis

Marcus Hadfield[1]; David Parkinson[1]; Stephen Ames[1]; Brad Naylor[1]; Lavender Lin[1]; Colette Quinn[2]; Lee Hansen[1]; John Price[1]
[1]Brigham Young University, Provo, UT; [2]TA Instruments, Draper, UT

Rheumatoid arthritis (RA) is a progressive auto-immune disease characterized by chronic inflammation of the body's joint tissues. Because RA often develops slowly over long periods of time, many patients are not diagnosed until advanced stages of the disease. While treatment for those in advanced stages of the disease is available, many interventions show limited efficacy. In addition, the mechanism for the development of RA remains poorly understood. The current diagnostic procedures, based upon detection of a protein marker (Rheumatoid Factor), are complicated by its low abundance in serum. These factors all contribute to the pressing need for novel strategies in further researching RA.

One such strategy our lab utilized in gaining additional insight into this disease is obtaining isothermal melting curves for patients with and without RA. Analysis of these curves revealed differences in the melting points of serum proteins between study groups. We supplemented this strategy by using mass spectrometry to investigate changes to the in vitro reactivity and in vivo modification of suface amino acids. We found in vivo modifications that seem to occur more at specific sites, in conjuction with changes in surface amino acid reactivity that suggests changes in secondary structure. These post-translational modifications can thus provide valuable insight in explaining why differences exist in our isothermal melting curves. Our current hypothesis is that post translational modifications perturb the structure of some very abundant protein's within the plasma proteome, resulting in differences within isothermal melting curves, and thus serve as an innovative biomarker for diagnosis which may lend insight into the etiology of RA.

Poster 51: MetaMorpheus for Enhanced Global PTM Discovery (G-PTM-D) and Quantitative Proteomics

Stefan Solntsev; Michael Shortreed; Brian Frey; Lloyd Smith
University of Wisconsin, Madison, WI

Correct identification of protein post-translational modifications (PTMs) is crucial to understanding many aspects of protein function in biological processes. G-PTM-D is one of a few tools for global identification and localization of PTMs. We enhanced G-PTM-D in three ways: 1) calibrating spectral files prior to applying G-PTM-D, 2) using multi-notch searches, and 3) enabling identification of co-isolated peptides. These enhancements are demonstrated by identification of numerous types of PTMs, including high-mass modifications such as glycosylations. The changes described in this work lead to a 20% increase in the number of identified modifications and an order of magnitude decrease in search time. The complete workflow is implemented in MetaMorpheus, a freely-available software tool.

Poster 52: Big Data: Ensuring Integrity of Differential Analyzes from Large Scale DIA-MS Experiments

Andrea Matlock[1]; Erin Crowgey[2]; Vidya Venkatraman[1]; Victoria Dardov[1]; Jennifer Van Eyk[1]
[1]Cedars-Sinai Medical Center, Los Angeles, CA; [2]Nemours Children's Health System, Wilmington, DE

Two large scale multi-omic projects underway, NeuroLINCS (http://neurolincs.org/) and Answer ALS (http://answerals.org/), seek to reveal the mechanisms associated with disease onset and progression in motor neuron disorders using patient derived iPSCs and their differentiated motor neuron cultures. We found a subset of samples within the differential analysis incorrectly cluster on a PCA plot due to misalignment of peptide abundances during normalization rather than true biological differences. This become clear when the protein and peptide abundance distributions were plotted before and after MS2 signal normalization. Further insight into the characteristics of the samples was observed in a plot of the number of protein or peptide identifications over MS2 TIC for the entire peptide elution region of the chromatogram. When changes in the technical aspects of the instrumentation shift the signal to peptide abundance curve within a dataset, an alternative method of normalization is required. One valid course of action would be to exclude all samples from the differential analysis that do not align in their peptide abundance distribution after normalization. However, given the quality of the spectral data itself and the fact that these samples only lack depth in the number of peptide identifications above the limit of quantitation, we began to explore alternative methods to correct the discrepancies in the alignment, i.e. normalization, of analyte abundances across a dataset. Here we define the steps taken to assess the quality of each sample within the dataset to ensure the integrity of the downstream differential analysis for which an accurate biological interpretation is dependent.

Poster 53: Complement C3 in Individual Plasma Lipoproteome as a Potential Biomarker of Alzheimer's Disease

Fangying Huang; Danni Li
University of Minnesota, Minneapolis, MN

Alzheimer's disease (AD) is the sixth-leading cause of death in the United States while currently no effective treatment or prevention strategies for AD exist. Although Cerebrospinal Fluid (CSF) and neuroimaging biomarkers are used in research studies to diagnose AD, problems such as safety, costs, and specialized facilities have limited their use clinically. Blood-based biomarkers represent a clinically applicable alternative that would be minimally invasive, cost effective and easily measured in community settings. Plasma lipoproteome is a sum of proteomes, from each plasma lipoprotein very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL). Because of the heterogeneous distribution of proteins in plasma lipoproteins, we hypothesize that plasma lipoproteome, measured in individual plasma lipoproteins, has the potential to increase the accuracy in diagnosing AD compared to that measured in unfractionated plasma. Our study compared AD diagnostic accuracy of plasma lipoproteome by applying targeted protein quantitation with selected reaction monitoring (SRM) method to measure 79 proteins in VLDL, IDL, LDL and HDL (fractionated by sequential gradient ultracentrifugation) and in unfractionated plasma (removed of albumin and IgG by immunoaffinity depletion). In this case-control study of five AD cases and five sex and age-matched controls, the results showed that complement C3, when measured in plasma fractions, had significantly lower p values distinguishing between cases and controls than they did when measured in unfractionated plasma. Our findings suggest that complement C3 could be a potential biomarker of Alzheimer's disease and plasma lipoproteome in individual plasma lipoproteins may substantially increase the accuracy of plasma lipoproteome in diagnosing AD.

Poster 54: Changes in Glycan and Protein Expression in Aging Brain Nigrostriatal Pathway for Adeno-Associated Virus Mediated Gene Therapy In Parkinson's Disease

Rekha Raghunathan[1]; Nicole Polinski[2]; Joshua Klein[1]; John D. Hogan[1]; Chun Shao[1]; Kshitij Khatri[1]; Deborah Leon[1]; Mark McComb[1]; Caryl Sortwell[2]; Joseph Zaia[1]
[1]Boston University, Boston, MA; [2]Michigan State University, Grand Rapids, MI

Introduction:Parkinson's disease is a neurological disorder characterized by the degeneration of dopaminergic neurons in the brain nigrostriatal pathway.Current treatments provide only symptomatic relief.Gene therapy has the potential to restore lost neuronal function.Adeno-associated viruses are vectors of choice in gene therapy due to their well-characterized safety and efficacy profiles.Significantly, all primary receptors of the viral serotypes used in gene therapy are glycans.A conundrum is that aging brain has demonstrated low efficacy in viral vector-mediated gene delivery while Parkinson's disease primarily occurs with age.We hypothesize that the age-related changes in glycan receptors of heparan sulfate (HS) proteoglycan, N-glycans with terminal galactose and protein co-receptors of the virus results in poor binding, affecting gene delivery.Methods:We used fresh-frozen 10 μm coronal tissue sections from young(3 month) and aged(20 month) rat brain.We released glycans and peptides by treating the tissue surface with glycosidase enzymes followed by trypsin.Glycan classes were analyzed using negative polarity HILIC-MS and tryptic peptides using reversed phase LC-MS.All analyses were performed using Waters NanoAcquity chromatographs interfaced with a Thermo-Fisher Q Exactive HF or Orbitrap-XL mass spectrometer.Results:We detected an average of

POSTER ABSTRACTS

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1250 proteins in the striatum and 1375 in the substantia nigra.We observed brain region-specific changes in striatum versus substantia-nigra with age.In particular, the receptor for the virus AAV2 was lower in aged compared to young animals.Further studies in humans will enable mass spectrometry guided design of viral vectors for success in gene therapy.

Poster 55: A Multi-omics Analysis of Lifespan Extension in Mice Through Short-term Calorie Restriction Reveals Mechanisms of Post-Transcriptional Proteome Regulation

Richard Carson; Bradley Naylor; John Price
Brigham Young University, Provo, UT

While calorie restriction is one of the best-studied methods for extending longevity in laboratory animals, the mechanism remains incompletely understood. Our lab undertook a multi-omics approach to study calorie restriction in a large-scale metabolic labeling experiment using adult mice. Cohorts of mice were subjected to calorie restriction under both low and high protein conditions in order to provide another axis of dietary signaling for study. We then combined RNA-Seq, RNA kinetics, quantitative proteomics, and kinetic proteomics in an unbiased shotgun application to liver tissue samples. Analysis of our data revealed that the majority of proteome perturbations from calorie restriction resulted from changes in post-transcriptional regulation. An overall slowing of protein synthesis consistent with previous literature was observed, with evidence suggesting that alterations in tRNA metabolism are responsible. Specifically, aminoacyl tRNA synthetase metabolism was found to differ significantly between calorie restriction and *ad libitum* diets, indicating changes in the available tRNA pool for protein synthesis. We hypothesize that, by decreasing the pool of charged tRNA available for translation, the cell simultaneously lowers its energy consumption and protein synthetic burden, allowing an improvement of protein quality in the cell through multiple mechanisms. These alterations should ultimately result in the improved cellular and organismal health observed under calorie restriction.

Poster 56: Investigating Mechanisms which Support Improved Proteostasis

John Price
Brigham Young University, Provo, UT

Aging is poorly understood, yet is the number one risk factor for disease and death. One of the few consistently observed age-dependent biochemical processes is that each cell of our body loses the ability to maintain the quality and concentration of the 1000's of individual proteins within the cell (proteostasis). Slowing this loss of quality, slows the aging rate and extends lifespan. The major cellular activities controlling protein quality are synthesis, folding, and degradation. We and others have shown that conditions which reduce the aging rate consistently reduce synthesis and degradation (turnover) of most proteins across the proteome. This raises some important questions about how slower turnover could improve quality. For example, one might expect that slower turnover could result in accumulation of damaged proteins, which reduces proteome quality. We have previously shown that slower general protein synthesis is associated with greater exchange of ribosomal proteins between the cytosol and the assembled ribosome structure. Therefore, slower proteome scale turnover might promote more accurate function of the ribosome during protein synthesis by removing damaged subunits. Here, I will present our recent work investigating the signal-specific controls of proteome-wide turnover and how synergistic changes in the proteostatic machinery effect the 'quality' of the proteome.

Poster 57: Quantification of the Proteins in Pathways Affected by Autophagy Inhibition in Rat Myoblasts

Edgar Arriaga; Katherine Muratore; Omprakash Nacham
University of Minnesota, Minneapolis, Minnesota

Autophagy is a highly conserved intercellular self-defense and recycling mechanism aims to prevent the toxic accumulation of cellular components (e.g. damaged organelles, bacteria, and misfolded protein aggregates) via lysosome mediated degradation. Although autophagy plays a crucial role in regulating the metabolic homeostasis, impaired autophagy often results in human pathogenesis and age-related diseases, including sarcopenia (a skeletal muscle disorder, marked by low mass of skeletal muscle in elderly individuals). The investigation of autophagy with MS-based proteomics can potentially provide insights to the complex intercellular signaling pathways that are implicated in defective autophagy. Previously, we have applied untargeted MS to evaluate the changes in protein abundance of autophagy deficient L6 rat myoblasts. Combining the results of three biological replicates, a total of 1898 non-redundant proteins which are involved in a variety of cellular pathways were determined. Stable isotope labeling by amino acids in cell culture (SILAC) technique was employed to assess the relative change in protein abundances of control and autophagy deficient cells. The obtained MS results were subjected to ingenuity pathway analysis (IPA), suggesting that the cholesterol biosynthesis pathway was represented by proteins with altered abundance after autophagy inhibition. To validate the findings of untargeted MS-based proteomics of impaired autophagy, we have selected 4 unique proteins which are involved in cholesterol biosynthesis pathway and attempted to determine their relative abundances via targeted MS technique, using triple quadruple mass spectrometer. A systematic optimization of mass spectrometric parameters was undertaken and subsequently important parameters that involve in multiple reaction monitoring (MRM) were optimized for selected peptides. Under optimized conditions, the developed method demonstrated good linear range as well as better intra-day reproducibility. This enabled us to employ the developed method to examine the abundance of endogenous peptide samples.

Poster 58: Investigation of the Effect of Reduced Synthesis and Degradation on in vivo Protein Stability

Lavender Hsien-Jung Lin; Marcus Hadfield; Nathan Zuniga; John C Price
Brigham Young University, Provo, Utah

As medicine and technology progress, the mortality rate has been greatly decreased. However, longevity is not the only thing people want from life. People want to live a full disease free life without the handicaps of age. Aging itself is then the next factor we need to overcome. It has been shown that calorie restriction slows down the aging process. Our lab had showed that the slowing down of dietary restriction (DR) is associated with the protein turnover rate in the cell. Protein folding not only contributes to protein turnover rate, but also directly impacts protein function and aggregation. Understanding the protein folding changes during aging and DR can help complete the story of how protein turnover effect (slowdown) aging. We are building on previous work by Fitzgerald and Colleagues to measure folding stability in vivo, by modifying surface amino acids. Here we show which amino acids are the best reporters and the change (difference) of protein folding free energies due to changes in aging rate.

Poster 59: The Effects of Apolipoprotein E Isoforms on the Metabolism of Lipid and Protein in Alzheimer's Disease Related Metabolic Pathways

Russell Denton; Joseph Creery; Joshua Chamberlain; John Price
BYU, Provo, UT

A strong genetic predictor of Alzheimer's disease (AD) risk is the lipid transporter Apolipoprotein E (ApoE). Relative to the ApoE isoform 3, isoform 2 provides protection against AD, but isoform 4 confers an increased risk when considering both their transport function and their effects on transcription. Differences in cognition and memory are also apparent between phenotypes, even in young healthy subjects. Importantly, there is also a functional difference in lipid, and in some cases protein, transport between these isoforms. Thus, a leading hypothesis is that the transport of lipid and protein creates a bias in healthy subject's brain activity and sensitizes or protects the brains of these subjects against different stresses. In support of this, lifestyle choices like exercise or diet modify the risk of AD because both affect lipid availability and their metabolism. The complex interplay of genetic and environmental risk factors indicates that the preexisting metabolic condition of the brain is the key initiating variable. Understanding this interlay is crucial to finding a viable method for treating or preventing AD. We are considering which AD related metabolic pathways are most effected by the ApoE isoforms. We are using kinetic and quantitative methods to compare protein and lipid metabolism observed in the brains of ApoE isoform variant mice. The goal is to

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identify metabolic biases which protect against or predispose individuals to AD.

Poster 60: Phosphoproteomic Investigation of the Mechanism of Calorie Restriction Attenuation by Dietary Protein Signaling

Joshua Mcphie[1]; Marco Hadisurya[2]; Aaron Moss[3]; Richard Carson[1]; John Price[1]

[1]Brigham Young University, Provo, UT; [2]Brigham Young University - Hawaii, Laie, HI; [3]Brigham Young University - Idaho, Rexburg, ID

Calorie restriction has been shown to significantly increase lifespan and provide health benefits associated with the prevention of aging-related diseases such as cancer and Alzheimer's disease. Interestingly, when dietary protein is also increased, the benefits of calorie restriction are largely reduced to baseline health. Even though calorie restriction has been studied for decades, the mechanisms behind it remain unknown. Likewise, although several hypotheses have been proposed, none have yet been able to explain why dietary protein inhibits calorie restriction benefits. The kinase mTOR is a nutrient sensor, capable of recognizing both cell stress caused by lack of caloric intake as well as dietary protein. We hypothesized that dietary protein intake and cell stress caused by lack of caloric intake would have opposite effects on these proteins therefore causing a deviation from normal activity. Using mice that had undergone calorie restriction in low and high protein conditions, we determined the relative concentration of a modified downstream target of mTOR called rpS6 via western blotting. By comparing the levels of modified rpS6 under low and high protein conditions, we inferred the relative activity of mTOR. Our results showed a higher prevalence of phosphorylated rpS6 in the low protein diet. Unexpectedly, this implies that mTOR was more active in the low protein diet rather than the high protein diet, which was contrary to what we originally supposed. To further investigate our findings, we determined the relative activity of mTOR through a broader inspection of its substrates via phosphoproteomics. We also used this approach to evaluate how the regulatory mechanisms of mTOR interact in the different diets. We here present the results of our phosphoproteomics assays. This information provides a greater insight into calorie restriction and our understanding of the mechanisms that govern it.

Poster 61: Dynamics of Proteomics Changes in Presence of ER Stress in ALS Relevant Mouse Neuronal Model

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[1]Center for Genomics and System biology, NYU, New York, NY; [2]Department of Biology, NYU, New York, NY

During environmental fluctuations, the relative contributions of transcriptional and translational regulation modulate the overall proteomic changes that maintain cellular homeostasis. Stress response pathways play a major role in controlling the transcriptional, translational and posttranslational processes to maintain a specific cellular state. A number of pathophysiological insults leading to accumulation of misfolded or unfolded proteins inside endoplasmic reticulum (ER) cause ER stress. In response to ER stress, cells activate very specific signal events collectively called Unfolded Protein Response (UPR). The UPR of ER is a multi-layered process that occurs over multiple stages and co-ordinates a variety of pathways, many of which leading to dysregulation of proteostasis. ER stress has been implicated to different pathophysiological conditions such as neurodegenerative diseases, diabetes etc. Amyotrophic Lateral Sclerosis (ALS) is an adult-onset fatal neurodegenerative disease with a selective, highly progressive loss of both spinal and upper motor neurons (Hardiman et al. 2011). Inability of the cells to respond appropriately to ER stress is thought to be one of the major reasons for the ALS progression. The dynamics of proteomic profiles over time in presence of ER stress condition is poorly understood. For this, a novel embryonic stem cell (ESC) derived ALS relevant system has been used to decipher the relation of transcriptional and translational changes in presence of ER stress. We have established a condition where ER stress shows differential vulnerability of stem cell derived cranial (cMN-NIP) and spinal motor (sMN-NIL) neurons. Using TMT based quantitative proteomics, we investigated the changes over time in presence of ER stress. Our analysis shows high expression of proteins from proteasomal and ubiquitin pathways cMN-NIP. This could probably help to remove toxic misfolded proteins efficiently in cMN-NIP than sMN-NIL resulting in differential vulnerability of these two types of neurons during ALS and open up the possibility of therapeutic strategies.

Poster 62: Utilizing Multi-Omic Network Analysis to Rapidly Assess the Biological Mechanism of Action of Methotrexate

Nicole Muszynski[1]; Melissa Farrow[2]; Danielle Gutierrez[1]; Stacy Sherrod[1]; Eric Spivey[1]; Tina Tsui[1]; James Pino[1]; Michael Ripperger[1]; Randi Gant-Branum[1]; Matthew Hensen[2]; Kaycei Moton-Melancon[2]; Jeremy Norris[1]; D. Borden Lacy[2]; John McLean[1]; Eric Skaar[2]; Carlos Lopez[1]; John Wikswo[1]; Richard Caprioli[1]

[1]Vanderbilt University, Nashville, Tennessee; [2]Vanderbilt University Medical Center, Nashville, TN

The widespread application of systems biology tools to characterize biological mechanism of action (MOA) has been limited by the time required to discern biological relevance in large, complex datasets. To address this limitation, we developed a platform that allows us to combine and analyze measurements from transcriptomics, proteomics, and metabolomics assays enabling de novo comprehensive characterization of toxic agent MOA in 30 days or less. On day one, we were provided with an unknown, white powder. We then used nuclear magnetic resonance spectroscopy (NMR) and high resolution mass spectrometry to decipher the chemical makeup of the agent. These analyses led us to characterize the toxin as Methotrexate (MTX), an S-phase inhibitor primarily used in treatment of cancer and rheumatoid arthritis. Data from all platforms were integrated and parsed for significantly changed, unique species for construction of the global mechanism. Global association and networking analyses were validated experimentally. We identified known MTX-induced molecular changes and additional molecular insights not previously recognized. Using this integrative approach, we were able to rapidly identify and add molecular resolution to the MOA by confirming the existing species in the literature as well as expanding molecular species contributing to the MOA by four fold. Our highly iterative, integrated, cross-platform approach to MOA has been immensely successful in identifying MOA for small molecule intoxicants. Current and future work involves exposure to bacterial toxins and infection with live bacteria. By applying systems biology tools to big data collected from our high-throughput multi-omics platform, we can characterize and significantly expand upon the mechanisms of action of chemical and biological agents in 30 days or less.

Poster 63: Large Datasets and You: Using Proteomics to Design a Biosensor

John B[1]; Minervo Perez[2]; Laurie L. Parker[3]

[1]UMN, Minneapolis, MN; [2]University of Minnesota, Minneapolis, MN; [3]University of Minnesota Twin Cities, Minneapolis, MN

The development of novel peptide substrates has become a powerful line of attack in the creation and testing of kinase inhibitors. Our lab hopes to use artificial substrates which are specific for individual kinases to study these kinases and kinase inhibitors in vivo instead of just in vitro. To create highly specific kinase biosensors our lab created the KINATEST-ID workflow, which used manual curation of published phosphorylated sequences to mathematically determine a kinase's most preferred sequence. Two difficulties with this process are that the dataset available can be small, especially with poorly known kinases, and the process is labor intensive. An additional tool was thus added called KALIP (Kinase Assay LInked with Phosphoproteomics) which creates a large array of random sequences phosphorylated by a kinase of interest, can be used in the KINATEST-ID workbook. This however created a problem: using small datasets and a workbook is labor intensive, using big datasets and a workbook is even moreso. Thus I created an automated script which could take KALIP data and perform the KINATEST-ID math on it in order to discover novel, highly specific biosensors. Ancillary scripts were also developed to help process the KALIP datasets for use in KINATEST-ID.

POSTER ABSTRACTS

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Poster 64: Determining Peptide Substrates for Bruton's Tyrosine Kinase Biosensors via the KALIP Process

Lindsay Breidenbach[1]; Minervo Perez[2]; John B[3]; Laurie L. Parker[4]
[1]University of Minnesota, Maple Grove, MN; [2]University of Minnesota, Minneapolis, MN; [3]UMN, Minneapolis, MN; [4]University of Minnesota Twin Cities, Minneapolis, MN

Chronic Lymphocytic Leukemia (CLL) causes cancerous growth in mature B lymphocytes and B cells. B-cell antigen receptor (BCR) creates pro-cancer microenvironments by secreting cytokines, signaling growth for nearby cells, and promoting cells adhesion. BTK is a crucial downstream kinase to BCR, involved directly in adhesion and found exclusively in B-cells. Because this protein is specific to B cells and play such an important role in CLL proliferation, it is a prime target for inhibition. In fact, inhibitors for BTK, like Ibrutinib, already succeeded in slowing disease progression. However, CLL cells mutate quickly and overcome these drugs. Terbium biosensors chelate only in the presence of phosphate. Thus, terbium biosensors can determine how effectively a kinase will be inhibited in a high-throughput setting. A substrate that BTK specifically phosphorylates is needed for this biosensor to target and measure it's activity, however no substrates are currently known. Kinase Assay Linked with Phosphoproteomics (KALIP) will be employed to find BTK substrates. This method exposes multiple peptides to the desired kinase. The resulting phosphopeptides are pulled out of solution with magnetic phosphopeptide enrichment beads. The phosphopeptides are then analyzed on the mass spectrometer. From here the KINATEST-ID pipeline analyzes the KALIP data to find which amino acids at what given position would form the best substrate. It also compares how likely other kinases can phosphorylate the peptides. This is to predict a substrate more exclusive to BTK. Substrates predicted from KINATEST-ID will be created and tested. Overall, using the KALIP and KINATEST-ID processes, a substrate for a terbium biosensor can be found for BTK. This creates a high-throughput assay for testing BTK inhibitors.

Poster 65: Application for Nanofluidic Devices Towards Single-Cell Proteomics: A Study with Xenopus Laevis Embryos

Anumita Saha-Shah[1]; Melody Esmaeili[1]; Peter Klein[1]; Benjamin A. Garcia[2]
[1]University of Pennsylvania, Philadelphia, Pennsylvania; [2]University of Pennsylvania School of Medicine, Philadelphia, PA

Nanofluidic technologies have enabled mass spectrometric analysis of single cells and study of inter-cellular heterogeneity. However, most of these technologies have been applied to metabolomics and lipidomic studies because lipids and small molecules are highly ionizable and predominate the mass spectra. Here, we have combined a micropipette (pulled glass capillary) based sample collection strategy with offline sample preparation and LC-MS/MS to analyze proteins through bottom-up proteomic strategy. Micropipettes (nanofluidic sample collection devices) are fabricated in-house through commercially available pipette puller and sample collection is controlled pneumatically. Post-sampling the samples are delivered into a small tube and prepared for LC-MS/MS. This strategy has enabled analysis of > 1200 proteins per Xenopus laevis embryonic cell. Xenopus laevis embryos were chosen as a model system for this study because 1) cell sizes are large and easy to manipulate, 2) the embryos divide after fertilization without increase in size, as a result, cell sizes vary from 1.2 mm to < 100 µm during development and serves as a good system for sample limited studies such as single cell analysis, 3) enables study of inter-cellular heterogeneity and relate it to development of various parts of a developed organism. Preliminary studies have shown that at developmental stage 2, more than 60 proteins are differentially expressed in dorsal versus ventral cells. The number of differentially expressed proteins increased to > 80 at stage 3 of development. Investigations are currently underway to look at intercellular heterogeneity among cells at later stages of development but essentially this tool will enable study of cellular differentiation during development and relate it to fate map of a developed organism.

Poster 66: Proteome-wide Structure-Based Function Prediction Reveals Roles of Proteins Responsible for *E. coli* Fitness

Chengxin Zhang; Mehdi Rahimpour; Peter Freddolino; Yang Zhang
University of Michigan, Ann Arbor, MI

We have developed a protein function prediction pipeline, COFACTOR, which can be applied for proteome-wide structure-based function annotation. Starting from a query sequence, the pipeline first uses I-TASSER and QUARK to generate a structure model of the protein, which is then used as a probe to detect functional analogs from the BioLiP structure-function database by global-fold and local-motif based structure alignments. Functional insights are obtained by combining annotations from these analogs with information deduced from sequence homologs and protein-protein interaction networks. This protocol has been applied to predict the structure and function of all 4,306 proteins from the *E. coli* K12 proteome. In addition to recapitulating known function on 1,244 well-annotated proteins, the pipeline provided informative annotation for a significant fraction of uncharacterized proteins in *E. coli* proteome, including many non-homologous targets whose function could not be obtained from the traditional sequence-homology based approaches. Among them, three uncharacterized proteins, yaiP, yhaJ, and ymgC, which are responsible for polysaccharide synthesis regulation, antibiotic resistance, and biofilm formation respectively, were experimentally validated and found highly consistent with the COFACTOR predictions.

Poster 67: Development of Hydrogen-Deuterium Exchange Methodology Coupled to Top- and Middle-Down Mass spectrometry Enables High-Resolution Analysis of Histone Dynamics

Kelly Karch; Mariel Coradin; ZhongYuan Kan; Ben Black; Benjamin Garcia
University of Pennsylvania School of Medicine, Philadelphia, PA

Nucleosomes, as the smallest repeating unit of chromatin, are critical for many nuclear processes. Nucleosomes are comprised of 147 base pairs of DNA wrapped around two copies of H2A, H2B, H3, and H4. Nucleosome dynamics are difficult to study, especially the N-terminal tail domains that are crucial for their function. Hydrogen-deuterium exchange (HDX) coupled to bottom-up mass spectrometry (BU-MS) has provided some insight, but fails to provide detailed analysis of tail domains due to the length of the tail peptides. Here, we develop HDX coupled to top-down (TD) and middle-down (MD) MS methodology for analysis of histone proteins to enable the first detailed view of histone dynamics.

For TD-HDX experiments, we analyzed H4 monomers and (H3/H4) 2 tetramers followed by TD-MS with ETD on our Thermo Fusion instrument. We achieved extremely high resolution across the entire sequence of H4, highlighting the power of this method to obtain very detailed information. However, this method cannot be used for very complex samples. We therefore optimized MD-HDX methodology to accommodate (H3/H4)^2 tetramers and intact nucleosomes on our Thermo Fusion instrument, representing the first analysis of a protein-DNA complex with MD-HDX. The tails were fragmented with ETD, and we were able to achieve highly resolved exchange information, up to single residue resolution, for each tail. We found that each tail exhibits increased protection, albeit to different degrees, upon incorporation into the nucleosome, which has important biological implications. Data was analyzed using HDExaminer software and ExMS2, which we modified to accommodate ETD data. The TD- and MD-HDX results are highly similar and match previous BU-HDX and crystallography data, indicating that both methods are highly accurate and reproducible. These methods can be easily adapted to accommodate a variety of proteins and therefore represent a powerful and versatile tool to study protein dynamics in solution in unprecedented detail.

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Poster 68: Antibody-antigen Interaction Characterization using Stopped Flow Assisted Hydrogen Deuterium Exchange Mass Spectrometry

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Antibody-antigen interaction is the fundamental reaction of immune system. The clear characterization of paratope/epitope of an immune complex can help the understanding of immune response and possibly the mechanisms of related diseases. Hydrogen deuterium exchange mass spectrometry (HDX-MS) has become a promising technique for studying structures of proteins. However, traditional HDX-MS suffers from poor reproducibility due to back exchange of deuterium and complicated protein complex structures. In our study, we introduced the stopped flow device (bio logic SFM 400) which can provide ultra-fast sample mixing and precise reaction time control (ms level) to improve the reproducibility of HDX-MS.

It is important to find an optimal time where both the antigen and antibody are not fully deuterated but have enough deuterium to locate the binding sites, thus, we first optimized the HDX reaction time for proteins of different sizes. After HDX, the immobilized pepsin beads were used to digest the samples. The digestion conditions were evaluated for efficient digestion and to prevent back exchanges during the digestion. To further minimize the back exchange, the LC-MS buffers, connecting capillaries, and nano-LC column were kept on ice. To evaluate the platform, protective antigen (PA), anti-PA, and their complex were deuterated with the optimal reaction time and digested for LC-MS /MS analysis. Samples in H_2O other than D_2O also underwent the same process as references. A mass error based matching algorism was developed to locate the binding site of the immune complex. The reference samples were also used for the identification of the peptides. The binding site was well characterized with highly reproducible results from three parallel experiments.

To summary, we have developed a stopped flow assisted hydrogen deuterium exchange mass spectrometry platform for charactering antibody-antigen interaction and successfully applied to the characterization of the binding site of PA and anti-PA complex.

Poster 69: Expanding Proteoform Identifications and Constructing Proteoform families in Top-Down Proteomics using Proteoform Suite

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Many proteoforms, the defined forms of a protein with specific sequences of amino acids and localized post-translational modifications, are detected in the precursor scan (MS1) but are not selected for fragmentation by the instrument. Therefore, they remain unidentified in a typical top-down proteomics workflow. Our laboratory has developed the open source software program Proteoform Suite to analyze MS1-only intact proteoform data. We adapted it to provide identifications of proteoform masses in precursor MS1 spectra of top-down data, supplementing the top-down identifications obtained using the MS2 fragmentation data. Proteoform Suite performs mass calibration using high-scoring top-down identifications and identifies additional proteoforms using calibrated, accurate intact masses. Proteoform families, the set of proteoforms from a given gene, are constructed and visualized from proteoforms identified by both top-down and intact-mass analysis. Using this strategy, we constructed proteoform families and identified 1861 proteoforms in yeast lysate, yielding an approximately 40% increase over the original 1291 proteoform identifications observed using traditional top-down analysis alone.

Poster 70: Development of A High-Performance Sheathless CZE-MS Interface for Top-Down Proteomics

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Recent developments in protein separation and mass spectrometry (MS) instrumentation have paved the way toward high throughput top-down proteomics. Specifically, capillary zone electrophoresis (CZE) has become a promising separation technique because of its ultrahigh sensitivity and high separation power (i.e., the theoretical plate number of CZE is 50,000~500,000). Sheath-flow interfaces and sheathless interfaces have been developed to couple CZE and MS for high-throughput proteomics. Sheath-flow interfaces provide the feasibility for post column chemistry but the sensitivity is largely depended on the fine control of the relative position between the outer tip and inner capillary. On the other hand, sheathless interfaces are easy to setup without any dilution effect, thus providing higher sensitivity. However it is relatively unstable.

Here we presented a novel sheathless interface of CZE/MS using a two-step hydrofluoric acid (HF) based etching process. The high resolution inverted microscope was applied to monitor the etching process. The first step etching was applied on the capillary tip to get a porous segment for the efficient and stable electric contact. The surface porous degree measured under the microscope is calculated and correlated with the measured current to select the optimal etching time. One interesting observation is that the emitter tip after the first step etching is flared with larger inner diameter at the end, which may cause the loss of sensitivity during the ESI process. The second step etching is to generate the non-flared emitter for improving the sensitivity as well as the intermitter reproducibility. As a result, we can routinely detect standard intact proteins such as Cytochrome C at low fmole detection limit. We further applied it to analyze complex samples such as intact E. coli cell lysate. Our results also suggested that the interface is less sensitive to the relative position between the outer tip and inner capillary.

Poster 71: Finding a Needle in a Haystack: Novel Top-Down Proteomics Tools for Serum Antibody Analysis

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Autoimmune disease is a leading cause of death and disability, affecting more than 23.5 million Americans, which are of chronic conditions with no cure. Thus, detecting autoimmune disease at an early stage is crucial for effective treatment and disease management to slow down disease progression and prevent irreversible organ damage. In many autoimmune diseases, serum disease-specific autoantibodies are produced by B cells in response to specific soluble auto-antigens, starting at a very early stage in disease development, thus holding a great potential as biomarkers for autoimmune disease diagnosis.

In this study, we established a high-throughput, sensitive, intact serum autoantibody analysis platform based on the optimization of a one dimensional ultra-high-pressure liquid chromatography high-resolution top-down mass spectrometry platform (1D UPLC-TD-HRMS). Combined with our customized sequencing software, this approach has been successfully applied to a 12 standard monoclonal antibody Fab mixture, demonstrating the feasibility to separate and sequence intact antibodies with high sequence coverage and high sensitivity. We then applied the optimized platform to characterize potential serum antibody based biomarkers in longitudinal SLE patient samples against healthy control samples, showing that 80+ dominant antibodies are only observed in SLE samples, which is the first top-down demonstration of serum antibody pool analysis. Among them, a potential antibody biomarker found in SLE serum samples was confirmed that strongly bound to SmD, a known SLE antigen. Thus, our proposed approach holds great promise for discovering novel serum antibody biomarkers that are of interest for diagnosis, prognosis, drug targets, and for our understanding of the various disease processes.

Poster 72: Improved Protein Sequence Analysis by 21 Tesla FT-ICR MS/MS and Advanced Mass Spectral Interpretation

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Intact protein sequence analysis is complicated by incomplete fragmentation, slow spectral acquisition rate, low S:N, and high data

POSTER ABSTRACTS

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complexity. Spectra typically contain many overlapping fragments that are difficult to resolve, deconvolve, and interpret, which typically requires spectral averaging to improve S:N and sequence coverage. Development of activation methods that achieve extensive fragmentation (e.g., EThcD, AI-ETD, UVPD) has been an active research area in top-down proteomics. More sequence-informative fragments are generated, but are we prepared to fully harness the data our instruments are capable of generating? The NHMFL 21 T FT-ICR mass spectrometer currently provides the highest data quality for top-down proteomics. However, the improved data quality is not fully reported by current analysis software. As this software was designed prior to the high resolution and high mass accuracy data now readily achievable at 21 T, algorithms must be revised or replaced by new methodologies to reap the full benefit of such state-of-the-art mass spectrometers . Here, we will discuss a single, fully annotated, ETD MS/MS spectrum of intact bovine CAII (29 kDa) in unparalleled detail. The spectrum has been exhaustively manually interpreted, and the results were compared to deconvolution with Xtract or Thrash with fragments matched by ProSight Lite. Manual examination of the data accounts for >90% of the total ion current observed in the spectrum. Conversely, we found that current software algorithms report inflated mass error, monoisotopic misassignments, and an inaccurate account (e.g. 5% fragments explained) of the total ion current within the spectrum. We will provide specific examples of software misinterpretation, define what is and is not currently possible within a given LC MS/MS analysis, and offer solutions moving forward (e.g. ion-ion proton transfer reactions coupled with ETD improve sequence coverage following Xtract deconvolution/ProSight Lite from 32% to 52% from 50 summed transients for a 50 kDa protein).

Poster 73: Examination of the Specific Binding of Cytochrome C On MPA-Coated Gold Nanoparticles via Protein Footprinting

Emily Tollefson[1]; Xi Zhang[2]; Nikita Rozanov[3]; Caley Allen[3]; Rigoberto Hernandez[3]; Catherine Murphy[2]; Erin Carlson[1]
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The rapidly growing field of nanotechnology centers on nanomaterials, which possess size-dependent properties not achievable with their larger counterparts. Increasing, nanoparticles are being utilized in a wide variety of applications, such as energy storage and bioimaging. Upon contact with the environment, nanoparticles immediately form a corona, which is composed of proteins and other biomolecules. The components of the corona significantly impacts the biological and ecology fate of a nanoparticle. Therefore, the assessment of, and ultimately the ability to predict, the biomolecule properties associated with nanoparticle interactions is of particular importance to the field of nanotechnology. This work will present the application of protein footprinting to examine the specific binding sites of the model system cytochrome c and 4 nm MPA-coated gold nanoparticles. Protein footprinting examines protein interactions by labeling solvent accessible residues of the protein alone and complexed with nanoparticles. Residues involved in the protein-nanoparticle interactions are no longer exposed, and therefore are unlabeled; these specific differentially labeled amino acids are identified by comparative peptide mapping via LC-MS/MS. In collaboration with computational chemists in the Center of Sustainable Nanotechnology, we will address the predictive power of computational systems in relation to experimental data.

Poster 74: Tumor-specific HLA-Peptide Identification for Cancer Immunotherapy using Accurate Epitope Prediction and Targeted Mass Spectrometry

Jennifer Busby[1]; Michele Busby[1]; Tyler Murphy[1]; Brendan Bulik-Sullivan[1]; Matthew Davis[1]; Lauren Young[1]; Andrew Clark[1]; Fujiko Duke[1]; Firaz Mohideen[1]; Corinne Gustafson[2]; Assunta De Rienzo[2]; William G. Richards[2]; Nhien T. Dao[2]; Hyeong R. Kim[3]; Jamie E. Anderson[2]; Chang-Min Choi[3]; Vincent De Montpreville[4]; Se Jin Jang[3]; Olaf Mercier[4]; Raphael Bueno[2]; Elie Fadel[4]; Joshua Francis[1]; Roman Yelensky[1]
[1]Gritstone Oncology, Cambridge, MA; [2]Division of Thoracic Surgery and Lung Center, BWH, Boston, MA; [3]Asan Medical Center, Seoul, Korea; [4]Centre Chirurgical Marie Lannelongue, Le Plessis-Robinson, France

A key goal in immuno-oncology is the identification of tumor-specific antigens recognized by CD8 T cells. Immune modulators such as PD-1 inhibitors indirectly promote T-cell attack against such HLA-peptide antigens. Other potent therapeutic classes (adoptive cell therapy, bi-specific mAb) also require tumor-specific HLA-peptide targets, which are known for only a minority of patients with solid cancers today.

We developed a novel approach for tumor-specific HLA-peptide target identification comprising three steps: (1) comprehensive identification of functionally tumor-specific genes by analysis of large RNASeq datasets of tumor and normal tissues; (2) high specificity prediction of candidate epitopes for the most common HLA alleles using a deep learning model of antigen presentation; and (3) epitope validation in human tumor samples using targeted MS. We applied the method to 50 lung tumor samples, assaying 1691 predicted epitopes for 51 HLA alleles across 211 tumor-specific genes. These included both well-known antigens (e.g., MAGE family) and uncharacterized genes. Aliquots of the same samples were analyzing using the standard DDA method to compare the breadth of epitopes obtained.

600 epitopes putatively presented by 46 HLA alleles in 140 genes were detected by targeted MS. These included both novel tumor-specific HLA-peptide targets in indications where few are known, as well as new epitopes for additional HLA alleles in well-studied genes (e.g., 20 epitopes in CT83, expressed in 40% of lung cancer patients). In contrast, DDA of the same samples yielded only 42 epitopes in 29 of the 211 genes at q<0.01, highlighting the large possible sensitivity gain of our approach.

Conclusions: We present an integrated genomic, informatic and proteomic strategy for tumor specific HLA-peptide identification and demonstrate that it significantly outperforms prior approaches, enabling broad target discovery for cancer immunotherapy.

Poster 75: Integrated Proteogenomic Analyses Reveal Extensive Tumor Heterogeneity and Validate Expression of Somatic Mutations in Lung Adenocarcinoma

Xu Zhang[1]; Paul Rudnick[2]; Shaojian Gao[1]; Shivangi Awasthi[1]; David Fenyo[3]; Udayan Guha[1]
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Tumor heterogeneity affects targeted treatment response in most cancers, including lung cancer, the commonest cause of cancer death in both men and women. We undertook a comprehensive analysis at the level of somatic variants, copy number, transcriptome, proteome and phosphoproteome on sequentially acquired lung and lymph node metastatic sites from an African American never-smoker lung adenocarcinoma patient who had survived with metastatic disease for over seven years while being treated with single or combination HER2-directed therapies. Whole genome sequencing (WGS) revealed unprecedented tumor heterogeneity between the lung and lymph node metastatic sites. Only 1% of somatic variants were common between the two sites. Here, we report how mass spectrometry-based quantitative proteomics complements the genomics analysis to reveal the extent of proteo-genomic heterogeneity and provides direct evidence of expressed somatic or germline variants. We employed "Super-SILAC" and TMT quantitative strategies to quantify the global proteome and phosphoproteome of the lung (L), two metastatic lymph nodes (LN1, LN2) acquired more than two years apart and five lymph nodes (MET1-5) obtained at autopsy. Patient-specific databases were built incorporating all somatic variants identified by WGS to interrogate the mass spectrometry data and an extensive validation pipeline was built for confirmation of variant peptides. We identified around 5000 proteins and significant heterogeneity was observed in global protein expression between the three sites. Interrogation of our patient-specific database identified 78 and 23 mutant peptides from the "super-SILAC" and TMT experiments, respectively. We further validated 360 spectra corresponding to 55 germline and 6 somatic variants. MRM assays were developed to detect and quantify the two somatic mutant peptides, CDK12-G879V (LADFVLAR) and FASN-R1439Q

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(GILADEDSSQPVWLK) that were detected in the lung and LN1, respectively. CRISPR-Cas9-mediated knockdown of CDK12 increased chemotherapy sensitivity in lung adenocarcinoma cells, suggesting a nonfunctional mutant CDK12-G879V may increase responsiveness to chemotherapy in lung adenocarcinoma.

Poster 76: Using HUPO Proteomics Standards With Software Inputs and Outputs for Galaxy-based Multi-Omic Informatics

Thomas Mcgowan; James Johnson; Subina Mehta; Praveen Kumar; Pratik Jagtap; Tim Griffin
University of Minnesota, Minneapolis, MN

The Galaxy-P project is developing tools that aggregate, process and visualize multi-omic data. Each of the Galaxy tools can process variously formatted and pre-processed files-- from proprietary binary files to text files adhering to standards created by time and habit rather than deliberation. We have created the Multi-omic Visualization Platform (MVP) (a Galaxy visualization plugin) to use mzIdentML and mzML files as standard inputs. We have also created Galaxy workflows that translate non-standard data files into standards compliant XML files. While XML files excel at defining data types, they make querying aggregate data difficult. In our applications, we are using the power of open standards developed at HUPO along with an easily queried relational database schema.

The MVP application is a Galaxy visualization plugin allowing researchers to inspect and verify multi-omic datasets. In addition, we have enabled the application to orient a user between an MS/MS scan, a peptide, a protein and a genomic exon location all on one screen.

We have built a data manipulation application to transform mzIdentML, mzML and MGF (Mascot generic format) files into a portable SQLite database. From the database, a JavaScript visualization tool can present peptide, protein and genome information graphically to the user. We have also built a Galaxy SQLite query tool allows an advanced user direct access to the SQLite database. When accessing the database, the user is interacting with the combined, transformed mzIdentML / mzML / MGF data.

In 2018, we will extend our toolset to allow a user the ability to generate schema verified mzIdentML / mzML files from a subset of the original data. So, after visual inspection a researcher may generate an XML output based on the peptides of interest for use in further data processing in multi-omics research.

Poster 77: Active Kinase Characterization using an Isobaric Labeling Activity-Correlated Protein Profiling Platform (TMT-ACPP)

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Kinase is known to play crucial functional regulation roles in many biological processes, like cell growth, differentiation, and apoptosis. It is considered as one type of important drug targets for cancer, tuberculosis, and other diseases. Traditional kinase characterization often requires target protein overexpression and purification, which is limited by the low throughput (i.e., it often takes months to study a single protein). Therefore, there is a crucial need to develop a high-throughput approach to functionally characterize active kinases at the systems level in the biological systems such as human cancer cells. Proteomics has been applied to study kinase in complex samples. However, the kinase candidates were identified through comparing their expression levels with the whole cell enzymatic activity. Therefore, it may not provide sufficient resolution of enzyme mixtures because the expression level of each kinase cannot be correlated to its own activity.

We have developed a novel functional proteomics tool Activity-Correlated Protein Profiling Platform (ACPP) that systematically correlates protein-level enzymatic activity patterns with hundreds of protein elution profiles from one-dimensional "native" LC separation using a label-free quantitative proteomics approach. Here, we applied the ACPP on the characterization of phosphoglycerate kinases (PGK) in cell lysates. Firstly, we developed and optimized a 96-well plate based kinase activity assay using Escherichia coli phosphoglycerate-

2-kinase (PGK2). The micro-scale sample consumption (i.e., 1-50ul) allows for multiple kinase characterizations in the same sample. Secondly, we developed an Isobaric Labeling Activity-Correlated Quantitative Proteomics Platform (TMT-ACPP) that directly correlates reporter ion profiles with enzyme activity patterns based on the well-established ACPP method. A predominant advantage is that multidimensional separation on the combined peptides prior to the MS study enabled the characterization of low-abundant "active" enzymes. Our results suggested that the TMT-ACPP hold great potential to precisely define each active kinase in biological samples such as cancer cell lysate.

Poster 78: Galaxy-Based Multi-Stage Two-Step Database Searching Pipeline for Improved Multi-Omics Analysis

Praveen Kumar[1]; James Johnson[1]; Thomas McGowan[1]; Matthew Chambers[2]; Mohammad Heydarian[3]; Subina Mehta[1]; Caleb Easterly[1]; Joel Rudney[1]; Pratik Jagtap[1]; Timothy Griffin[1]
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Multi-omic studies like proteogenomics employ high-throughput next-generation genomics, transcriptomics, and proteomics technologies, which generate copious amounts of data. The protein sequence databases, generated from the translation of genome and/or transcriptome data, are matched with the tandem-mass-spectrometry (MS/MS) spectra to identify the peptide-spectrum-matches (PSMs). A PSM confirms the presence of the translated proteins in the sample. However, databases used for matching MS/MS data in proteogenomics are large, leading to loss of sensitivity for identifying true PSMs. Additionally, these translated databases are typically concatenated with the reference proteins database, precluding False Discovery Rate (FDR) estimation specific to identified variant types (e.g. missense, splice isoforms etc.). One solution is a multi-stage, two-step database searching method, where, the MS/MS spectra are first matched sequentially with each reference and variant-specific database, by removing the spectra that resulted in a confident PSM at each previous database searching stage. A database of reduced size is generated composed only of proteins identified from the above multi-stage database searching, providing a database which can be searched again in a second step and increase the sensitivity of true PSMs. Preliminary results show an increase of 5 to 10% in PSM identifications. Identifying more PSMs in a proteogenomic study has helped in identifying and characterizing more novel variant peptide sequences, as well as providing variant-specific FDR estimations. The multi-stage two-step database searching method has also helped improve results in a metaproteomics analysis, increasing confident PSMs and thereby improving proteome coverage and confidence in reporting taxonomy and functional analysis results. We have implemented our method in the flexible and user-friendly Galaxy platform for proteomics (Galaxy-P), which is ideally suited to facilitate workflows such as this containing multiple steps and software tools. Here, we will describe the tools composing this workflow, and show its value in these multi-omic approaches.

Poster 79: A High-Throughput Omics Pipeline for Comprehensive Analysis of Challenged Human Cells

Danielle Gutierrez[1]; Carrie Romer[1]; Jamie Allen[1]; Yuan-Wei Nei[2]; Melissa Farrow[3]; Randi Gant-Branum[1]; Stacy Sherrod[1]; Nicole Muszynski[1]; Eric Spivey[1]; Salisha Hill[1]; Kristie Rose[1]; John Wikswo[1]; John McLean[1]; Eric Skaar[3]; D. Borden Lacy[3]; Jeremy Norris[1]; Richard Caprioli[1]
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Integrated multi-omics analysis has emerged as a powerful approach for comprehensive interrogation of complex biological questions, such as host-microbe interactions, therapeutic resistance, and multifaceted pathologies. These investigations require temporal resolution, analysis of multiple conditions, and adequate replicates to gain insight into the key mechanisms involved, making these studies time and resource intensive and potentially limiting their utility. To overcome these barriers and facilitate the application of multi-omics studies, we have developed a high-throughput pipeline for the analysis of proteins, metabolites, and RNA. HL60 cells were treated with 25 μM

POSTER ABSTRACTS

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methotrexate over a time course of 20 seconds to 60 hours. Sample preparation was performed using a rapid workflow approach that incorporated a solvent buffer for digestion (making the post-precipitation supernatant compatible with downstream metabolomics analysis), a 45 minute digestion, and fast data acquisition methods. For comparison, proteomic data were assessed that had been previously collected using a typical strategy of detergent based lysis buffer, overnight digestion, and longer data acquisition methods. The results for label-free, SILAC, and phSILAC analyses show that the high-throughput multi-omics workflows perform equally or better than the more time consuming traditional proteomic protocols. Central components of this platform include the unification of sample preparation and efficient data acquisition. Approaches such as this, which maintain data quality and provide global views of cellular response in a high-throughput manner, are essential to facilitate the practical acquisition of temporally resolved, multi-condition datasets to gain understanding of currently challenging biological and clinical issues.

Poster 80: Multi-Omics Analysis of Stem Cell Neural Differentiation - Processing SWATH® Acquisition Data in the Cloud with the OneOmics™ Project

Christie Hunter[4]; Katherine Williams[2, 3]; Hao Chen[3]; Joshua Robinson[3]; Arianna Jones[1]

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A quantitative proteomics study was performed and analyzed using the OneOmics™ project suite of applications. Undifferentiated human embryonic stem cells (hESCs) were compared to their neuronal derivatives, i.e., neuronal progenitor cells (NPCs), to examine common and unique differences at the transcriptional and protein level. hESCs were differentiated into NPCs using a previously developed method, which involves cells growing in suspension and the addition of neural promoting factors. RNA and protein fractions from both cell populations were collected and analyzed.

Quantitative proteomics was performed using SWATH® Acquisition and data were processed using the suite of tools in the OneOmics™ Project in the SCIEX Cloud. Using a library generated from a pool of NPC and hESC cells, 2278 proteins were reliably quantified using SWATH® Acquisition across the 2 sample types (3 biological replicates of each). Transcriptomic data was analyzed using standard procedures, then both the protein and RNA data was loaded into iPathwayGuide (Adviata) for comparison. SWATH data identified significant differential expression of 280 proteins with 2 or more peptides from SWATH® Acquisition data using 70% fold change confidence. For the proteins/genes identified to significantly different between NPCs and hESCs, good correlation in differential expression was observed, especially for the molecules involved in neuronal development-related biological processes. Many of these proteins/genes were involved in neurogenesis as expected. There were an additional 197 proteins that had significant differential expression at the protein level but not at the RNA level; 26 of these proteins also mapped to neuronal development processes, highlighting the importance of measuring changes at the protein level.

Poster 81: A Comparison of Quantitative Reproducibility Between DDA Precursor and DIA Fragment Quantification Techniques

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A common LC-MS/MS workflow for unlabeled proteomics experiments relies on Data Dependent Acquisition (DDA) and uses precursor intensity values for quantification. However, precursor peaks often exhibit interference, producing inaccurate quantitative results. Data Independent Acquisition (DIA) coupled with fragment-based quantification may alleviate this problem. To compare the reproducibility of these two methods, we analyzed multiple replicate

injections of a HeLa digest using precursor quantification from state-of-the-art DDA data and fragment-based quantification from DIA data.

Replicates of a commercially available tryptic HeLa cell digest were injected. A C18 HPLC column was coupled to a Thermo Fisher Orbitrap Fusion Lumos mass spectrometer. Results were first obtained in DDA mode using the Top Speed approach. The instrument was then run in DIA mode. HeLa injections were captured in DIA mode using sets of 4-m/z windows to build a DIA reference library. Experimental DIA data was captured using sets of 12-m/z windows. DDA data was searched against the UniProt Human FASTA using Mascot version 2.6.0. Precursor intensity values were calculated using Mascot Distiller version 2.6.3.0. Mascot search results were loaded into Scaffold 4.8.4 for visualization and probability assignments. Scaffold DIA was used to create a narrow-window DIA reference library and to search the wide-window HeLa experimental data and perform fragment-based quantification.

Preliminary data indicated that quantitative variability between replicate injections is reduced when the instrument is operated in DIA mode and fragment-based quantification is used.

Poster 82: Comprehensive Benchmarking for Label-Free Quantitative Proteomics

Thevaa Chandereng[2]; Shengchao Liu[2]; John Denu[1]; Anthony Gitter[2]; James Dowell[1]

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Accurate protein quantification is one of the most important applications of mass spectrometry-based proteomics. Recently, label-free quantification has gained popularity and is now in wide-spread use within the proteomics community. However, the direct impact of instrument acquisition technique, signal processing, and statistical approach on quantification accuracy in label-free proteomics has not been thoroughly examined. In this study, we have used a spike-in protein standard in conjunction with a high-throughput computing platform to benchmark these variables. Specifically, we have assessed the difference between data dependent acquisition (DDA) and data independent acquisition (DIA) as well as the accuracy of downstream data processing. More importantly, we have examined the accuracy of different statistical approaches in discerning significant changes when varying the number of sample replicates. Using this approach, we have found that workflows employing DIA are generally superior to those employing DDA and that statistical approaches vary widely in their ability to accurately discern differential protein expression with small sample sizes. These results underscore the importance of careful experimental design based on both available instrumentation and the available number of samples.

Poster 83: Statistical Model for Detecting Differentially Abundant Proteins in Isobaric Labeling-Based Protein Quantification Experiments

Ting Huang[1]; Meena Choi[1]; Manuel Tzouros[2]; Nikhil Pandya[2]; Balazs Banfai[2]; Tom Dunkley[2]; Olga Vitek[1]

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Isobaric labeling (iTRAQ and TMT) is a popular technology for protein quantification since it allows multiple samples to be analyzed simultaneously in a single mass spectrometry experiment. However, the design of multiple iTRAQ or TMT experiments can be very complex due to different allocations of samples and groups to channels and experiments. Different designs introduce distinct sources of variance in the reporter ion intensity. Statistical models for accurately estimating the components of variance and incorporating them to identify differentially abundant proteins are still lacking.

In this study, we develop a statistical model for detecting differentially abundant proteins in isobaric labeling proteomic experiments. The model first measures relative protein abundance by robustly summarizing reporter ion intensities in a sub-plot model. This summarization addresses the issue of missing values in multiple experiments, where many peptides are only identified in a subset of

POSTER ABSTRACTS

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experiments. Next, protein abundances are normalized based on masterpool channels to allow comparison in samples among multiple iTRAQ or TMT experiments. Then different linear models are fitted based on the underlying experimental design, including one or multiple experiments with or without technical replicates and fractionation. Finally, model-based inference is carried out to identify differentially abundant proteins. In particular, we extend the model by applying an empirical Bayes shrinkage when the sample size is small.

The model is evaluated using a controlled mixtures dataset comprising 15 TMT 10-plex experiments with 48 Sigma UPS1 spiked-in proteins. The results show that our model produces best sensitivity with controlled FDR. It also demonstrates the benefit of using robust summarization to improve the accuracy of fold change estimation. We also illustrate the application of the proposed model using two publicly available biological datasets. We implement the model in an open-source R package, which supports statistical inference of iTRAQ and TMT.

Poster 84: Cross-Species Comparison of Proteome Turnover Kinetics

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[1]University of Rochester, Rochester, NY; [2]University of Rochester Mass Spectrometry Lab, Rochester, NY

The constitutive process of protein turnover plays a key role in maintaining cellular homeostasis. Recent technological advances in mass spectrometry have enabled the measurement of protein turnover kinetics across the proteome. However, it is not known if turnover kinetics of individual proteins are highly conserved or if they have evolved to meet the physiological demands of individual species. Here, we conducted systematic analyses of proteome turnover kinetics in primary dermal fibroblasts isolated from eight different rodent species. Our results highlighted two trends in the variability of proteome turnover kinetics across species. First, we observed a decrease in cross-species correlation of protein degradation rates as a function of evolutionary distance. Second, we observed a negative correlation between global protein turnover rates and maximum lifespan of the species. We propose that by reducing the energetic demands of continuous protein turnover, long-lived species may have evolved to lessen the generation of reactive oxygen species and the corresponding oxidative damage over their extended lifespans.

Poster 85: Label-Free Quantitative Proteomics of Active Replication Forks

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DNA replication is coordinated with other metabolic processes, such as transcription and DNA repair, but it is still unclear how closely the transcriptional and repair machineries associate with replication forks. Recently, the isolation of proteins on nascent DNA (iPOND) has been combined with quantitative proteomics, allowing for the systematic study of the proteome associated with active replication forks. We utilized a novel proteomics algorithm, RIPPER, which enables label-free relative quantification. Different from common quantitative approaches that utilize data dependent acquisition (DDA), RIPPER quantifies peptides that are differentially abundant in two samples and subjects them to directed mass spectrometry (directed RIPPER or DRIPPER). In conjunction with iPOND, we have employed DRIPPER and reproducibly identified 462 proteins enriched at active replication forks. The majority of proteins are involved in DNA replication as well as different repair pathways, including mismatch, nucleotide excision, and base excision repair. Components of the spliceosome were also found to be associated with active forks, suggesting that a sizable fraction of replication complexes moves through actively transcribed regions of the genome. In contrast, histone proteins were enriched in post-replicative chromatin.

When we compared the performance of DRIPPER with a DDA-based, label-free quantification technology, MaxLFQ, DRIPPER identified more than twice as many proteins as MaxLFQ. DRIPPER also revealed more peptides per protein than MaxLFQ, with an average of 5.61 compared to 2.92 in MaxLFQ. Furthermore, DRIPPER uncovered 69 proteins that were not identified by other DDA-based labeling approaches, such as SILAC or iTRAQ. These proteins include PARP1, APLF, and DDB1 repair factors, the NuRD chromatin remodeling complex, and the NELF complex, which suppresses transcriptional elongation. These results provide insight into the complexity of eukaryotic replication complexes and suggest that regulators of transcription and DNA repair are intimately associated with replication forks.

Poster 86: Accurate, Sensitive, and Precise Multiplexed Proteomics using the Complement Reporter Ion Cluster

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Quantitative analysis of proteomes across multiple time points, organelles, and perturbations is essential for understanding both fundamental biology and disease states. The development of isobaric tags (e.g. TMT) have enabled the simultaneous measurement of peptide abundances across several different conditions. These multiplexed approaches are promising in principle because of advantages in throughput and measurement quality. However, in practice existing multiplexing approaches suffer from key limitations. In its simple implementation (TMT-MS2), measurements are distorted by chemical noise leading to poor measurement accuracy. The current state-of-the-art (TMT-MS3) addresses this, but requires specialized quadrupole-iontrap-Orbitrap instrumentation. The complement reporter ion approach (TMTc) produces high accuracy measurements and is compatible with many more instruments, like quadrupole-Orbitraps. However, the required deconvolution of the TMTc cluster leads to poor measurement precision. Here, we introduce TMTc+, which adds the modeling of the MS2-isolation step into the deconvolution algorithm. The resulting measurements are comparable in precision to TMT-MS3/MS2. The improved duty cycle, and lower filtering requirements make TMTc+ more sensitive than TMT-MS3 and comparable with TMT-MS2. At the same time, unlike TMT-MS2, TMTc+ is exquisitely able to distinguish signal from chemical noise even outperforming TMT-MS3. Lastly, we compare TMTc+ to quantitative label-free proteomics of total HeLa lysate and find that TMTc+ quantifies 8.0k versus 3.8k proteins in a 5-plex sample. At the same time the median coefficient of variation improves from 13% to 6%. Thus, TMTc+ advances quantitative proteomics by enabling accurate, sensitive, and precise multiplexed experiments on more commonly used instruments.

Poster 87: Bayesian Confidence Intervals for Multiplexed Proteomics Integrate Ion-Statistics with Peptide Quantification Concordance

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Multiplexed proteomics has emerged as a powerful tool to measure protein expression levels across multiple conditions. The relative protein abundances are inferred by comparing the signal generated by isobaric tags, which encode the samples origins. Intuitively, the trust associated with a protein measurement depends on the similarity of ratios from different peptides and the signal level of these measurements. Up to this point in the field, peptide-level information has not typically been integrated into confidence, and only the most likely results for relative protein abundances are reported. If confidence is reported, it is based on protein-level measurement agreement between replicates. Here we present a mathematically rigorous approach that integrates peptide intensities and peptide-measurement agreement into confidence intervals for protein ratios (BACIQ). The main advantages of BACIQ are: 1) it removes the need to threshold reported peptide signal based on an arbitrary cut-off, thereby reporting more measurements from a given experiment; 2) confidence can be assigned without replicates; 3) for repeated experiments BACIQ

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provides confidence intervals for the union, not the intersection, of quantified proteins; 4) for repeated experiments, BACIQ confidence intervals are more predictive than confidence intervals based on protein measurement agreement. Therefore, our method drastically increases the value obtained from quantitative proteomics experiments and will help researchers to interpret their data and prioritize resources. To make our approach easily accessible we distribute it via an R/Stan package.

Poster 88: Intact Protein Quantitation in Complex Samples using Protein-Level TMT Labeling and Top-Down Mass Spectrometry
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The isobaric chemical tags (e.g., iTRAQ and TMT) have been widely applied to quantify peptides and proteins in the bottom-up MS. However, till now, it only has limited successes on several known purified proteins in the top-down proteomics, and has not yet been applied on labeling and quantifying complex intact protein samples. In this study, we reported a TMT top-down MS platform for confidently identifying and quantifying intact proteoforms with molecular weight less than 35 kDa in complex biological samples. Intact protein TMT labeling in complex samples is often challenging because different size proteins often have different optimal labeling conditions. To reduce the sample complexity to remove large proteins in complex sample, we here developed a "filter-SEC" approach that combined a molecular weight cutoff step with a high-performance size exclusion chromatography (SEC) separation, which allows for the efficient enrichment of the intact proteome with molecular weight less than 35 kDa. We further combined the filter-SEC, the optimized intact protein-level TMT labeling, and the top-down MS to quantify and identify 408 intact proteoforms in the E. coli cell lysate from two LC-MS/MS runs. Our results suggest that the optimized TMT top-down MS platform enables a high-throughput quantitation of intact proteoforms in complex protein samples. To our knowledge, it represents a first high-throughput TMT-labeling based quantitative top-down MS analysis of complex biological samples such as the E. coli cell lysate.

Poster 89: Evaluation of Precursor-Based Peptide Quantification Software Tools Within the Galaxy Framework
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Mass Spectrometry (MS) based quantitative proteomics provides information regarding protein expression and abundance in a given sample. Protein/peptide level quantitation (either labeled or label-free) is routinely used in analysis of shotgun proteomics data. For multi-omics studies such as proteogenomics and metaproteomics, peptide detection and quantitation is essential. Proteogenomics identifies variant sequences at the peptide level, while in metaproteomics 'metapeptides' may be assigned to taxa or functions; in both cases quantification at the peptide-level is critical. Label-free quantification based on the precursor peak (MS1) intensities are considered reliable because of their reproducibility, efficiency and ease in peptide identification. In this study, we evaluate moFF (DOI:10.1038/nmeth.4075) and FlashLFQ (DOI:10.1021/acs.jproteome.7b00608), which are label-free quantification tools that provide accurate and fast peptide quantification. These open source quantification tools were evaluated after implementation in the Galaxy platform, which is an open, web based multi-omic informatics platform, which has proven valuable for proteogenomic (DOI:10.1158/0008-5472.CAN-17-0331) and metaproteomic (DOI:10.1002/pmic.201500074) informatics. Samples from ABRF-PRG (Association of Biomolecular Research Facilities - Proteomics Research Group) study were used for this evaluation. RAW files (single and fractionated) generated from samples with 4 proteins spiked into a human cell lysate at 20-200 fmol concentrations were used for this study. Outputs generated from moFF and FlashLFQ were compared with MaxQuant (DOI:10.1038/nprot.2016.136) to test the

efficacy of these tools. Ratios estimated from these measurements were compared with the actual amounts of spiked-in proteins. Results from this evaluation will be presented and will be used as a reference point for future proteomics, proteogenomics and metaproteomics quantification studies utilizing the Galaxy platform.

Poster 90: High-Throughput Untargeted Data-Independent Analysis of Misoprostol Challenged HL60 Cells as a Model for Chemical Threat Assessment
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Large-scale analyses to interrogate complex biological questions and elucidate pathways that result from the introduction of exogenous materials usually requires the analysis of replicate samples over multiple time points and various conditions. This necessitates a higher throughput mode of analysis than is typically used in traditional discovery proteomics. To begin removing the barrier of lengthy analytical requirements for large-scale analyses, this study evaluates fast chromatographic separation followed by data-independent acquisition as a platform for high-throughput proteomics.

HL60 cells were left untreated or treated with misoprostol for 30 min (4 treated, 4 control) and 18 h (4 treated, 4 control). The samples were lysed in a solvent buffer, and 100 micrograms were precipitated at -80°C for 2 h in acetone/methanol. Samples were then reduced and alkylated, and digested with Rapid Trypsin/Lys-C (Promega). The samples were desalted on the Agilent AssayMAP Bravo and dried down. Prior to analysis, samples were reconstituted in 0.1% formic acid. LC-MS analysis was performed using fast gradients (30 min run time, 22 min reverse phase gradient) on 0.3 x 150 mm analytical columns or on a 0.15 x 100 mm microfluidic tile and using SONAR data independent analysis on a Q-TOF mass spectrometer. Data were analyzed in Progenesis QIP.

Misoprostol is a synthetic analogue of PGE1 and an agonist of the PGE2 receptor. PGE2 is synthesized via the arachidonic acid cascade and is involved in many cellular processes, including inflammatory signaling. PGE2 is known to have both pro- and anti-inflammatory effects. Using both technical and biological replicates, qualitative and quantitative assessment at multiple time points indicated protein expression changes are consistent with perturbations to arachidonic acid metabolism/regulation at 30 minutes and inflammatory signaling at 18 hours.

This study will investigate the chromatographic separation, data acquisition and analysis protocols that will further enable robust, rapid assays for chemical exposure.

Poster 91: Protein Turnover in a Whole Mouse Model
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Proteostasis is vital for maintaining protein abundance and preventing a buildup of damaged proteins. The decline of protein turnover rates is a key hallmark of aging and results in abnormal proteins in the form of protein inclusions or aggregates. These and other disruptions to proteostasis contribute to a growing list of disorders including neurodegenerative diseases, cancer, diabetes, and cardiovascular diseases. Since changes in turnover rates often do not results in a change in protein abundance, but instead alter protein temporal dynamics, they usually elude capturing in conventional quantitative proteomic experiments. Characterizing turnover rates on a large-scale will provide a unique opportunity to increase our understanding of the mechanisms of age related diseases and recycling needs of the proteome.

We address this need using metabolic labeling and mass spectrometry to build a global repository of protein half-lives collected from nine mouse organs. We then leveraged this resource to interrogate turnover rates for differences due to tissue of origin as well as functional and

POSTER ABSTRACTS

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biological characteristics. Most studies of turnover on the proteome scale have relied on cell culture and single-celled organisms for their relative ease of labelling with SILAC and sample collection. Of those conducted on a whole animal model most are limited to 3-4 tissues. By analyzing a large number of mouse tissues we have the unique ability to compare the half-life of a single protein between numerous tissues. We calculated rates in nine tissues from mouse totaling 8,745 unique proteins and analyzed their dependence on physical and biological properties. Many of these proteins were measured across multiple tissues revealing the effect of tissue localization on protein half-life. This study catalogs a property of proteins which is regularly absent from proteomic analyses and thus provides a valuable resource to researchers studying diverse areas of biology.

Poster 92: Plasma Proteomics using Label-Free Quantification and Tandem Mass Tags in Non-Depleted and Immune-Depleted Samples

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The objective of this study is to globally quantify proteins from 24 patient plasma samples in order to find significantly changed proteins between disease and control groups. Plasma proteome profiling using a shot-gun proteomic workflow has been of great interest in finding protein biomarkers. A big challenge is the high dynamic range of proteins abundances in plasma. In our label-free approach, we compared non-depleted and immune-depleted samples using a GeLC method. Our preliminary data showed that, for the non-depleted samples, a total of 523 unique proteins were identified. Of these, 40% were identified in all 24 samples and 16% of were only identified in one sample. The same plasma samples were also immune-depleted using a Seppro IgY 14 LC5 column. A total of 783 unique proteins were identified in the LC-MS/MS analysis of the depleted samples. Of these, 45% were identified in all 24 samples and only 2% of the proteins were only identified in one sample.

For the isobaric tagging experiments, the non-depleted and depleted samples will be digested with trypsin and labeled with TMT10plex. A total of 5 multiplexed samples will be created allowing technical replicates and a pooled internal control. The samples will be off-line fractionated using a high-pH reverse phase HPLC method after labeling.

A ThermoScientific Fusion Lumos system running 3s-cycle data dependent HCD acquisition was used in our label-free study. For TMT-labeled samples, we will take advantage of the Synchronous Precursor Selection (SPS) capability of the Lumos instrument, and use a SPS-MS3 data acquisition method.

To validate the methods of data analysis, human protein mixtures UPS1 and UPS2 with known ratios were spiked into an E. Coli background to make standard samples. LCMS Data was analyzed and validated using MaxQuant and Proteome Discovery for label-free samples and TMT-labeled samples, respectively.

AUTHOR INDEX

AUTHOR INDEX