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PROGRAM OVERVIEW

SUN, MARCH 13	MON, MARCH 14	TUES, MARCH 15	WED, MARCH 16
8 am - 7 pm **Registration** *Concourse Level* **Full-day Short Course** 9:00 am - 4:00 pm **Design and Analysis of Quantitative Proteomics Experiments** *Commonwealth C* **Afternoon-only Short Courses** 1:00 – 4:00 pm **Cross-Linking Mass Spectrometry** *Commonwealth A* 1:00 - 4:00 pm **Stable and Transient Protein-Protein Interactions** *Commonwealth B*	**8:00 – 8:30 am** Early Morning Coffee *Exhibits & Posters*	**8:00 – 8:30 am** Early Morning Coffee *Exhibits & Posters*	**8:00 – 8:30 am** Early Morning Coffee *Grand Foyer Foyer*
	8:30 – 9:20 am **Plenary Lecture** Masanori Aikawa *Grand A*	**8:30 – 9:20 am** **Awards Talks** Paola Picotti Brendan MacLean *Grand A*	**8:30 – 9:20 am** **Tips & Tricks Talks** *Grand A*
	9:20 – 9:50 am Coffee Break, *Exhibits & Posters*	**9:20 – 9:50 am** Coffee Break, *Exhibits & Posters*	**9:50 – 10:10 am** Coffee Break, *Palm Foyer*
	9:50 – 11:10 am Parallel Sessions (2) **Drug Development** *Grand A* **Top-Down Proteomics** *Grand B*	**9:50 – 11:10 am** Parallel Sessions (2) **Cross'Omics** *Grand A* **Pediatric Proteomics** *Grand B*	**9:50 – 11:10 am** Parallel Sessions (2) **Biomarkers** *Grand A* **Neuroproteomics** *Grand C*
	11:10 am – 12:00 pm Plenary Session **Lightning Talks – Round I** *Grand A*	**11:10 am – 12:00 pm** Plenary Session **Lightning Talks – Round II** *Grand A*	**11:10 am – 12:00 pm** **Plenary Lecture** Sangeeta Bhatia *Grand A*
	12:00 – 1:30 pm **Lunch Seminars** Agilent, *Commonwealth B* Bruker, *Commonwealth A* Thermo, *Commonwealth C*	**12:00 – 1:30 pm** **Lunch Seminar** SCIEX, *Commonwealth C*	
	1:30 - 3:00 pm **Poster Session I** *Exhibits & Posters*	**1:30 - 3:00 pm** **Poster Session II** *Exhibits & Posters*	
	3:00 – 4:20 pm Parallel Sessions (2) **Computational Proteomics** *Grand A* **Structural Proteomics** *Grand C*	**3:00 – 4:20 pm** Parallel Sessions (2) **Microbiology** *Grand A* **Proteogenomics** *Grand C*	
	4:30 – 5:50 pm Parallel Sessions (2) **Proteomics of Aging** *Grand A* **Imaging** *Grand C*	**4:30 – 5:50 pm** Parallel Sessions (2) **Cancer** *Grand A* **New Developments** *Grand C*	
6:00 – 7:15 pm **Opening Session** **Plenary Lecture** Matthias Mann *Grand A* + **Intro to hPOP project** **7:15 – 8:45 pm** **Opening Reception** *Exhibits & Posters* Food & Drinks All are welcome!	**5:50 – 6:30 pm** **Mixer** *Exhibits & Posters* Munchies & Drinks All are welcome! Grab something to eat before the workshops.	**6:30 – 8:30 pm** **Offsite Social Event at the Barking Crab** Make sure to have your ticket from Conference Registration. Walking instructions available at Registration.	
	6:30 – 8:00 pm Evening Workshops (4) **ABC's and XYZ's of Starting Your Own Mass Spec Lab**, *Commonwealth A* **Elevator Pitch – Sharing Your Science**, *Commonwealth C* **How to Obtain a Faculty Position and Keep It**, *Commonwealth B* **Computation and Statistics for Quantitative Proteomics**, *Grand C*		

GENERAL INFORMATION

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 program code of presentations.

POSTERS. Posters are located in Exhibits & Posters between the lecture rooms. All posters should be mounted Monday morning by 10:00 am. Posters must be removed at the conclusion of the Tuesday poster session (3:00 pm).

All posters present on Monday and Tuesday.

- Odd-numbered boards are attended 1:30 – 2:15 pm
- Even-numbered boards are attended 2:15 – 3:00 pm

TALKS. All Plenary and Parallel sessions are located in sections A or C of the Grand Ballroom.

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.

WIFI. There is wifi for conference attendees in the meeting areas. Look for walk-in slides and signage for details on network ID and password.

EVENING WORKSHOPS. Four workshops are scheduled for Monday 6:30 – 8:00 pm immediately following an informal mixer. All conference attendees are welcome to attend the workshops, there is not a separate registration.

LUNCH SEMINARS. Free lunch seminars are hosted on Monday and Tuesday. All attendees are invited to attend, but are encouraged to RSVP at host company exhibit booths. See pages 10 and 16 for details.

CAREER CENTER. Located in Grand Foyer. Job boards available for postings. Job seekers may leave CVs for employer perusal. Interview rooms available for sign-up.

CELL PHONES. Please **TURN OFF** all wireless devices (cell phones, smartphones, etc) when in session rooms. This courtesy is requested on behalf of presenters as well as fellow audience members.

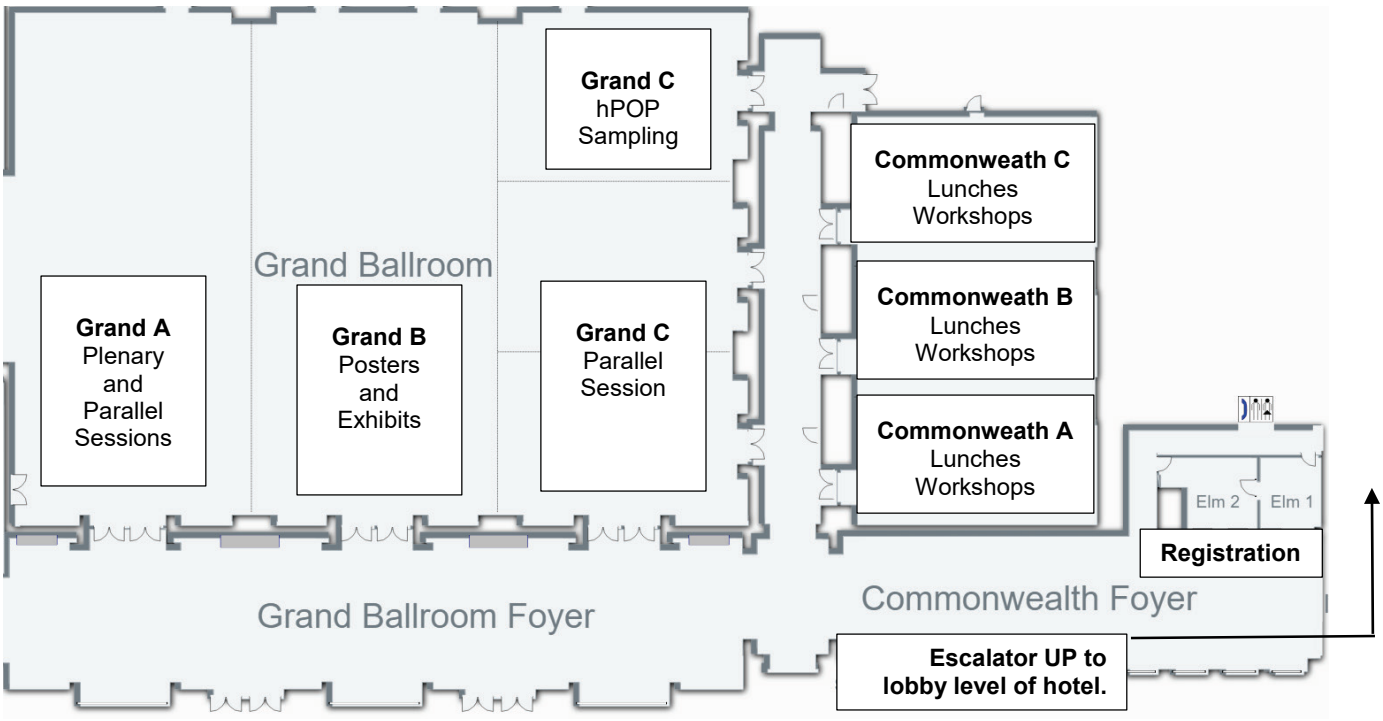
NO PHOTOGRAPHY or RECORDING of posters or talks is permitted.

SOCIAL EVENT AT THE BARKING CRAB. Tuesday evening, 6:30 – 8:30 pm is a social event for all registered attendees (tickets included in your registration envelope. The Barking Crab is walking distance from the hotel. Enjoy the stunning views of Boston harbor with your colleagues! Includes light supper and drinks.

hPOP (human Personal Omics Profiling) project is a study that will launch at US HUPO in Boston! Attendees will learn how they can become a participant in this ground-breaking study.

WESTIN WATERFRONT HOTEL – CONCOURSE LEVEL

All conference functions are located on the Concourse Level of the hotel.
From the hotel lobby take escalators down one level to reach the Concourse.
There are street level doors that exit onto D Street (at Fargo) and access the Concourse level directly.



EXHIBITORS

US HUPO is pleased to acknowledge the support of conference exhibitors. Exhibit booths are located Grand B along with the technical posters. Opening reception, coffee breaks, and mixer will be located here with the exhibitors.

Agilent Technologies

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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, 12:00 – 1:30 pm *See page 10 for more information.*	**Tuesday, 12:00 – 1:30 pm** *See page 16 for more information.*
Agilent Technologies, *Commonwealth B* **Bruker**, *Commonwealth A* **Thermo Scientific**, *Commonwealth C*	**SCIEX**, *Commonwealth C*

AWARDS

US HUPO now has two awards with the new addition of the computational proteomics award. Both awards were created to honor the name and contributions of leaders in the field and of the Society.

ROBERT J. COTTER NEW INVESTIGATOR AWARD

2016 Recipient: **Paola Picotti**
ETH Zurich

The New Investigator award honors an individual early in his or her career, in recognition of significant achievements in proteomics.

Paola Picotti is recognized for her early career development of a targeted proteomics workflow based on SRM-MS. Her current research applies a combination of unbiased and targeted proteomic techniques and biochemical tools to the study of pathological protein aggregation.

GILBERT S. OMENN COMPUTATIONAL PROTEOMICS AWARD

2016 Recipient: **Brendan MacLean**
University of Washington

The Computational Proteomics award recognize the specific achievements of scientists that have developed software tools used by proteomics community

Brendan MacLean is honored for his development of Skyline. Initially created to fill a critical software tool to enable targeted proteomics experiments it now supports a variety it now supports a broad range of mass spectrometry-based experiments. There are now over 6,300 registered users of the Skyline platform.

STUDENT AND POST-DOC TRAVEL STIPENDS

US HUPO supports graduate students and post-doctoral fellows with stipends to support their participation at the conference. We are pleased to announce the 2016 recipients.

Hassan Alamri, *Cleveland State University*

Zhe Cheng, *New York University*

Yaojun Li, *Houston Methodist Research Institute*

Megan Maurer, *West Virginia University*

Bradley Naylor, *Brigham Young University*

Phuong Nguyen, *University of California, Los Angeles*

Minervo Perez, *University of Minnesota*

Brendan Powers, *Purdue University*

Prahlad Rao, *Texas Biomedical Research Institute*

Julia Roberts, *Duke University*

Monique Speirs, *Brigham Young University*

Shisheng Sun, *Johns Hopkins University*

Adam Swenson, *Brigham Young University*

Hannah Trassati, *Rensselaer Polytechnic Institute*

Randi Turner, *University of Maryland*

Kun-Hsing Yu, *Stanford University*

9:00 AM – 4:00 PM: FULL-DAY SHORT COURSE, *Commonwealth C*

DESIGN AND ANALYSIS OF QUANTITATIVE PROTEOMIC EXPERIMENTS: INTRODUCTION TO STATISTICAL METHODS AND PRACTICAL EXAMPLES USING SKYLINE

Olga Vitek, *Northeastern University*; Brendan MacLean, *University of Washington*; and Meena Choi, *Purdue University*

Modern quantitative mass spectrometry-based proteomic workflows require computational and statistical tools for experimental planning and data analysis. This course is designed for experimentalists looking to enhance their skills in this area. The aim of the course is two-fold. First, we will introduce the fundamental concepts of statistical experimental design, as well as statistical models used to summarize the spectral signals into protein-level conclusions. Particular attention will be paid to the assumptions underlying various analysis steps, and to the generalizability of the results. Second, using the computational framework offered by Skyline and its external tools, we will conduct several detailed and practical case studies with real-life experimental datasets. Although the examples will focus on relative peptide and protein quantification in label-free SRM experiments, and in SRM experiments with stable isotope-labeled reference peptides, we will also highlight the capabilities of Skyline and its eternal tools when working with other workflows, such as data-independent spectral acquisition.

1:00 – 4:00 PM, HALF-DAY SHORT COURSE, *Commonwealth A*

CROSS-LINKING MASS SPECTROMETRY: PRACTICAL USES IN STUDYING PROTEIN INTERACTIONS AND STRUCTURES

Lan Huang, *University of California, Irvine* and Robert Chalkley, *University of California, San Francisco*

Protein-protein interactions are fundamental to the assembly, structure and function of protein complexes. Aberrant protein interactions can have drastic impacts on cellular functions and thus lead to various human diseases. Mapping protein interactions and their binding interfaces in living cells is critical not only for understanding protein function, but also for therapeutic interventions. Cross-linking mass spectrometry represents a powerful and emergent technology which possesses unparalleled capabilities for studying protein interactions. The identification of cross-linked peptides by mass spectrometry provides direct molecular evidence describing the physical contacts between and within proteins. This information can be used for generating experimentally derived protein interaction network topology maps and for computational modeling to establish architectures of large protein complexes. This course will cover basic principles and practical uses of various cross-linking mass spectrometry approaches for studying protein interactions and structures. Specially, we will discuss about 1) sample preparation; 2) experimental workflows with conventional and MS-cleavable cross-linking reagents; 3) data analysis for identifying cross-linked peptides; 4) result interpretation, validation and usage.

1:00 – 4:00 PM, HALF-DAY SHORT COURSE, *Commonwealth B*

STABLE AND TRANSIENT PROTEIN-PROTEIN INTERACTIONS

Ileana Cristea, *Princeton University* and Alexey Nesvizhskii, *University of Michigan*

Dynamic protein interactions carry out the majority of the processes within a cell, including cellular responses to environmental stimuli and pathogens. Isolation of protein complexes and characterization of protein-protein interactions provide critical insights into their biological functions. An ideal isolation would maintain the protein-protein interaction or the protein assembly as close as possible to the original state in the cell. Therefore, proteomic-based methodologies that can access stable and transient interactions are invaluable for diverse studies, such as those of cell cycle or pathogen infection that require characterization of temporal and spatial protein interactions. This course will cover fundamental and practical aspects of studying protein interactions. Topics discussed will include:

1. protein function considerations for workflow design,
2. cell lysis methods for efficient protein extraction,
3. critical choices for optimizing an immunoaffinity purification experiment, including resin type and speed of isolation,
4. denaturing and non-denaturing methods of eluting captured protein complexes,
5. assessing the specificity of interactions using bioinformatics approaches, metabolic labeling with stable isotopes, or peptide labeling with isobaric tags,
6. challenges for assessing direct or indirect interactions,
7. aspects of data analysis and generation of interaction networks.

SUNDAY, MARCH 13

Welcome to the Opening Session
Proteomics: From New Technology to New Biology

6:00 – 6:10 pm Opening Remarks: Joshua LaBaer

6:10 – 7:00 pm **Proteomics for Translational Research**; Matthias Mann, *Max-Planck Institute for Biochemistry and The Novo Nordisk Foundation Center for Protein Research*

7:00 – 7:15 pm **hPOP (human Personal Omics Profiling): Introduction to the Study** and brief presentation on how attendees can sign up to participate while in Boston.

6:00 - 7:15 AM: **OPENING RECEPTION**, *Grand B*

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

MONDAY, MARCH 14

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

8:30 - 9:20 AM: **PLENARY LECTURE**, *Grand A*
Jeff Agar, presiding

8:30 - 9:20 am **A New Model of Multidisciplinary Drug Discovery for Cardiovascular Disease: Establishing a Unique International Academia-Industry Partnership**; Masanori Aikawa, *Brigham & Women's Hospital, Harvard Medical School*

9:20 - 9:40 AM: **COFFEE BREAK**, *Exhibits & Posters*
Refresh and visit with the exhibitors.

9:50 – 11:10 AM: PARALLEL SESSION
DRUG DEVELOPMENT, *Grand A*
Markus Schirle, presiding

9:50 am - 10:15 am **Drug Action in the Context of the Proteome**; Marcus Bantscheff; *Cellzome a GSK company, Heidelberg, Germany*

10:15 am - 10:40 am **Investigating Protein-Protein Interaction Networks to Fuel Drug Discovery at Genentech**; Erik Verschueren; *Genentech, South San Francisco, CA*

10:40 am - 10:55 am **gMODs: An Open Source Data Analysis Tool for Quantifying the Differential Site Occupancy of Therapeutic Protein Modifications**; Tsung-Heng Tsai[1]; Zhiqi Hao[2]; Benjamin Moore[2]; Qiuting Hong[2]; Cinzia Stella[2]; Jeffrey Zhang[2]; Yan Chen[2]; Michael Kim[2]; Theo Koulis[3]; Erik Verschueren[4]; Fred Jacobson[2]; Olga Vitek[1]; William Haskins[2]; *[1]Northeastern University, Boston, MA; [2]Protein Analytical Chemistry, Genentech, South San Francisco, CA; [3]Nonclinical Biostatistics, Genentech, South San Francisco, CA; [4]Protein Chemistry, Genentech, South San Francisco, CA*

10:55 am - 11:10 am **Improving Drug Target Space Coverage of Chemical Proteomics with Photoaffinity Labeling-Based Approaches**; Jason Thomas; Scott Brittain; Jennifer Lipps; Markus Schirle; *Novartis, Cambridge, MA*

9:50 – 11:10 AM: PARALLEL SESSION
TOP-DOWN PROTEOMICS, *Grand C*
Ying Ge, presiding

9:50 am - 10:15 am **Heavy Sugar or Water Create Arbitrary Changes in Isotope Distribution (ACID) for Quantitative MS of any Biomolecule, Feed, and Organism**; Jeniffer V. Quijada; Joseph P. Salisbury; Jared R. Auclair; Jeffrey N. Agar; *Northeastern University, Boston, MA*

10:15 am - 10:35 am **Top-Down Mass Spectrometry-Based Proteomics: Challenges and Opportunities**; Ying Ge; *University of Wisconsin-Madison, Madison, WI*

10:40 am - 10:55 am **Analyzing Histone Tail Dynamics using Hydrogen-Deuterium Exchange Coupled to Top-Down Mass Spectrometry**; Kelly Karch; Ben Black; Benjamin A. Garcia; *University of Pennsylvania, Philadelphia, PA*

10:55 am - 11:10 am **Elucidating Proteoform Families from Proteoform Intact Mass and Lysine Count Measurements**; Michael Shortreed; Brian Frey; Mark Scalf; Rachel Knoener; Anthony Cesnik; Lloyd Smith; *University of Wisconsin, Madison, WI*

11:10 AM – 12:00 PM: PLENARY SESSION
LIGHTNING TALKS I, *Grand A*
Robert Moritz and Robert Rivers, presiding
High-energy (and brief) presentations selected from poster presentations. 2 minutes max or face the music!

Presentation Order

Mon 01 **Quantitative Proteomic Approaches for Identifying Urinary Biomarkers in Lupus Nephritis**; Veronica Anania; *Genentech, Inc., South San Francisco, CA.* ***Visit Poster 005.***

Mon 02 **A New Approach for Understanding Cancer and Chemoresistance: Metabolic Labeling and LC-MS to Measure Protein Turnover in Breast Cancer;** Monique Paré Speirs; Michael Porter; Bradley Naylor; John Price; *Brigham Young University, Provo, UT.* ***Visit Poster 015.***

Mon 03 **Integrative Proteomic and Glycomic Profiling of Metastatic Signatures in Breast Tumor;** Le Meng; *Boston University, Boston, MA.* ***Visit Poster 019.***

Mon 04 **Accumulated Ion Monitoring (AIM) Improves Sensitivity and Quantitative Dynamic Range for Precision Functional Proteomics;** Paolo Cifani; Zheng Ser; Avantika Dhabaria; Alex Kentsis; *Sloan-Kettering Institute, New York, NY.* ***Visit Poster 025.***

Mon 05 **PRM Coupled to an Intensity-based Quantification Strategy Accurately Measures D3-Leu Tracer of less than One Percent in apoA-I/HDL Clinical Samples;** Lang Ho Lee[1]; Brett Pieper[1]; Allison Andraski[2]; Frank Sacks[2]; Masanori Aikawa[1]; Sasha Singh[1]; *[1]Brigham and Women's Hospital, Boston, MA; [2]T.H. Chan Public Health Harvard University, Boston, MA.* ***Visit Poster 031.***

Mon 06 **Parameterization of Averagine Composition Improved Feature Detection of Oligonucleotides**; Samuel Wein[1]; Ben Garcia[2]; *[1]University of Pennsylvani, Philadelphia, PA; [2]University of Pennsylvania School of Medicine, Philadelphia, PA.* ***Visit Poster 040.***

Mon 07 **Integrative Systems Biology Approach to Identify Mechanisms of Action;** Akos Vertes[1]; Andrew Korte[1]; Camille Lombard-Banek[1]; Peter Nemes[1]; Lida Parvin[1]; Ziad Sahab[1]; Bindesh Shrestha[1]; Sylwia Stopka[1]; Wei Yuan[1]; Deborah Bunin[2]; Merrill Knapp[2]; Ian Mason[2]; Denise Nishita[2]; Andrew Poggio[2]; Carolyn Talcott[2]; Maneesh Yadav[2]; Brian Davis[3]; Adriana Larriera[3]; Christine Morton[3]; Christopher Sevinsky[3]; Maria Zavodszky[3]; Nicholas Morris[4]; Heather Anderson[4]; Matthew Powell[4]; Trust Razunguzwa[4]; *[1]George Washington University, Washington, DC; [2]SRI International, Menlo Park, CA; [3]GE Global Research, Niskayuna, NY; [4]Protea Biosciences Inc., Morgantown, WV.* ***Visit Poster 051.***

Mon 08 **A Strategy to Study the Mitochondrial Proteome from Genetically Defined Cell Classes in the Nervous System**; Gulcin Pekkurnaz; Thomas L. Schwarz; *Boston Children's Hospital, Harvard Medical School, Boston, MA.* ***Visit Poster 060.***

Mon 09 **Identification of Missing MHC Class I HIV Epitopes**; Marijana Rucevic[1]; Renata Blatnik2[2]; Georgio Kourjian1[1]; Matthew J. Berberich[1]; Angelika B. Riemer[2]; Sylvie LeGall[1]; *[1]Ragon Institute of MGH, MIT and Harvard, Cambridge, MA; [2]German Cancer Research Center, DKFZ, Heidelberg, Germany.* ***Visit Poster 062.***

Mon 10 **A Novel Tandem Isobaric Tag (QUANTITY) for Quantitative Analysis of N-glycans;** Shuang Yang[1]; Meiyao Wang[2]; Lijun Chen[1]; Bojiao Yin[1]; Guoqiang Song[3]; Illarion V. Turko[2]; Karen W. Phinney[4]; Michael J. Betenbaugh[1]; Shuwei Li[2]; Hui Zhang[1]; *[1]Hopkins, Baltimore, Maryland; [2]University of Maryland, College Park, MD; [3]Changzhou University, Jiangsu, China; [4]NIST MML, Rockville, MD.* ***Visit Poster 068.***

Mon 11 **Phosphoproteomic Analysis of *in vivo* Cdc14 Phosphatase Substrate Specificity by SWATH-MS;** Brendan Powers; Mark Hall; *Purdue University, West Lafayette, IN.* ***Visit Poster 067.***

Mon 12 **Detecting Cysteine Modifications in Methanogen Methanosarcina Mazei Gö1**; Phuong Nguyen[1, 2]; Hong Hanh Nguyen[1]; Robert Gunsalus[1]; Joseph A Loo[1]; Rachel Loo[1]; *[1]University of California, Los Angeles, California; [2]University of Science, Ho Chi Minh City, Vietnam.* ***Visit Poster 066.***

Mon 13 **A Comprehensive Temporal Analysis of Differentiating Pancreatic β-Islet Cells from Human Embryonic Stem Cells Provides insights into Maturation;** A. Ertugrul Cansizoglu[1]; Quinn Peterson[3]; Shaojun Tang[1]; Douglas Melton[3]; Judith Steen[2]; *[1]Harvard Medical School / BCH, Boston, MA; [2]Boston Children's Hospital, Boston, MA; [3]Harvard Dep. of Stem Cell and Regenerative Biology, Cambridge, MA.* ***Visit Poster 085.***

MONDAY, MARCH 14

Mon 14 **A Proteogenomic Approach to Identify Surface Proteins in Acute Myeloid Leukemia**; Hossein Fazelinia[1]; Kian Huat Lim[1]; Tina Glisovic-Aplenc[1]; Lynn A. Spruce[1]; Ian R. Smith[1]; Sarah K. Tasian[1]; Saar Gill[2]; Richard Aplenc[1]; Steven H. Seeholzer[1]; *[1]The Children's Hospital of Philadelphia, Philadelphia, PA; [2]University of Pennsylvania School of Medicine, Philadelphia, PA.* ***Visit Poster 086.***

Mon 15 **The Signature Exosome Map for Bone Marrow Stromal Cells: The Development of an Optimal Sample Preparation Approach**; Qin (Stefanie) Liang; Michail A. Alterman; *FDA, Silver Spring, MD.* ***Visit Poster 088.***

Mon 16 **The Characterization of IFIX as an Anti-Viral Factor during Infection with DNA Viruses;** Marni S. Crow; Michelle-Ann R. Tan; Tuo Li; Benjamin A. Diner; Ileana M. Cristea; *Princeton Univeristy, Princeton, NJ.* ***Visit Poster 094.***

Mon 17 **Proteome and Secretome Profiling Reveals Temporal Differences in the Toll-like Receptor Responses to Different Ligands**; Marijke Koppenol-Raab; Virginie Sjoelund; Bhaskar Dutta; Zachary Benet; Iain Fraser; Aleksandra Nita-Lazar; *NIH/NIAID, Bethesda, MD.* ***Visit Poster 093.***

Mon 18 **Proteomic Level Identification of Degradation Resistant Proteins, Complexes & Aggregates in Human Plasma**; Hannah Trasatti; Ke Xia; Wilfredo Colon; *RPI, Troy, NY.* ***Visit Poster 097.***

Mon 19 **The Nuclear Proteome of a Vertebrate**; Martin Wühr[1]; Thomas Güttler[2]; Leonid Peshkin[2]; Graeme C. McAlister[2]; Matthew Sonnett[2]; Keisuke Ishihara[2]; Aaron C. Groen[2]; Marc Presler[2]; Brian K. Erickson[2]; Timothy J. Mitchison[2]; Marc Kirschner[2]; Steven P. Gygi[2]; *[1]Princeton University, Princeton, NJ; [2]Harvard Medical School, Boston, MA* ***Visit Poster 114.***

Mon 20 **IEF-SPLC-MS for Generalized High Resolution Intact Glyco-Proteoform Analysis and Top-Down Proteomics**; Steven Patrie; *UT Southwestern Medical Center, Dallas, TX* ***Visit Poster 118.***

LUNCH SEMINARS

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

12:00 – 1:30 PM: **AGILENT TECHNOLOGIES**, *Commonwealth B*

Agilent Technologies

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

Technologies and Insights from Robust and Scalable Targeted Proteomics Assays; Jacob D. Jaffe, *Director - LINCS Proteomic Characterization Center for Signaling and Epigenetics, Associate Director - Proteomics Platform, The Broad Institute*

Innovations for Proteomics Research, Christine Miller, *Agilent Technologies*

12:00 – 1:30 PM: **BRUKER**, *Commonwealth A*

BRUKER

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

Novel LC/MS Strategies for Top-down Proteomics, Ying Ge, *University of Wisconsin-Madison*

In depth Proteomics with the Impact II and Beyond, Matthias Mann, *Max Planck Institute of Biochemistry*

12:00 – 1:30 PM: **THERMO SCIENTIFIC**, *Commonwealth C*

Thermo SCIENTIFIC

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

Ultrasensitive Quantitative Profiling of Single FFPE Tumour Sections from Ovarian Cancers using TMT-MS3 on an Orbitrap Fusion for Clinical Research, Gregg B. Morin, *Michael Smith Genome Sciences Centre, British Columbia Cancer Agency, Department of Medical Genetics, University of British Columbia*

1:30 – 3:00 PM: **POSTER SESSION I**, *Exhibits & Posters*
Odd-numbered boards present **1:30 – 2:15 pm**; **even**-numbered boards present **2:15 – 3:00 pm.**

3:00 – 4:20 PM: PARALLEL SESSION
COMPUTATIONAL PROTEOMICS, *Grand A*
Olga Vitek, presiding

3:00 pm - 3:25 pm	**Discovering and Modeling Relationships among Antibody Properties, Functional Activities, and Protection, using Multidimensional Assays of Polyclonal Samples**; Chris Bailey-Kellogg; *Dartmouth College, Hanover, NH*
3:25 pm – 3:50 pm	**The Perseus Computational Platform for Comprehensive Analysis of Large-scale (Prote)Omics Data**; Juergen Cox, *Max-Planck Institute for Biochemistry*
3:50 pm - 4:05 pm	**Stochastic Modeling of Protein Turnover in Metabolic Labeling**; Mahbubur Rahman; Jayant Avva; Rovshan Sadygov; *UTMB, Galveston, Texas*
4:05 pm - 4:20 pm	**Quantifying Homologous Proteins and Proteoforms**; Nikolai Slavov; *Northeastern University, Boston, MA*

3:00 – 4:20 PM: PARALLEL SESSION
STRUCTURAL PROTEOMICS, *Grand C*
Juri Rappsilber, presiding

3:00 pm- 3:25 pm	**Protein Structure Determination by Mass Spectrometry**; Juri Rappsilber[1, 2]; *[1]Wellcome Trust Centre for Cell Biology, Edinburgh, Scotland, United Kingdom; [2]TU Berlin, Berlin, Germany*
3:25 pm – 3:50 pm	**Environment Affects Structure: Membranes, Binding, and Modification**; John R. Engen; *Northeastern University, Boston, MA*
3:50 pm - 4:05 pm	**A Systematic Exploration of the Human Interactome**; Edward Huttlin; Joao Paulo; Raphael Bruckner; Lily Ting; J. Wade Harper; Steve Gygi; *Harvard Medical School, Boston, MA*
4:05 pm - 4:20 pm	**Development and Application of a Covalent-Labeling Strategy for the Large-Scale Thermodynamic Analysis of Protein Folding and Ligand Binding**; Yingrong Xu; Ryenne Ogburn; Michael C. Fitzgerald; *Duke University, Durham, NC*

4:30 – 5:50 PM: PARALLEL SESSION
PROTEOMICS OF AGING, *Grand A*
Rena Robinson, presiding

4:30 pm - 4:55 pm	**Stratifying Dementia Patients using FLEXITau and Machine Learning Approaches**; Judith Steen; *Boston Children's Hospital, Boston, MA*
4:55 pm - 5:20 pm	**Enhanced Multiplexing Proteomics and the Role of the Periphery in Alzheimer's Disease**; Renã Robinson; *University of Pittsburgh, Pittsburgh, PA*
5:20 pm - 5:35 pm	**Protein Co-Expression Network Analysis Reveals Cell Type Changes Linked to Alzheimer's Disease Risk**; Nicholas Seyfried[1]; Eric Dammer[1]; Vivek Swarup[2]; Divya Nandakumar[1]; Duc Duong[1]; Luming Yin[1]; Qiudong Deng[1]; Tram Nguyen[1]; Marla Gearing[1]; Madhav Thambisetty[3]; Juan Troncoso[4]; Daniel Geschwind[2]; James Lah[1]; Allan Levey[1]; *[1]Emory University School of Medicine, Atlanta, Georgia; [2]UCLA School of Medicine, Los Angeles , CA; [3]National Institute on Aging, Baltimore, MD; [4]Johns Hopkins School of Medicine, Baltimore, MD*
5:35 pm - 5:50 pm	**Identification of Age-Related Protein Folding Stability Differences in the Mouse Proteome**; Julia Roberts; Michael C. Fitzgerald; *Duke University, Durham, North Carolina*

4:30 – 5:50 PM: PARALLEL SESSION
IMAGING, *Grand C*
Jeffrey Spraggins, presiding

4:30 pm – 4:55 pm **Next-Generation MALDI Imaging Mass Spectrometry Capabilities for Spatial Proteomics**; Jeffrey Spraggins, *Vanderbilt University*

4:55 pm - 5:20 pm **Lipid Imaging for Cancer Diagnosis by Ambient Ionization Mass Spectrometry**; Livia Eberlin; *University of Texas at Austin, Austin, TX*

5:20 pm - 5:35 pm **Investigation of the Molecular Pathology of Traumatic Brain Injury by Imaging Mass Spectrometry**; Bo Yan[1]; Andrew Fisher[2]; Mark Wojnarowicz[1]; Olga Minaeva[3]; Yi Pu[1]; Mark E. McComb[1]; Lee E. Goldstein[1]; Catherine E. Costello[1]; *[1]Boston University School of Medicine, Boston, MA; [2]College of Engineering, Boston University, Boston, MA; [3]Boston University Photonics Center, Boston, MA*

5:35 pm – 5:50 pm **Supervised and Unsupervised Analysis of Mass Spectrometry Imaging Experiments Using Cardinal**; Kyle Bemis[1]; April Harry[1]; David Calligaris[2]; Armen Changelian[2]; Sandro Santagata[2]; Nathalie Agar[2]; Olga Vitek[3]; *[1]Purdue University, West Lafayette, IN; [2]Brigham & Women's Hospital, Boston, MA; [3]Northeastern University, Boston, MA*

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

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

MONDAY, MARCH 14

EVENING WORKSHOPS

There are four concurrent workshops. All attendees are invited to participate in these informal and more interactive sessions.

6:30 – 8:00 PM: PARALLEL EVENING WORKSHOP
ELEVATOR PITCH –SHARING YOUR SCIENCE, *Commonwealth C*
Organizer: Rob Rivers, *NIH*
All conference attendees are invited to participate in any of the four parallel evening workshops.

We all know the feeling after several months of hard work there is finally enough data to begin to piece together a coherent message to present at US HUPO. Now What?

- How best to engage with people that stop by your poster?
- How can you engage with other researchers in order to maximize the opportunity and hopefully improve your work through productive conversations?

In the Elevator Pitch – Sharing your Science Workshop participants will learn key tools and strategies that allow them to share their work in clear and coherent ways. The workshop will be a mixture of a short presentation and opportunities to apply concepts with other participants. At the end of the workshop you will be able to convey your work and its purpose in less than 90 seconds.

6:30 – 8:00 PM: PARALLEL EVENING WORKSHOP
THE ABC's AND XYZ's OF STARTING YOUR OWN MASS SPEC LAB *Commonwealth A*
Organizer: Sasha Singh and John Froelich, *Brigham & Women's Hospital*
All conference attendees are invited to participate in any of the four parallel evening workshops.

Many of you are currently working in a proteomics group or collaborating with one. Either way, the instruments are up and running and any concern as to how they got there luckily does not impact your research.

But what does it take to set up a mass spectrometry lab? What if you are applying for a faculty position that is providing the candidate the opportunity to build a proteomics program? How ready are you to pitch a program that includes a start-up and installation phase? These points often deter researchers from taking on such a challenge.

This workshop will provide general considerations and specific to-do lists for a timeline that starts from instrument research and ends at acquiring your first MS run. We will also provide estimated costs for budgetary and funding purposes. Be as prepared as you can be to start your lab on the right track. The workshop is built from contributions by researchers that have recently started their own labs, and from instrument vendors and funding agencies. Participants will have the opportunity to ask questions.

6:30 – 8:00 PM: PARALLEL EVENING WORKSHOP
HOW TO OBTAIN AN ACADEMIC FACULTY POSITION AND KEEP IT!, *Commonwealth B*
Organizer:Ben Garcia, *University of Pennsylvania*
All conference attendees are invited to participate in any of the four parallel evening workshops.

This workshop is targeted at the next generation of MS scientists to give information, advice and support for those interested in an academic career. The workshop will focus on how to keep on the path to an academic position (doing exciting research, networking, putting together an academic application, securing good letters of recommendation, preparing the research statement and applying and interviewing for faculty positions) for graduate students/postdocs. Additionally, advice on how to also keep on the path to tenure (hiring the right people, building good collaborations, writing grants and papers, presenting your work, etc.) will also be presented. The workshop will be composed of an informal lecture presentation to provide information for beginning academic scientists, paired with a panel discussion (made up of current established young to mid-range academic faculty) to give attendees a chance to ask questions.

6:30 – 8:00 PM: PARALLEL EVENING WORKSHOP (4 of 4)
COMPUTATION AND STATISTICS FOR QUANTITATIVE PROTEOMICS, *Grand C*
Organizer: Olga Vitek, *Northeastern University*
All conference attendees are invited to participate in any of the four parallel evening workshops.

This evening workshop is dedicated to recent developments in computational and statistical methods for quantitative proteomics. The workshop will include brief presentations from 3 speakers: Brendan MacLean from University of Washington, the lead developer of Skyline and the recipient of the Gilbert S. Omenn Computational Proteomics Award, Juergen Cox from Max Planck Institute Munich, the lead developer of MaxQuant, and Meena Choi from Purdue University, the lead developer of MSstats. The presentations will be followed by an informal discussion of open problems.

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

8:30 – 9:25 AM: PLENARY SESSION
AWARD PRESENTATIONS AND LECTURES, *Grand A*
Gil Omenn, presiding

8:30 – 8:55 am **Robert J. Cotter New Investigator Award: Paola Picotti**, *ETH Zurich*
Award presentation followed by 20 minute talk.

8:55 – 9:20 am **Gilbert S. Omenn Computational Proteomics Award: Brendan MacLean**, *University of Washington*
Award presentation followed by 20 minute talk.

9:20 – 9:25 am **Poster Awards** – Best Graduate Student Poster, Best Undergraduate Poster Award

9:25 - 9:40 AM: **COFFEE BREAK**
Coffee and pastries with the exhibitors.

9:50 – 11:10 AM: PARALLEL SESSION
CROSS'OMICS, *Grand A*
Wilhelm Haas, presiding

9:50 am - 10:15 am **The Role of Transcriptomics and Proteomics in Defining Functional Protein Networks**; Wilhelm Haas; *Massachusetts General Hospital, Charlestown, MA*

10:15 am – 10:40 am **Victoria D'Souza**, Harvard University

10:40 am - 10:55 am **Introducing Epigenomics in Systems Biology: Cross-Talk between Cell Signal Transduction and Epigenetic Mechanisms**; Simone Sidoli; Pau Pascual Garcia; Katarzyna Kulej; Maya Capelson; Benjamin A. Garcia; *University of Pennsylvania, Philadelphia, Pennsylvania*

10:55 am - 11:10 am **Characterization of Human Sirtuin 2 Interactome and substrates**; Hanna Budayeva; Ileana Cristea; *Princeton University, Princeton, New Jersey*

9:50 – 11:10 AM: PARALLEL SESSION
PEDIATRIC PROTEOMICS, *Grand C*
Hanno Steen, presiding

9:50 am - 10:15 am **Developmental Proteomics: Unravelling Age Specific Differences in the Human Proteome**; Vera Ignjatovic[1, 2]; *[1]Murdoch Childrens Research Institute, Melbourne, Victoria; [2]University of Melbourne, Parkville, Victoria, Australia*

10:15 am – 10:40 am **Towards Comprehensive and Quantitative Proteomics for Diagnosis and Therapy of Childhood Disease**, Alex Kentsis, *Memorial Sloan-Kettering Cancer Center*

10:40 am – 10:55 am **Adjuvant-Induced Human Monocyte Secretome Profiles Reveal Adjuvant- and Age-Specific Protein Signatures**; David Dowling[1, 2]; Djin-Ye Oh[3, 4]; Spencer Brightman[1]; Sebastian Berger[1, 2]; Hanno Steen[1, 2]; Ofer Levy[1, 2]; *[1]Boston Children's Hospital, Boston, MA; [2]Harvard Medical School, Boston, MA; [3]Boston Childrens Hospital / Harvard Medical School, Boston, MA; [4]New York University Medical School, New York, NY*

10:55 am - 11:10 am **Serum Proteomes Distinguish Children Developing Type-1 Diabetes**; D Goodlett[1]; R Moulder[2]; S Bhosale[2]; J Mykkänen[2]; T Erkkilä[3]; E Laajala[2]; J Salmi[2]; E Nguyen[7]; H Kallionpaa[2]; H Hyöty[4]; R Veijola[5]; J Ilonen[6]; T Simell[2]; J Toppari[2]; M Knipp[2]; H Lähdesmäki[4]; O Simell[2]; R Lahesmaa[2]; *[1]University of Maryland, Baltimore, USA; [2]University of Turku, Turku, Finland; [3]University of Aalto, Espoo, Finland; [4]University of Tampere, Tampere, Finland; [5]University of Helsinki, Helsinki, Finland; [6]Hospital District of Southwest Finland, Turku, Finland; [7]Monash University, Melbourne, Australia*

11:10 AM – 12:00 PM: PLENARY SESSION
LIGHTNING TALKS II, *Grand A*
Robert Moritz and Robert Rivers, presiding
High-energy (and brief) presentations selected from poster presentations.

Presentation Order

Tue 01 **Characterization of Ubiquitin Trimers by Top-down Mass Spectrometry**; Catherine Fenselau; Amanda E. Lee; Lucia Geis-Asteggiante; Emma K. Dixon; Yeji Kim; Tanuja R. Kashyap; Yan Wang; David Fushman; *University of Maryland, College Park, MD.* ***Visit Poster 116.***

Tue 02 **Quantitative Proteomic Profiling of PANDER Transgenic Mice Reveals Increased Lipogensis and Fatty Acid Synthesis Modulated by the Liver X Receptor**; Mark Athanason; Stanley Stevens; Brant Burkhardt; *University of South Florida, Tampa, FL.* ***Visit Poster 115.***

Tue 03 **Rigorous MRM Quantitation of a Multiplexed Panel of Salivary Proteins for Biomarker Assessment Studies;** Andrew Percy[1]; Darryl Hardie[1]; Juncong Yang[1]; Armando Jardim[2]; Yassene Mohammed[3]; Christoph H. Borchers[1]; *[1]University of Victoria/Genome BC Proteomics Centre, Victoria, BC; [2]McGill University, Montreal, QB; [3]Leiden University Medical Center, Leiden, Netherlands.* ***Visit Poster 111.***

Tue 04 **Understanding the Network Signaling Capacity of HBx in HBV Host Infection**; Emanuela Milani; Charlotte Nicod; Bernd Wollscheid; *ETH Zurich, Zurich, Switzerland.* ***Visit Poster 096.***

Tue 05 **Getting a Grip on What Determines the Composition of Urinary Proteomes;** Jan Muntel[1, 2]; Sebastian T. Berger[1, 2]; Jennifer K. Cheng[1]; Sarah D. de Ferranti[1, 2]; Nirav K. Desai[1, 2]; Tracy K. Richmond[1, 2]; Kendrin R. Sonneville[3, 4]; Stavroula K. Osganian[1, 2]; Hanno Steen[1, 2]; *[1]Boston Children's Hospital, Boston, MA; [2]Harvard Medical School, Boston, MA; [3]Harvard T.H. Chan School of Public Health, Boston, MA; [4]University of Michigan School of Public Health, Ann Arbor, MI.* ***Visit Poster 032.***

Tue 06 **Src-Family Kinase Signaling Mediating Gemoitabine Resistance in Gallbladder Cancer Revealed by Quantitative Phosphoproteomics**; Patricia García[1]; Jun Zhong[2]; Carolina Bizama[1]; Jaime Espinoza[1]; Juan Carlos Roa[1]; Pamela Leal[3]; *[1]Pontificia Universidad Católica de Chile, Santiago, Chile; [2]Delta Omics Biotechnology, Rockville, MD; [3]Universidad de La Frontera, Temuco, Chile.* ***Visit Poster 022.***

Tue 07 **Inter-grade Comparative Proteomic Analysis of Gliomas using Cerebrospinal Fluid**; Nikita Gahoi[1]; Darpan Malhotra[1]; Aliasgar Moiyadi[2]; Sanjeeva Srivastava*[1]; *[1]Indian Institute of Technology, Bombay, Mumbai, India; [2]Department of Neurosurgery, ACTREC, Mumbai , India.* ***Visit Poster 020.***

Tue 08 **The Comparison of the Serum Proteome in Individuals with Cancers versus Those without Cancer, and Its Application to Wellness**; Haiyan Zheng[1]; Caifeng Zhao[1]; Swapan Roy[2]; Devjit Roy[3]; Amenah Soherwardy[2]; Ravish Amin[2]; Matthew Kuruc[2]; *[1]Rutgers Center for Integrative Proteomics, Piscataway, NJ; [2]Biotech Support Group LLC, Monmouth Junction, New Jersey; [3]Wyoming Medical Center, Casper, WY.* ***Visit Poster 017.***

Tue 09 **Analysis of Vascular Endothelial Growth Factor Receptor 2 (VEGFR-2) N-Glycosylation Sites using PNGase F/18O Labeling and Tandem Mass Spectrometry**; Kevin Chandler; Deborah Leon; Rosana Meyer; Nader Rahimi; Catherine Costello; *Boston University School of Medicine, Boston, MA.* ***Visit Poster 078.***

Tue 10 **Phosphoproteomic Comparison of Osteoblasts Stimulated with Forteo or Biased PTH1R Ligand as Determined via SILAC**; Grace Williams; *MUSC, Charleston, SC.* ***Visit Poster 080.***

Tue 11 **Identifying Host Factors Associated with Replicating Viral DNA**; Emigdio D. Reyes; Katarzyna Kulej; Daphne C. Avgousti; Lisa Akhtar; Daniel Bricker; Neha Pancholi; Sarah Koniski; Benjamin A. Garcia; Matthew D. Weitzman; *University of Pennsylvania, PA.* ***Visit Poster 061.***

Tue 12 **Cerebral Organoid Proteomics to Study Central Nervous System Development in Down Syndrome**; Tristan Mcclure-Begley; Christopher Ebmeier; Michael Klymkowsky; Kerri Ball; William Old; *University of Colorado, Boulder, Boulder, CO.* ***Visit Poster 058.***

Tue 13 **Differential Dynamics of the Mammalian mRNA and Protein Expression Response to Misfolding Stress;** Zhe Cheng[1]; Guoshou Teo[2]; Sabrina Krueger[3]; Tara Rock[1]; Hiromi Koh[2]; Hyungwon Choi[2]; Christine Vogel[1]; *[1]New York University, New York, NY; [2]National University of Singapore, Singapore, Singapore; [3]Max-Delbruck-Center, Berlin, Germany.* ***Visit Poster 052.***

Tue 14 **Species Identification using Bayesian Modeling and Mass Spectrometry;** Jennifer Teubl; *NYU Langone Medical Center, New York, NY.* ***Visit Poster 043.***

Tue 15 **Rapid Changes in the Proteome of Gut Microbiota in Response to Short-Term Dietary Changes in Baboons**; Prahlad Rao; Kimberly Spradling-Reeves; Vicki Mattern; Laura Cox; Anthony Comuzzie; Michael Olivier; *Texas Biomed Res Inst., San Antonio, TX.* ***Visit Poster 092.***

TUESDAY, MARCH 15

Tue 16 **A Biomimetic, Synthetic RNA platform for *in vivo*, Co-Translational Labeling of Proteins;** Randi Turner; Daniel Dwyer; *University of Maryland, College Park, MD.* ***Visit Poster 091.***

Tue 17 **Quantitative Proteomics & Network Analysis Identifies Retinoic Acid Therapy to Bypass Impaired Vitamin A Metabolism in Heart Failure;** Ni Yang[1]; TIng Liu[1]; Brian O'Rourke[1]; Maureen Kane[2]; D. Brian Foster[1]; *[1]Johns Hopkins School of Medicine, Baltimore, Maryland; [2]University of Maryland Medical Center, Baltimore, MD.* ***Visit Poster 089.***

Tue 18 **Monitoring Signal-Specific Changes in Ribosome Maintenance and Biosynthesis *in vivo***; Andrew Mathis; Bradley Naylor; John Price; *Brigham Young University, Provo, UT.* ***Visit Poster 087.***

Tue 19 **When Can Glycopeptides Be Assigned Based Solely on Tandem Mass Spectrometry Data?;** Kshitij Khatri; Joshua Klein; Joseph Zaia; *Boston University, Boston, MA.* ***Visit Poster 074.***

Tues 20 **A Cross-Sectional Study on Dietary Factors and their Correlation with Body Mass Index among School-going Teenagers in Karachi**; Arshma Zuberi; *Dow University of Health Sciences and Jinnah Unive, Karachi, Pakistan.* ***Visit Poster 035.***

LUNCH SEMINARS

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

12:00 – 1:30 PM: **SCIEX**, *Commonwealth C*

If you have not already registered in advance, please look for SCIEX representative to RSVP.

New Innovations Towards Industrializing Proteomics

Next-generation Solutions to Industrialize Large-Scale Proteomics Studies and Advance Precision Medicine
Mark Cafazzo, Director Academic Business, SCIEX

From PCT-HD to SWATH® Data Acquisition in Half a Day
Vera Gross, Ph.D., Senior Scientist, Pressure Biosciences, Inc.

Overview of iPathwayGuide For Next-Gen Proteomics
Andrew Olson, VP of Business Development, Advaita Corporation

1:30 – 3:00 PM: **POSTER SESSION II**, *Exhibits & Posters*
Odd-numbered boards present **1:30 – 2:15 pm**; **even**-numbered boards present **2:15 – 3:00 pm**.

3:00 – 4:20 PM: PARALLEL SESSION
MICROBIOLOGY IN PROTEOMICS, *Grand A*
Ileana Cristea, presiding

3:00 pm - 3:25 pm **Spatial-Temporal Dynamics of Host Organelle Morphology and Composition during Herpes Virus Infection**; Ileana Cristea; *Princeton University, Princeton, NJ*

3:25 pm - 3:50 pm **What Can MALDI-TOF MS Do for the Clinical Microbiology Laboratory?** Mark Fisher; *Univ. of Utah / ARUP Laboratories, Salt Lake City, UT*

3:50 - 4:05 pm **Application of UV and Chemical Cross-Linking in Combination with Mass Spectrometry and Deep Sequencing to Study Complex Interaction Networks**; Yu Qian[1, 2]; Catherine E Costello[1]; Ruslan Afasizhev[2]; *[1]Boston University School of Medicine, Boston, MA; [2]Boston University School of Dental Medicine, Boston, MA*

4:05 pm - 4:20 pm **What the Acetylomes Tell Us about Sirtuin Promiscuity: Lessons from Bacterial and Yeast Sirtuins**; Brian Weinert; Chuna Choudhary; *University of Copenhagen, Copenhagen, Denmark*

3:00 – 4:20 PM: PARALLEL SESSION
PROTEOGENOMICS, *Grand C*
Alexey Nesvizhskii, presiding

3:00 - 3:25 pm **Revisiting FDR Estimation and Protein Inference in Large Proteomics and Proteogenomics Datasets;** Alexey Nesvizhskii, *University of Michigan*

3:25 pm - 3:50 pm **Insights into Dynamic Gene Expression Regulation from Integrative Analyses**; Christine Vogel; *New York University, New York, New York*

3:50 pm - 4:05 pm **Integration of PTM Knowledge Networks with Multi-Level Omics Data for Analysis of PTMs in Cancer**; Karen E. Ross[1]; Cathy H. Wu[2]; *[1]Georgetown University Medical Center, Washington, DC; [2]University of Delaware, Newark, DE*

4:05 pm - 4:20 pm **Comprehensive Genomics and Proteomics Analyses Reveal Extensive Tumor Heterogeneity in Lung Adenocarcinoma**; Xu Zhang; Shaojian Gao; Constance Cultraro; Romi Biswas; Tapan Maity; Udayan Guha; *CCR, NCI, NIH, Bethesda, MD*

4:30 – 5:50 PM: PARALLEL SESSION
CANCER PROTEOMICS, *Grand A*
Thomas Kislinger, presiding

4:30 pm - 4:55 pm **Membrane Proteomics: Surface Markers & Horizontal Signaling**; Thomas Kislinger; *University of Toronto, Toronto, Canada*

4:55 pm – 5:20 pm **Global Ubiquitylome Profiling for the Identification of Drug Targets in Cancer**, Namrata Udeshi, *Broad Institute of MIT and Harvard*

5:20 pm - 5:35 pm **Predicting Ovarian Cancer Patients' Clinical Response to Platinum-based Chemotherapy by their Tumor Proteomic Signature**; Kun-Hsing Yu[1]; Douglas Levine[2]; Hui Zhang[3]; Daniel Chan[3]; Zhen Zhang[3]; Michael Snyder[1]; *[1]Stanford University, Stanford, CA; [2]Memorial Sloan Kettering Cancer Center, New York City, NY; [3]Johns Hopkins Medical Institutions, Baltimore, MD*

5:35 pm - 5:50 pm **Affinity Proteomics Establishes a Bifurcated Signaling Cascade of NIMA-related Kinases that Regulate Cell Division**; Sierra Cullati; Rufus Hards; Lilian Kabeche; Scott Gerber; *Geisel School of Medicine at Dartmouth, Lebanon, New Hampshire*

4:30 – 5:50 PM: PARALLEL SESSION
NEW DEVELOPMENTS IN PROTEOMICS, *Grand C*
Sasha Singh, presiding

4:30 pm - 4:55 pm **High Resolution/Accurate Mass Parallel Reaction Monitoring to Measure Stable Isotope Enrichment of Proteins in Kinetic Studies with Endogenous Labeling**; Sasha Singh; *Brigham and Women's Hospital, Boston, MA*

4:55 pm - 5:20 pm **Ion Mobility Spectrometry Coming of Age**; Melvin Park; *Bruker Daltonics, Billerica, MA*

5:20 pm - 5:35 pm **N-Linked Glycoproteomic Analysis using N-linked Glycans And Glycosite-containing (NGAG) Method**; Shisheng Sun; Punit Shah; Shadi Toghi Eshghi; Hui Zhang; *Johns Hopkins University, Baltimore, MD*

5:35 pm - 5:50 pm **SIMPLEX: a Combinatorial Multimolecular Omics Approach for Systems Biology**; Cristina Coman; Fiorella Andrea Solari; Andreas Hentschel; Rene Peiman Zahedi; Albert Sickmann; Robert Ahrends; *ISAS, Dortmund, Germany*

6:30 – 8:30 PM: **SOCIAL EVENT AT THE BARKING CRAB**
Tickets included with your registration envelope.
The Barking Crab is walking distance from the Westin Waterfront. Look for walking instructions at Registration window.

Relax and enjoy a casual dinner and drinks with your colleagues at this Boston instituation. Fantastic views of the city and bay.

Sponsored by
PBI Pressure BioSciences Inc.

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

8:30 – 9:20 AM: PLENARY SESSION
TIPS & TRICKS, *Grand A*
Technology (relaxed) Lightning Session
High-energy, 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

01 **Application OF Microfluidic/Tandem Quadrupole LC-MS/MS for MRM Based Translational Research ANALYSIS of Putative Heart FAILURE Peptide Biomarkers in Human Plasma;** Richard Mbasu[1, 2]; Liam Heaney[2]; Billy Molloy[3]; Chris Hughes[3]; Roy Martin[4]; Leong Ng[2]; Johannes Vissers[3]; James Langridge[3]; Don Jones[1, 2]**;** *[1]Department of Cancer Studies, RKCSB, University of, Leicester, UK; [2]Department of Cardiovascular Sciences and NIHR, Leicester, UK; [3]Waters Corp, Wilmslow, UK; [4]Waters, Beverly, Massachusetts*

02 **OQ-STrap Technology for Processing of Large Protein Loads**; John Wilson[1]; Darryl Pappin[1]; Rosamonde Banks[2]; Alexandre Zougman[2]; *[1]Protifi, LLC, Huntington, NY; [2]University of Leeds, Leeds, UK*

03 **Quantitative Analysis of AKT/mTOR Pathway Using Multiplex-Immunoprecipitation and Targeted Mass Spectrometry**; Bhavin Patel[1]; Alex Behling[1]; Leigh Foster[1]; Ryan Bomgarden[1]; Carrie Clothier[1]; Kay Opperman[1]; Rosa Viner[2]; Andreas Huhmer[2]; John Rogers[1]*; [1]Thermo Fisher Scientific, Rockford, IL; [2]Thermo Fisher Scientific, San Jose, CA*

04 **Protein-Based PTM Quantitative Analysis with PEAKS Software**; Baozhen Shan; Lei Xin; *Bioinformatics Solutions Inc, Waterloo, Canada*

05 **Industrializing SWATH Proteomics with Microflow LC**; Christie Hunter[1]; Ken Hamill[2]; *[1]SCIEX, Redwood City, CA; [2]SCIEX, Framingham, MA*

06 **Demystifying Proteolytic Digestion under High Pressure Cycling: Enzyme Specificity, Efficiency and Synergy of Pressure with Acid-Labile Detergents**; Vera Gross[1]; John Wilson[2]; Alexander Lazarev[1]; Darryl Pappin[2]; *[1]Pressure BioSciences, Inc, Medford, MA; [2]Protifi, LLC, Huntington, NY*

07 **Efficient Enrichment of SUMOylated Peptides from Alpha-lytic Protease Digest Using K-Ɛ-GG Remnant Immuno-affinity Purification** ; Hongbo Gu[1]; Xiaoying Jia[1]; Jianmin Ren[1]; Elizabeth Komives[2]; Matthew Stokes[1]; *[1]Cell Signaling Technology, Danvers, Massachusetts; [2]Department of Chemistry & Biochemistry, UCSD, La Jolla, CA*

08 **MRM Assays and Tools for Quantifying Multiplexed Panels of Proteins in 4 Mouse Tissues**; Andrew Percy[1]; Sarah Michaud[2]; Nicholas Sinclair[1]; Yassene Mohammed[3]; Christoph Borchers[1]; *[1]UVic-Genome BC Proteomics Centre, Victoria, Canada; [2]MRM Proteomics, Victoria, BC; [3]Leiden University Medical Center, Leiden, Netherlands*

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

9:50 – 11:10 AM: PARALLEL SESSION
BIOMARKERS, *Grand A*
Jennifer Van Eyk, presiding

9:50 am - 10:15 am **Proteomics-based Biomarker Discovery: Mirage or Emerging Reality?** Steven A. Carr; *Broad Institute of MIT and Harvard, Cambridge, MA*

10:15 am – 10:40 am **Jennifer Van Eyk**, *Cedars-Sinai Medical Center*

10:40 am - 10:55 am **An Affinity Proteomics Strategy for Plasma Biomarker Validation**; Claudia Fredolini; Sanna Byström; Elin Birgersson; Peter Nilsson; Mathias Uhlén; Jochen Schwenk; *Science for Life Laboratory - KTH, Solna, Sweden*

10:55 am - 11:10 am **Application and Optimization of MStern Blot for Translational Proteomics**; Sebastian T. Berger[1, 2]; Saima Ahmed[1, 2]; Jan Muntel[1, 2]; Michaela Helmel[1, 2]; Richard Bachur[1]; Alex Kentsis[3]; Hanno Steen[1, 2]; *[1]Boston Children's Hospital, Boston, MA; [2]Harvard Medical School, Boston, MA; [3]Sloan Kettering Institute / Cornell University, New York, NY*

9:50 – 11:10 AM: PARALLEL SESSION
NEUROPROTEOMICS, *Grand C*
Judith Steen, presiding

9:50 am – 10:15 am **Interrogation of Signaling Pathways in Parkinson's Disease**, Jarrod Marto, *Dana-Farber Cancer Institute, Harvard University*

10:15 am – 10:40 am **Lessons Learnt from a Cell Type– and Brain Region–Resolved Brain Protein Atlas**, Kirti Sharma *Max-Planck Institute for Biochemistry*

10:40 am - 10:55 am **Characterization of Depolarization-Dependent Signaling Pathways in the Active Zone in Isolated Nerve-Terminals**; Martin R. Larsen[1]; Simone Sidoli[2]; Katarzyna Kulej[2]; Jing Xue[3]; Maria Ibanez Vea[1]; Mark Graham[3]; Phillip J. Robinson[3]; *[1]University of Southern Denmark, Odense M, Denmark; [2]Perelman School of Medicine, University of Pennsyl, Philadelphia , PA; [3]Chrildren´s Medical Research Institute,, Wentworthville NSW 2145, Australia*

10:55 am - 11:10 am **Proteomic Analysis to Identify Molecular Regulators of Nerve Regeneration**; Ajay Yekkirala[1, 2]; Hui Chen[1, 2]; Kristina Hempel[1, 2]; Judith Steen[1, 2]; Clifford Woolf[1, 2]; *[1]Boston children's Hospital, Boston, MA; [2]Harvard Medical School, Boston, MA*

11:10 AM – 12:00 PM: **PLENARY LECTURE + CLOSING SESSION**, *Grand A*
Hanno Steen, presiding

11:10 am – 11:55 am **Sangeeta Bhatia**, *Massachusetts Institute of Technology*

11:55 am – 12:00 pm Closing Remarks: Hanno Steen

MONDAY 9:50 – 11:10 AM
DRUG DEVELOPMENT, *Grand A*

MOB 9:50 am - 10:15 am: Drug action in the Context of the Proteome

Marcus Bantscheff
Cellzome a GSK company, Heidelberg, Germany

In the last few years, our ability to study drug action on the proteome has increased remarkably in dimensionality. Robust quantitative technologies enable the study of protein abundances, activation state, turnover and many other parameters with spatiotemporal resolution. These approaches allow to study cellular and tissue phenotypes in a disease context and their modulation by bioactive molecules at an unprecedented level of detail. Direct target identification methodologies have diversified and now allow the comprehensive analysis of cellular targets of bioactive compounds in live cells and correlation of target engagement with effects on the proteotype.

This presentation describes experimental strategies in current chemical proteomics research, discusses recent examples of successful applications, and highlights areas in drug discovery where proteomics has impact.

MOB 10:15 am - 10:40 am: Investigating Protein-Protein Interaction Networks to Fuel Drug Discovery at Genentech

Erik Verschueren
Genentech, South San Francisco, CA

In order to design therapeutics for the treatment of maladies such as cancer, auto-immunity and neuro-degeneration it is important to understand the basic molecular signaling mechanisms responsible for the onset of these etiologies. A number of discovery proteomic platforms at Genentech are engaged to investigate such signaling mechanisms including protein interactome studies, global post-translational modification analysis and chemo-proteomics. Here we present the application of our Affinity Purification Mass Spectrometry (AP-MS) platform to interrogate the protein interaction network associated with Protein Arginine MethylTransferase (PRMTs). Deregulation of this class of enzymes has recently been linked to carcinogenesis and metastasis and is likely implicated in the pathogenesis of several different diseases. To systematically compile the interaction network of the human PRMT family we collected data using complementary affinity tagging protocols and employed specialized AP-MS scoring algorithms to identify key interacting partners for all 9 PRMTs. In addition to technical challenges & details of these workflows we also highlight how we can empower findings of isolated proteomics studies in the context of orthogonal datasets.

MOB 10:40 am - 10:55 am: gMODs: An Open Source Data Analysis Tool for Quantifying the Differential Site Occupancy of Therapeutic Protein Modifications

Tsung-Heng Tsai[1]; Zhiqi Hao[2]; Benjamin Moore[2]; Qiuting Hong[2]; Cinzia Stella[2]; Jeffrey Zhang[2]; Yan Chen[2]; Michael Kim[2]; Theo Koulis[3]; Erik Verschueren[4]; Fred Jacobson[2]; Olga Vitek[1]; William Haskins[2]
[1]Northeastern University, Boston, MA; [2]Protein Analytical Chemistry, Genentech, South San Francisco, CA; [3]Nonclinical Biostatistics, Genentech, South San Francisco, CA; [4]Protein Chemistry, Genentech, South San Francisco, CA

We hypothesized that an open source data analysis tool for quantifying the differential site occupancy of chemical and post-translational modifications might improve our therapeutic protein characterization efforts. To test our hypothesis, we first collected liquid chromatography-mass spectrometry (LC/MS)- and liquid chromatography-tandem mass spectrometry (LC/MS/MS)-based peptide mapping data for reference samples, forced degradation and enriched minor variant samples of an antibody drug conjugate (ADC) and its monoclonal antibody intermediate (AI). Second, Skyline was used to construct digital libraries from LC/MS- and LC/MS/MS data and from qualitative chromatographic peak assignments based on sequence database searching. Third, a novel R tool called gMODs, an extension of MSstats, was developed to determine the site occupancy for each modification and amino acid residue in the AI and ADC. To achieve this, gMODs models the site occupancy of a modified peptide as its relative abundance, calculated from the integrated peak area of each peptide feature (charge state, isotope and modification), to all modified forms of that peptide. To develop an appropriate statistical framework for detecting differential site occupancy, we investigated statistical approaches by: i) performing statistical testing for each modification separately with subsequent summarization of all modified forms of a peptide, or ii) translating the abundance of modified forms of the peptide into compositions and performing compositional data analysis. We carried out a series of simulations to evaluate both approaches based on their ability to detect unaccompanied and complementary changes in modified peptides, in consideration of various experimental scenarios defined by effect size, measurement variability, number of replicates and number of modification forms. The insights and conclusions derived from these efforts are all integrated into gMODs. Based on comparisons to the results from orthogonal methods, gMODs greatly improved our confidence in quantifying statistically significant differences in the site occupancy of therapeutic protein modifications.

MOB 10:55 am - 11:10 am: Improving Drug Target Space Coverage of Chemical Proteomics with Photoaffinity Labeling-based Approaches

Jason Thomas; Scott Brittain; Jennifer Lipps; Markus Schirle
Novartis, Cambridge, MA

Quantitative chemical proteomics has emerged as a powerful affinity-based approach for the elucidation of cellular targets for hit compounds from phenotypic screens. Starting from the endogenously expressed proteome of a disease-relevant cell line, an interaction profile of proteins capable of binding to the compound of interest is generated. From this primary hit list, individual target hypotheses can be prioritized for cellular validation experiments by considering factors such as relative potency of binding and consistency of the binding profile with the cellular structure-activity relationship. Typical lysate-based experiments using reversible inhibitors have been highly successful for a wide variety of protein families. However, it has also become clear that lysate-based chemical proteomics often miss important classes of proteins which require a near native cellular environment to retain compound binding competence; for example, ion channels and G-protein coupled receptors. In these cases, covalent strategies such as photoaffinity labeling-based experiments using live cell treatment have proven to be successful but require careful experimental design and optimization. Our efforts towards improving live cell photoaffinity-labeling experiments will be presented.

MONDAY 9:50 – 11:10 AM
TOP-DOWN PROTEOMICS, *Grand C*

MOC 9:50 am - 10:15 am: Heavy Sugar or Water Create Arbitrary Changes in Isotope Distribution (ACID) for Quantitative MS of any Biomolecule, Feed, and Organism

Jeniffer V. Quijada; Joseph P. Salisbury; Jared R. Auclair; Jeffrey N. Agar
Northeastern University, Boston, MA

A labeled central metabolic precursor, here 13C-sugar or 2H-water, is spiked into any feedstock and incorporated into all biomolecules through central metabolic pathways. This results in (Arbitrary) Changes of Isotopic Distributions (ACID), creating “heavy” peaks for isotope dilution MS. This relatively inexpensive, multiplex technique is applicable to any model organism, bottom-up MS, and is shown here to be particularly effective for intact protein and lipid quantitation. Microbes and animals are problematic for SILAC, but are easily labeled using our technique. ACID is a partial metabolic labeling method that employs atom-replacement with heavy isotopes to increase the mass of biomolecules until their MS peaks can be distinguished from natural abundance peaks. An arbitrary (even imprecise) amount of labeled metabolic precursor – any applicable metabolic source of C, H, N, or O can be used – is added to or replaces part of the feed. The application of ACID to intact protein quantitation is demonstrated in bacteria, yeast, and an insect. ACID is shown to compare favorably to existing methods for intact protein quantitation. ACID avoids many of the weaknesses of existing metabolic labeling strategies, while having the following strengths i) cost (cheaper sources of label and less label used), ii) predictable peak shifts, iii) applicability to any model organism, iv) compatibility with partial-labeling proteomics workflows, v) no requirement for the identity of the molecules that are being quantified, and vi) fast and facile labeling.

MOC 10:15 am - 10:35 am: Top-Down Mass Spectrometry-based Proteomics: Challenges and Opportunities

Ying Ge
University of Wisconsin-Madison, Madison, WI

Proteomics is essential for deciphering how molecules interact as a system and for understanding the functions of cellular systems in human disease; however, the human proteome is extremely complex due to a plethora of post-translational modifications (PTMs) and sequence variations. The emerging top-down mass spectrometry (MS)-based proteomics, which is based on analysis of intact proteins, is arguably the most powerful method to comprehensively characterize proteoforms that arise from genetic variations, alternative splicing, and PTMs. We have shown that top-down MS has unique advantages for unraveling the molecular complexity, quantifying multiple modified protein forms, complete mapping of modifications with full sequence coverage, and discovering unexpected modifications. However, the top-down approach still faces significant challenges in terms of protein solubility, protein separation, the detection of low-abundance proteins, and the under-developed data analysis tools. Recently we are employing a multi-pronged approach to address these challenges in a comprehensive manner by developing new MS-compatible surfactants for protein solubilization, novel materials and new strategies for multi-dimensional chromatography separation of proteins, novel nanomaterials for enrichment of low-abundance proteins, and a new comprehensive software package for top-down proteomics. In this talk I will present our recent technology developments in top-down proteomics and their application to understand heart failure.

MOC 10:40 am - 10:55 am: Analyzing histone Tail Dynamics using Hydrogen-Deuterium Exchange Coupled to Top-Down Mass Spectrometry

Kelly Karch; Ben Black; Benjamin A. Garcia
University of Pennsylvania, Philadelphia, PA

Nucleosomes are the fundamental repeating unit of chromatin and are therefore vital in maintaining many nuclear processes. Nucleosome dynamics have been difficult to assess, especially for the N- or C-terminal tail domains of each histone which are critical for their function. Hydrogen-deuterium exchange (HDX) coupled to bottom-up mass spectrometry (BU-MS) has provided some insight but fails to characterize tail peptides because they do not perform well in reverse-phase chromatography. Here, we develop HDX coupled to top-down (TD) MS methodology, which has several advantages over BU-HDX-MS: (1) full coverage of the protein is ensured, including tail domains; (2) up to single amino acid resolution is possible; (3) it is technically more facile.

One challenge of TD-HDX-MS, however, is that amide hydrogen and deuterium atoms can migrate along the protein backbone in the gas phase, effectively randomizing deuterium signal in a process called scrambling. We used a synthetic peptide (sequence: HHHHHHIIKIIK) to monitor scrambling and determine allowable instrument settings as done previously. Another challenge is that back-exchange can occur, where deuterium atoms on the protein exchange with hydrogen atoms in the quench buffer, reducing overall deuterium signal. We minimized back-exchange by optimizing the pH and composition of the quench buffer and cooling the sample to subzero temperatures during infusion using a home-made cooling device. We applied this method to recombinant H3/H4 tetramers. Tetramers were infused into a Thermo Fusion instrument, and each histone was isolated and fragmented using ETD (10ms). Data were analyzed manually using Excel and XCalibur software. We obtained near complete fragment ion coverage of the tail region, demonstrating the power of this method to monitor histone tail dynamics. We are also exploring middle down HDX-MS to monitor tail peptides, using ExMS software, and are working with the Englander lab to adapt this software to automate TD-HDX-MS data analysis.

MOC 10:55 am - 11:10 am: Elucidating Proteoform Families from Proteoform Intact Mass and Lysine Count Measurements

Michael Shortreed; Brian Frey; Mark Scalf; Rachel Knoener; Anthony Cesnik; Lloyd Smith
University of Wisconsin, Madison, WI

Proteomics is presently dominated by the “bottom-up” strategy, where proteins are enzymatically digested into peptides for mass spectrometric identification. While this approach is highly effective at identifying large numbers of proteins present in complex samples, the digestion into peptides renders it impossible to identify the proteoforms from which they were derived. We present here a powerful new strategy for the identification of proteoforms and the elucidation of proteoform families (groups of related proteoforms) from experimental determination of the accurate proteoform mass and number of lysine residues contained. Accurate proteoform masses are determined by standard LC-MS analysis of undigested protein mixtures in an Orbitrap mass spectrometer, and the lysine count is determined using the recently developed NeuCode isotopic tagging method. We demonstrate the approach in analysis of the yeast proteome, revealing 8,637 unique proteoforms and 1,178 proteoform families. The elucidation of proteoforms and proteoform families afforded here provides an unprecedented new perspective upon proteome complexity and dynamics.

MONDAY 3:00 – 4:20 PM
COMPUTATIONAL PROTEOMICS, *Grand A*

MOD 3:00 pm - 3:25 pm: Discovering and Modeling Relationships among Antibody Properties, Functional Activities, and Protection, using Multidimensional Assays of Polyclonal Samples

Chris Bailey-Kellogg
Dartmouth College, Hanover, NH

A detailed understanding of antibody properties associated with effective immune responses may both provide basic scientific insights and guide vaccine design efforts. We have been focusing on the non-neutralizing role of antibodies in driving innate immune responses, which, for example, may have been a key aspect of the protection observed in the RV144 HIV vaccine trial. Using both unsupervised and supervised machine learning techniques, we have extensively investigated relationships in a variety of datasets ranging from nonhuman primate vaccinees to naturally infected human subjects. We have identified associations among antibody features, effector functions, and protection from infection, and demonstrated that classification and regression models can effectively use antibody properties to robustly predict qualitative and quantitative outcomes. This integration of antibody data within a machine learning framework demonstrates a new approach to understanding and potentially guiding a protective immune response.

MOD 3:50 pm - 4:05 pm: Stochastic Modeling of Protein Turnover in Metabolic Labeling

Mahbubur Rahman; Jayant Avva; Rovshan Sadygov
UTMB, Galveston, Texas

The continuous degradation and synthesis of proteins plays an important role in maintaining cellular homeostasis. The dynamic equilibrium of cellular protein abundances can change due to, for example, external stimuli, developmental programs, onset of diseases, or ageing. Mass spectrometry based proteomics combined with metabolic labeling is a widely used technology for high-throughput protein turnover studies. Statistical models are needed to describe the protein turnover kinetics and extract the degradation rate constants. Here we describe a stochastic model, Gaussian Process, for protein turnover. We show that the often used one- and two-compartment non-stochastic models allow explicit solutions from the corresponding stochastic differential equations. The resulting stochastic process is a Gaussian Process with Ornstein-Uhlenbeck covariance matrix. We applied the model to a large scale data set from 15N labeling and compared its performance metrics with that of a non-stochastic model. The comparison showed that for more than 99% of proteins the stochastic model produced better fits to the experimental data (based on residual sum of squares). The model was used for extracting protein decay rate constants from mouse brain (slow turnover) and liver (fast turnover) samples. We found that the most affected (compared to two-exponent curve fitting) results were those for liver proteins. The ratio of the median of degradation rate constants of liver proteins to those of brain proteins increased by four-fold in stochastic modeling compared to the two-exponent fitting. Stochastic modeling predicted stronger differences of protein turnover processes between mouse liver and brain than previously estimated.

Our model is independent of the labeling isotope. To show this we also applied the model to a protein turnover studied in induced heart failure

in rats where metabolic labeling was achieved by admin-istering heavy water. No changes in the model were necessary for adapting to heavy water labeling.

MOD 4:05 pm - 4:20 pm: Quantifying Homologous Proteins and Proteoforms

Nikolai Slavov
Northeastern University, Boston, MA

Many protein isoforms (proteoforms) -- arising from alternative splicing, post-translational modifications (PTMs), or paralogous genes -- have distinct biological functions. However, the accuracy of quantifying proteoforms and the stoichiometries among them by existing bottom-up mass--spectrometry (MS) methods remains limited because of noise due to variations in protein-digestion and in peptide-ionization. We eliminate the influence of this analytical noise by deriving a first-principles model (HIquant) for quantifying these stoichiometries only from corresponding-ion ratios. We prove the conditions under which HIquant has a unique solution, derive an algorithm for its optimal solution, and demonstrate experimentally unprecedented accuracy in quantifying fractional site occupancy of PTMs without using external standards, even in the challenging case of the histone modification code. We use HIquant to quantify for the first time the stoichiometries among paralogous core ribosomal proteins.

MONDAY 3:00 – 4:20 PM
STRUCTURAL PROTEOMICS, *Grand C*

MOE 3:00 pm - 3:25 pm: Protein Structure Determination by Mass Spectrometry

Juri Rappsilber[1, 2]
[1]Wellcome Trust Centre for Cell Biology, Edinburgh, Scotland, United Kingdom; [2]TU Berlin, Berlin, Germany

Chemical cross-linking combined with mass spectrometry (CLMS) has proven useful for studying protein-protein interactions and protein structure, however the low resolution of cross-linking has so far precluded its use in determining structures de novo. Cross-linking resolution has been typically limited by the chemical selectivity of the standard cross-linking reagents that are commonly used for protein cross-linking. We have implemented the use of a heterobifunctional cross-linking reagent sulfo-SDA, combining a traditional sulfo-NHS ester and a UV photoactivatable diazirine group. This diazirine yields a highly reactive carbene species, the net result being an order of magnitude more cross-links compared with homobifunctional, NHS-based cross-linkers. We combine the use of this high-density cross-linking with conformational space search to investigate the structure of proteins in their native environment. This approach enables the elucidation of protein structures in the context of human serum as demonstrated for albumin (RMSD to crystal structure of 3.38/6.13/3.98 Å for domains A/B/C). To blindly assess the general applicability of this approach, we participated as the first experimentalists in CASP, contributing our data to this community wide experiment to assess protein structure prediction tools. The results support our claims as well as clearly define a list of technical challenge that remain to be addressed before this approach may complement NMR, EM and crystallography as a fourth routine structure determination technology.

MOE 3:25 pm - 3:50 pm: Environment Affects Structure: Membranes, Binding, and Modification

John R. Engen
Northeastern University, Boston, MA

Protein structure can be sensitive to the local environment of the protein. This simple fact is critical for some proteins where proximity to membranes, ligands, or substrates induces structural changes that are critical for function. Protein modification (*e.g.*, PTMs such as phosphorylation, glycosylation etc.) or amino acid mutation can also alter the local protein environment and lead to structural changes. The occurrence and location of such changes can be ascertained for a wide variety of conformational states using hydrogen exchange mass spectrometry (HX MS). Recent studies of peripheral membrane proteins will highlight the development of HX MS methods involving Langmuir monolayers. Small-scale screening studies of DNA interactions, protein mutation, and comparisons of protein isoforms will illustrate what one can learn. Through the course of such examples, an update on the state-of-the-art in HX MS measurements will be provided.

MOE 3:50 pm - 4:05 pm: A Systematic Exploration of the Human Interactome

Edward Huttlin; Joao Paulo; Raphael Bruckner; Lily Ting; J. Wade Harper; Steve Gygi
Harvard Medical School, Boston, MA

Because a cell's phenotype reflects its underlying proteome, mass-spectrometry-enabled proteomics can provide essential biological insights. Whereas surveys of protein expression and select post-translational modifications have achieved near-comprehensive scope, mass-spectrometry-based protein interaction profiling has typically targeted small protein families. In an attempt to map the human protein interaction landscape more comprehensively, we have established a high-throughput pipeline capable of identifying interacting partners for several hundred baits per month and are now systematically mapping human protein interactions at unparalleled depth and breadth. The emerging interaction network is providing unique insights into both normal and pathological biological processes by revealing novel functions for familiar proteins and providing tantalizing insights into roles of unknown proteins.

Our platform for high-throughput protein interaction profiling relies upon expressing HA-tagged versions of human proteins within HEK293T cells; following immunoprecipitation, the baits and their interacting partners are identified using Q-Exactive mass spectrometers aided by customized data analysis techniques as described previously (Huttlin *et al.* **2015** *Cell* 162(2):425-440). While our original publication described the results of the first 2594 AP-MS experiments, we have since doubled our coverage of the human interactome: to date we have completed analysis of 5891 bait proteins and their interacting partners, defining an interaction network that spans 56,553 interactions among 11,782 proteins. The growing network of protein interactions reveals with steadily-increasing detail clusters that correspond to known sub-cellular structures such as the proteasome, signalsome, and mediator complexes, while suggesting biological functions for unknown proteins and highlighting shared functional and regulatory clusters across the interactome. Whether viewed individually or in aggregate, these interaction profiles offer unique insights into both known and unknown proteins while also illuminating larger patterns of proteomic regulation.

MOE 4:05 pm - 4:20 pm: Development and Application of a Covalent-Labeling Strategy for the Large-Scale Thermodynamic Analysis of Protein Folding and Ligand Binding

Yingrong Xu; Ryenne Ogburn; Michael C. Fitzgerald
Duke University, Durham, NC

Thermodynamic measurements on proteins and protein-ligand complexes can offer insights not only into the fundamental properties of protein folding reactions and protein functions, but also into the development of protein-directed therapeutic agents to combat disease. Conventional calorimetric or spectroscopic approaches for measuring protein stability typically require large amounts of purified protein. This requirement has precluded their use in proteomic applications. Here we report on a mass spectrometry-based protocol for making thermodynamic measurements of protein folding and ligand binding reactions on the proteomic scale. The protocol, which can be combined with quantitative, bottom-up, shotgun proteomics technologies, enables the evaluation of protein folding free energies using the denaturant dependence of the rate at which globally protected tryptophan and methionine residues are modified with dimethyl (2-hydroxyl-5-nitrobenzyl) sulfonium bromide and hydrogen peroxide, respectively.

Presented here will be the results of proteome-wide experiments, in which the above tryptophan and methionine labeling strategies were simultaneously used to evaluate the thermodynamic stability of proteins in lysates derived from yeast, human (MCF-7) and dust mite (D. Farinae) cells. The described protocol enabled the thermodynamic stability of ~1000 proteins in each cell lysate to be evaluated using ~2000 different peptide probes. The dual labeling strategy increased the proteomic coverage by 50%-100% compared to the coverage observed using the methionine modification strategy alone. Also reported will be results obtained using the described protocol to detect and quantify the binding of geldanamycin to Hsp90 in cell lysates. To date, we have successfully detected and quantified the binding of geldanamycin to one of its known protein targets, Hsp90, in MCF-7 cell lysate. The measured K_d, 0.62 μM, is in the range of literature values (0.08-0.6 μM) obtained using purified Hsp90. To our knowledge, this is

the first K_d value measurement of the human Hsp90-geldanamycin complex in a cell lysate.

MONDAY 4:30 – 5:50 PM
PROTEOMICS OF AGING, *Grand A*

MOF 4:30 pm - 4:55 pm: Stratifying Dementia Patients Using FLEXITau and Machine Learning Approaches

Judith Steen
Boston Children's Hospital, Boston, MA

The aggregation of tau protein in the brain is the hallmark of a diverse group of neurodegenerative diseases resulting in dementia called tauopathies. Currently, there is no accurate means of stratifying these diseases. Here, we present a classifier that can identify specific tauopathies using mass spectrometry data quantifying the post-translational modification (PTM) state of tau. A total of 129 post-mortem brain samples from 5 different brain banks encompassing patients with Alzheimer's Disease (AD), progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), Pick's Disease (PiD), and non-demented control subjects (ctrl) were analyzed to quantify tau modification and isoform patterns using a targeted MS assay. Supervised machine-learning approaches identified relevant peptide combinations and associated PTMs that distinguish each disease category using a training set of 68 patients. The classifier was then validated on an independent heterogeneous dataset of 61 patients. The final classifier developed achieved excellent diagnostic accuracy of 96.0% for AD, 94.0% for CBD and ctrl, and 92.0% for PiD. Good diagnostic accuracy was achieved for PSP with 80% accuracy. The majority of discriminating peptide features are located in the MT-binding region of tau, including exon 10 which is prone to alternative splicing leading to 3R and 4R isoforms. Tau modification and isoform abundance quantified at the peptide level provides a tauopathy specific barcode that can distinguish tauopathies and could be used as a novel diagnostic test.

MOF 4:55 pm - 5:20 pm: Enhanced Multiplexing Proteomics and the Role of the Periphery in Alzheimer's Disease

Renã Robinson
University of Pittsburgh, Pittsburgh, PA

The growing population of elderly persons, estimated at 50 million, that will be affected by Alzheimer's disease (AD) in the next thirty years, necessitates better disease understanding and the development of preventative, diagnostic, and therapeutic strategies to help delay and stop disease. It is becoming more widely accepted that Alzheimer's disease, while a neurodegenerative disorder, is also grossly affected by and affects bodily systems outside of the central nervous system (CNS). The periphery plays an important part of Alzheimer's disease pathogenesis however a lot of questions remain regarding the role of peripheral organs in AD and their contributions to and communications with the CNS. Energy and lipid metabolism and oxidative stress pathways are heavily implicated in the CNS of AD subjects and in animal models. Previously we have shown with an enhanced multiplexing quantitative proteomics approach that these pathways are also altered in peripheral tissue (i.e., liver) from a late-stage APP/PS-1 mouse model of AD. We believe that much remains to be understood about how these pathways are influenced in the periphery and with disease progression. This presentation will discuss the advances we have made in enhanced multiplexing proteomics in order to better facilitate high-throughput analyses of several tissues across genotypes and disease stages. Additionally, we will discuss our preliminary findings of alterations to energy and lipid metabolism and oxidative stress pathways in the periphery and how they correlate with changes in the CNS in the APP/PS-1 model.

MOF 5:20 pm - 5:35 pm: Protein Co-Expression Network Analysis Reveals Cell Type Changes Linked to Alzheimer's Disease Risk

Nicholas Seyfried[1]; Eric Dammer[1]; Vivek Swarup[2]; Divya Nandakumar[1]; Duc Duong[1]; Luming Yin[1]; Qiudong Deng[1]; Tram Nguyen[1]; Marla Gearing[1]; Madhav Thambisetty[3]; Juan Troncoso[4]; Daniel Geschwind[2]; James Lah[1]; Allan Levey[1]
[1]Emory University School of Medicine, Atlanta, Georgia; [2]UCLA School of Medicine, Los Angeles , CA; [3]National Institute on Aging, Baltimore, MD; [4]Johns Hopkins School of Medicine, Baltimore, MD

Cellular and molecular mechanisms linking the asymptomatic phase of Alzheimer's Disease (AD) to clinically evident AD are inadequately understood. Although recent network analyses of the transcriptome identified modules of co-expressed genes linked to cell type and neuropathology in AD, no such network-based analysis has been performed of the proteome. In this study, we performed a label-free mass spectrometry (MS)-based quantitative proteomic analysis of 2,735 proteins across both the dorsolateral prefrontal cortex and precuneus from 50 individuals clinically and pathologically defined as controls, asymptomatic AD (AsymAD) or AD. Differential and co-expression network analyses defined key proteins and pathways linked to specific molecular functions and cell types in brain. Protein networks enriched for neuronal and astrocyte/microglia proteins were decreasing and increasing, respectively, in both AsymAD and AD. We were able to confirm these findings in a second group of brains and identify common and distinct mechanisms shared across AD, Parkinson's Disease (PD) and amyotrophic lateral sclerosis (ALS), which confirmed the specific relationship of several astrocytic, microglial, and neuronal marker-enriched modules to AD. Together, these consensus protein networks offer new insights into the cellular mechanisms underlying cognitive decline in AD.

MOF 5:35 pm - 5:50 pm: Identification of Age-Related Protein Folding Stability Differences in the Mouse Proteome

Julia Roberts; Michael C. Fitzgerald
Duke University, Durham, North Carolina

Large-scale, molecular level analyses of aging have previously focused on the measurement of gene and protein expression levels to identify important biological pathways and proteins involved in the functional decline of model organisms over time. Here, a new mass spectrometry-based proteomics approach is used to study age-related differences in mammalian systems at the molecular level. The approach involves the proteome-wide measurement of protein folding stabilities in tissue cell lysates to identify proteins with age-related differences. Presented here are the results of studies using the Stability of Proteins from Rates of Oxidation (SPROX) technique to profile the thermodynamic stability of 300 to 800 proteins in brain (n=4), heart (n=2), and kidney (n=2) cell lysates derived from mice, aged 6- and 18-months. The thermodynamic stability profiles revealed that the biological variability of the protein stability measurements was low (i.e., <3%) and within the false discovery rate of the technique. Over 100 protein hits were detected with age-related protein folding stability differences in the brain samples, whereas few age-related stability differences were detected in the heart and kidney samples. Many of the brain protein hits with age related differences were previously linked to aging via pathways (e.g., glycolysis) and/or established targets of known post-translational modifications (e.g., carbonylations and phosphorylations). Remarkably, the large majority of the brain protein hits were destabilized in the old mice. These results not only validate the use of thermodynamic stability measurements to capture relevant age-related proteomic changes, but they also establish a new biophysical link between these proteins and aging. This new link creates the possibility of targeting the destabilized brain proteins via pharmacological chaperones to treat the adverse effects of aging.

MONDAY 4:30 – 5:50 PM
IMAGING, *Grand C*

MOG 4:55 pm - 5:20 pm: Lipid Imaging for Cancer Diagnosis by Ambient Ionization Mass Spectrometry

Livia Eberlin
University of Texas at Austin, Austin, TX

There is a clinical need for new technologies that would enable rapid disease diagnosis based on diagnostic molecular signatures. Spatial and chemical characterization of lipids in biological samples is of particular interest as their abnormal expression has been increasingly explored in a variety of diseases. Ambient ionization mass spectrometry has revolutionized the means by which lipid information can be obtained from tissue samples in real time and with minimal sample pretreatment. The latest developments in ambient ionization techniques applied to clinical research suggest that ambient ionization mass spectrometry will soon become a routine medical tool for tissue diagnosis. This talk will cover the main developments in ambient ionization techniques applied to lipid imaging and tissue analysis, with focus on the use of desorption electrospray ionization mass spectrometry for cancer diagnosis. Recent approaches to incorporate

this technology for routine, clinical use in the treatment and management of cancer patients will be discussed.

MOG 5:20 pm - 5:35 pm: Investigation of the Molecular Pathology of Traumatic Brain Injury by Imaging Mass Spectrometry

Bo Yan[1]; Andrew Fisher[2]; Mark Wojnarowicz[1]; Olga Minaeva[3]; Yi Pu[1]; Mark E. McComb[1]; Lee E. Goldstein[1]; Catherine E. Costello[1]
[1]Boston University School of Medicine, Boston, MA; [2]College of Engineering, Boston University, Boston, MA; [3]Boston University Photonics Center, Boston, MA

Traumatic brain injury (TBI) is a leading cause of disability and death in the world. TBI is associated with increased risk of developing age-related neurodegenerative diseases, e.g., Alzheimer disease (AD) and chronic traumatic encephalopathy (CTE). However, the TBI-related pathology remains largely unknown. Here, we report results obtained using matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) as the imaging tool to monitor multiple biomolecules in two mouse models of traumatic brain injury. Our hypothesis is that the specific information on biomolecular distributions generated by MALDI-MS imaging will provide critical insight into the pathology of TBI.

Male C57BL6 mice were subjected to impact and blast TBI according to IACUC-approved protocols. MALDI imaging MS experiments were performed on a Bruker solariX 12-T FT-ICR MS and a Bruker ultrafleXtreme TOF/TOF MS.

We mapped multiple biomolecules simultaneously using MALDI-MS imaging, carried out in both the positive- and negative-ion modes, supporting the assignments with MALDI-MS/MS and LC/MS/MS analysis after tryptic digestions were performed on the tissues and in vitro, respectively. The observed anatomical distribution of heme provided a biomarker for hemorrhagic vascular disruption after TBI. The measured lipid and protein distributions and their profile changes may be associated with the biological consequences of TBI.

These MALDI-MS imaging results, combined with immunohistochemical staining and other neuroimaging techniques, complement our parallel metallomic studies that employ inductively coupled plasma MS, to gain anatomical and pathological understanding of focal hemorrhage and blood-brain barrier disruption as clinically-relevant consequences of impact TBI.

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MOG 5:35 - 5:50 pm: Supervised and Unsupervised Analysis of Mass Spectrometry Imaging Experiments Using Cardinal

Kyle Bemis[1]; April Harry[1]; David Calligaris[2]; Armen Changelian[2]; Sandro Santagata[2]; Nathalie Agar[2]; Olga Vitek[3]; *[1]Purdue University, West Lafayette, IN; [2]Brigham & Women's Hospital, Boston, MA; [3]Northeastern University, Boston, MA*

Cardinal is an open-source R package for the pre-processing, visualization, and statistical analysis of mass spectrometry imaging (MSI) experiments. It supports importing from the open-source imzML and Analyze 7.5 data formats, and common pre-processing methods including normalization, baseline reduction, spectral smoothing, and peak picking. Most importantly, Cardinal provides methods for statistical analysis, including principal components analysis (PCA) and projection to latent structures (PLS), as well as spatial shrunken centroids, a novel method developed specifically for MSI experiments which incorporates the spatial relationships between pixels.

We showcase a Cardinal workflow for the pre-processing and supervised analysis of an experiment designed for the detection and delineation of pituitary tumors. By use of matrix-assisted laser desorption/ionization (MALDI) MSI, images were collected from six nonpathological human pituitary glands and 45 hormone secreting and nonsecreting pituitary adenomas. We use a subset of this dataset to demonstrate the pre-processing and subsequent statistical analysis using spatial shrunken centroids in Cardinal.

Additionally, we introduce new features which have been added to Cardinal recently or are in development for the near-future, including improved support for the 'processed' imzML format, limited support for working with larger-than-memory datasets, and support for the importing of 3D datasets and visualization of 3D ion images. We demonstrate some of these new features using publicly available benchmark 3D datasets.

TUESDAY 9:50 – 11:10 AM
CROSS'OMICS, *Grand A*

TOB 9:50 am - 10:15 am: The Role of Transcriptomics and Proteomics in Defining Functional Protein Networks

Wilhelm Haas
Massachusetts General Hospital, Charlestown, MA

The ability to multiplex quantitative mass spectrometry-based proteomics measurements is currently causing a huge shift in the experimental setups accessible to proteome analysis. We are routinely applying 10-plexed proteome measurements using tandem mass tag (TMT) reagent technology on an Orbitrap Fusion mass spectrometer that allows high accuracy multiplexed quantification through an Synchronous Precursor Selection (SPS) powered MS3 method. This allows us to quantify a full human proteome in less than 5 hours. This unprecedented throughput in proteomics is closing the historical performance gap between genomics methods – such as RNA sequencing – and proteomics and enables an insight into the many aspects of the proteome based on analyzing the number of samples required to understand complex biological associations.We have used this technology to quantitatively map the proteome of 41 breast cancer cell lines to a depth of 9,000 proteins per cell line. The availability of extensive other data on many of these cell lines in the form of genome-wide gene mutation maps and transcriptomics profiles generates a data set that allows an in depth comparison of individual omics data sets from a large number of samples. Drug screen data on the same cell lines allow a phenotypical assessment. We have analyzed these multiple omics data to evaluate their significance in attempting to correlate molecular and phenotypical properties and understanding functional networks of gene products.

TOB 10:40 am - 10:55 am: Introducing Epigenomics in Systems Biology: Cross-Talk between Cell Signal Transduction and Epigenetic Mechanisms

Simone Sidoli; Pau Pascual Garcia; Katarzyna Kulej; Maya Capelson; Benjamin A. Garcia
University of Pennsylvania, Philadelphia, Pennsylvania

Integrating –omics strategies is becoming a new frontier in systems biology, since disciplines like genomics and proteomics are now established. However, the traditional view of how these disciplines interplay, i.e. genomics → transcriptomics → proteomics → metabolomics, is too static to exhaustively represent a biological system. Events like early response to stimulus (protein phosphorylation) and structural gene regulation (epigenetic mechanisms) must enter in the equation.

We investigated the development of larvae from *Drosophila melanogaster* upon treatment with kinase inhibitors. By integrating proteomics, phosphoproteomics, histone modification analysis and chromatin immunoprecipitation coupled with DNA sequencing (ChIP-seq) we reconstructed links between drug treatment and phenotypic abnormalities during development. The success of this proof of principle paved the way to an analog characterization of drug treatment in human acute myeloid leukemia.

Larvae from wild type (OregonR) hatched and grew from eggs laid on food w/o inhibitors for the kinases EGFR and c-Met. Larvae growing in food containing inhibitors took up to one week longer to reach the third instar stage of development. Considering the phosphoproteome as indicative of early response to stimulus we characterized pathways of proteins with regulated phosphorylations connecting the inhibitor target with nuclear receptors and histone modifier enzymes (protein interaction retrieved from STRING database).

Specifically, we found downregulated phosphosites in both inhibitor treatments on the Ecdysone nuclear receptor and the interacting trithorax complex, which last catalyzes methylation on histone H3 lysine 4 (H3K4me). This modification, enriched in actively transcribed genes (Herz et al. Genes Dev. 2012), globally decreased upon inhibitor treatment. ChIP-seq analysis mapped H3K4me on genes coding for

proteins involved in translational initiation in wild type, which we found expressed in lower abundance in treated larvae.

Collectively, our preliminary data indicate how drug treatment might be related to developmental abnormalities, using epigenomics to link early response to proteome regulation.

TOB 10:55 am - 11:10 am: Characterization of Human Sirtuin 2 Interactome and Substrates

Hanna Budayeva; Ileana Cristea
Princeton University, Princeton, New Jersey

Sirtuin-2 (SIRT2) is a ubiquitously expressed and predominantly cytoplasmic NAD^+-dependent deacetylase. Although information about its substrates remains limited, the accumulated knowledge points to critical cellular regulatory functions for SIRT2, with its known substrates functioning in cytoskeletal organization (α-tubulin), cell cycle regulation (histone H4), and transcription regulation (NFκβ, p53). Recent studies point at new roles for SIRT2 in cancer progression, neuronal pathology, and viral infection. This emphasizes the necessity to better understand the means through which SIRT2 exerts its functions. Here, we combined proteomics with molecular biology to generate refined networks of SIRT2 interactions (interactome) and discover SIRT2-modulated deacetylation events (acetylome). We generated human fibroblasts expressing fluorescently-tagged SIRT2, and monitored its subcellular localization and deacetylation by microscopy and fluorometric assays, respectively. We used immunoaffinity purification (IP) of SIRT2-EGFP in conjunction with both label-free (SAINT) and metabolic labeling (I-DIRT) quantification to assess the specificity of the identified interactions. In addition to the expected associations with proteins functioning in transcription and cytoskeletal organization, we found previously uncharacterized associations with vesicular trafficking pathways. By confocal microscopy, we confirmed SIRT2 co-localization with the Endoplasmic Reticulum-Golgi Intermediate Compartment, and assessed its regulation.

To further determine which interactions are SIRT2 substrates, we designed an approach to identify SIRT2-dependent deacetylation events. Acetylated peptides were enriched by IP (using anti-acetyl antibodies) from SILAC-labeled cells stably expressing non-targeting shRNA or shRNA targeting SIRT2. Changes in total protein abundances upon SIRT2 knockdown were assessed in parallel. Proteins functioning in ER organization, hydrogen transport, and several receptor proteins were upregulated upon SIRT2 knockdown. By integrating the I-DIRT and SAINT interaction datasets with the quantitative acetylome results, information was gained about the relative stability of SIRT2 interactions with substrates or protein complexes. Overall, this represents the first global study of SIRT2 substrates and interactions, expanding the knowledge of SIRT2 enzymatic and non-enzymatic functions.

TUESDAY 9:50 – 11:10 AM
PEDIATRIC PROTEOMICS, *Grand C*

TOC 9:50 am - 10:15 am: Developmental Proteomics: Unravelling Age Specific Differences in the Human Proteome

Vera Ignjatovic[1, 2]
[1]Murdoch Childrens Research Institute, Melbourne, Victoria; [2]University of Melbourne, Parkville, Victoria, Australia

Proteomics studies utilizing human plasma have to date focused largely on specific disease settings, on detecting the highest number of proteins or on the effect of certain drugs on changes in plasma protein expression. In addition, the majority of such studies have focused on adults, with limited number of studies and hence limited knowledge of the plasma proteome in infants and children.

HUPO Plasma Proteome Project recognizes the importance of analyzing and understanding age related differences in the plasma proteome by identifying this as one of their scientific aims and research priorities. In addition, the recently established Paediatric Proteomics (PediOme) initiative, a part of HUPO, aims to advance the use of proteomics techniques to solve major issues in paediatric medicine through characterization of the paediatric proteome across a wide-variety of tissues and biological samples.

Developmental Proteomics is a new concept that focuses on age specific differences in the human proteome. This new biology will be described through the use of SWATH-MS data independent acquisition as a new technology that is particularly suitable for developmental proteomics studies. Specifically, SWATH-MS allows confident identification of peptides over a dynamic range of 4 orders of magnitude, making it a useful tool for the analysis of biological specimens such as plasma. The use of SWATH-MS in neonatal and paediatric plasma samples to determine variability and subsequent comparison to adult plasma remains an exciting research opportunity, and one that has, to date, not been explored.

This presentation will outline the results of a recent study, which utilised SWATH-MS to profile the expression of plasma proteins associated with healthy human development.

TOC 10:40 am - 10:55 am: Adjuvant-Induced Human Monocyte Secretome Profiles Reveal Adjuvant- and Age-Specific Protein Signatures

David Dowling[1, 2]; Djin-Ye Oh[3, 4]; Spencer Brightman[1]; Sebastian Berger[1, 2]; Hanno Steen[1, 2]; Ofer Levy[1, 2]
[1]Boston Children's Hospital, Boston, MA; [2]Harvard Medical School, Boston, MA; [3]Boston Childrens Hospital / Harvard Medical School, Boston, MA; [4]New York University Medical School, New York, NY

Adjuvants boost vaccine responses, enhancing protective immunity against infections that are most common among the very young. Many adjuvants activate innate immunity, some via Toll-Like Receptors (TLRs), whose activity varies with age. Accordingly, characterization of age-specific adjuvant-induced immune responses may inform rational adjuvant design targeting vulnerable populations. In this study, we use proteomics to characterize the adjuvant-induced secretome response of human newborn and adult monocytes to Alum, the most commonly used adjuvant in licensed vaccines; Monophosphoryl Lipid A (MPLA), a TLR4-activating adjuvant component of a licensed Human Papilloma Virus vaccine; and R848 an imidazoquinoline TLR7/8 agonist that is a candidate adjuvant for early life vaccines. Monocytes were incubated in vitro for 24 hours with vehicle, Alum, MPLA, or R848 and supernatants collected for proteomic analysis employing liquid chromatography-mass spectrometry (LC-MS). 1894 non-redundant proteins were identified, of which ~30 - 40% were common to all treatment conditions and ~5% were treatment-specific. Adjuvant-stimulated secretome profiles, as identified by cluster analyses of over-represented proteins, varied with age and adjuvant type. Adjuvants, especially Alum, activated multiple innate immune pathways as assessed by functional enrichment analyses. Release of lactoferrin, pentraxin 3, and matrix metalloproteinase-9 was confirmed in newborn and adult whole blood stimulated with adjuvants alone or adjuvanted licensed vaccines with distinct clinical reactogenicity profiles. MPLA-induced adult monocyte secretome profiles correlated in silico with transcriptome profiles induced in adults immunized with the MPLA-adjuvanted RTS,S malaria vaccine (Mosquirix). Overall, adjuvants such as Alum, MPLA and R848 induce distinct and age-specific monocyte secretome profiles, paralleling responses to adjuvant-containing vaccines in vivo. Age-specific in vitro modeling coupled with proteomics may provide fresh insight into the ontogeny of adjuvant action thereby informing targeted vaccine development for distinct age groups.

TOC 10:55 am - 11:10 am: Serum Proteomes Distinguish Children Developing Type-1 Diabetes

D Goodlett[1]; R Moulder[2]; S Bhosale[2]; J Mykkänen[2]; T Erkkilä[3]; E Laajala[2]; J Salmi[2]; E Nguyen[7]; H Kallionpaa[2]; H Hyöty[4]; R Veijola[5]; J Ilonen[6]; T Simell[2]; J Toppari[2]; M Knipp[2]; H Lähdesmäki[4]; O Simell[2]; R Lahesmaa[2]
[1]University of Maryland, Baltimore, USA; [2]University of Turku, Turku, Finland; [3]University of Aalto, Espoo, Finland; [4]University of Tampere, Tampere, Finland; [5]University of Helsinki, Helsinki, Finland; [6]Hospital District of Southwest Finland, Turku, Finland; [7]Monash University, Melbourne, Australia

The Finnish Type 1 Diabetes (T1D) Prediction and Prevention Study (DIPP) began in 1994 with collection of follow-up samples from children with increased genetic risk for T1D. Here we report the results of comparing serum proteomes during the early stages of life in children who progressed to develop T1D to identify potential changes that could be associated with disease onset or activity and detected before the appearance of T1D associated autoantibodies. Proteomic profiles were

compared between those who remained healthy to those who developed T1D, matched by age, gender, sample periodicity and risk group. Profiles were generated by a combination of iTRAQ and label free analyses on 26 subjects from approximately six time points per patient representing periods from early infancy, to seroconversion and diagnosis. Functional annotation enrichment analysis highlighted proteins related to lipid transport and inflammatory response. On the basis of top scoring pair analysis, classification of the T1D developing subjects was observed with a success rate of 91% indicating that we have identified new candidates whose levels change in children during the early development of T1D. A summary of the discovery aspects of this work appeared in 2015 (Moulder et al. Diabetes 2015 Jun;64(6):2265-78). Follow up work to confirm or refute the power of the putative markers is underway using a different patient cohort.

TUESDAY 3:00 – 4:20 PM
MICROBIOLOGY IN PROTEOMICS, *Grand A*

TOD 3:00 pm - 3:25 pm: Spatial-Temporal Dynamics of Host Organelle Morphology and Composition during Herpesvirus Infection

Ileana Cristea
Princeton University, Princeton, NJ

Viruses and host cells have established complex, dynamic interactions that function either in promoting virus replication and dissemination or in host defense against invading pathogens. Thus, viral infection triggers a drastic transformation in intracellular proteomes, including the reorganization of organelles. An important example is human cytomegalovirus (HCMV), a beta-herpesvirus that infects a large majority of the adult population worldwide, leading to life-threatening diseases in immunocompromised individuals. HCMV triggers an extensive reorganization of organelle structure for energy production, intracellular trafficking, and generation of the virion assembly compartment. However, the viral proteins that target distinct organelles or the specific organelle proteins recruited for these morphological changes remain in large part unknown. Here, we report the first global proteomic study of cellular organelles during viral infection. First, using time-lapse microscopy and fluorescent confocal microscopy, we monitored changes in organelle morphology during HCMV infection of primary human fibroblasts. We observed the signature mitochondria fission, rearrangement of the Golgi apparatus around the viral assembly complex, increased lysosome size, and increased density of the endoplasmic reticulum. Next, organelle densities were examined by ultracentrifugation, while changes in their compositions were determined by quantitative mass spectrometry. Labeling with tandem mass tags was combined with a label-free approach to gather spatial and temporal information of organelle composition. Dimensional reduction algorithms and supervised machine learning allowed us to confidently assign proteins to organelles throughout the time course of infection. Importantly, we discovered viral proteins with temporally-regulated localizations at different organelles, including the previously uncharacterized HCMV protein, pUL13. We next investigated the function of pUL13, demonstrating its requirement for viral replication and spread. Furthermore, using immunoaffinity purification and functional assays, we demonstrated that pUL13 targets and inhibits an important cell defense protein in the mitochondria. Altogether, our study highlights mass spectrometry as an important component of discoveries in virology.

TOD 3:25 pm - 3:50 pm: What Can MALDI-TOF MS Do for the Clinical Microbiology Laboratory?

Mark Fisher
Univ. of Utah / ARUP Laboratories, Salt Lake City, UT

Mass spectrometry is a very recent addition to the clinical microbiology laboratory. Rather than a strict proteomic approach of precisely identifying characteristic proteins or peptides, current systems rely on MALDI-TOF MS fingerprints that may differ substantially across microbial species. Pattern matching algorithms allow rapid identification of patient isolates by searching predetermined fingerprints from known microorganisms. This seemingly low-resolution approach has dramatically changed the landscape in the clinical microbiology laboratory by reducing time to identification as well as reagent costs. In our laboratory, MALDI-TOF MS has largely replaced both traditional biochemical approaches and DNA sequencing-based methods for the identification of most bacteria. This talk will focus on the broad applicability of MALDI-TOF MS in the clinical microbiology lab and discuss advanced applications of this method such as database customization, difficult to identify organisms, and rapid diagnosis of sepsis.

TOD 3:50 - 4:05 pm: Application of UV and Chemical Cross-Linking In combination with Mass Spectrometry and Deep Sequencing to Study Complex Interaction Networks

Yu Qian[1, 2]; Catherine E Costello[1]; Ruslan Afasizhev[2]
[1]Boston University School of Medicine, Boston, MA; [2]Boston University School of Dental Medicine, Boston, MA

Trypanosomes are parasitic protozoan hemoflagellates that cause serious diseases in developing countries. These important pathogens possess unusual RNA processing pathways which are important from the basic science perspective and represent potential targets for therapeutic intervention. The Trypanosome mitochondrion encloses a disc-shaped DNA structure composed of a few maxicircles and thousands of minicircles. Most mitochondrial genes encoded in maxicircles are encrypted and their transcripts require extensive uridine insertion/deletion mRNA editing to produce open reading frames. The RNA editing is directed by minicircle-encoded guide RNAs (gRNAs), which are stabilized by the gRNA binding complex (GRBC). Here, we apply UV and chemical cross-linking coupled with mass spectrometry (MS) to define protein-RNA and protein-protein interactions within the GRBC, to better understand its role in Trypanosome RNA editing. First, GRBC is purified using tandem affinity purification (TAP) and MS analysis is used to identify the proteins within the purified complex. Then the complex is cross-linked with isotopically labeled Bis-Sulfosuccinimidyl-Suberate (BS3-H12/D12). Cross-linked peptides are analyzed by the Agilent 6550 Q-TOF mass spectrometer using data dependent analysis. The xQuest/xProphet software is utilized to assign cross-linked peptides and their specific cross-linking residues. In the second stage, *in vivo* cross-linking is being performed to capture transient interactions. After cross-linking with disuccinimidyl glutarate (DSG), the GRBC complex is purified under denaturing conditions using an *in vivo* biotinylation system. The purified complex will be subjected to MS analysis to determine the complex network *in vivo*. Finally, the GRBC-bounded portion(s) of the RNA sequence will be identified through UV-crosslinking affinity purification and deep sequencing, using the same *in vivo* biotinylation system. This research is supported by NIH grants R01 AI091914 and P41 GM104603.

TOD 4:05 pm - 4:20 pm: What the Acetylomes Tell Us about Sirtuin Promiscuity: Lessons from Bacterial and Yeast Sirtuins

Brian Weinert; Chuna Choudhary
University of Copenhagen, Copenhagen, Denmark

Advances in mass spectrometry and acetylated peptide enrichment have enabled the identification and quantification of thousands of acetylation sites in diverse cells and tissues. NAD+-dependent Sirtuin deacetylases play important roles in regulating health and longevity, therefore studying Sirtuin-regulated acetylation sites is important for understanding Sirtuin function. By measuring the degree (stoichiometry) of acetylation we recently demonstrated that the mammalian mitochondrial Sirtuin SIRT3 suppresses acetylation at hundreds of sites, suggesting that SIRT3 is a protein repair factor that removes acetylation lesions. Here we measured bacterial acetylation stoichiometry using an improved method and show that the E. coli sirtuin CobB similarly suppresses acetylation at hundreds of sites. These results suggest that CobB may also act to repair acetylation lesions and counteract acetylation stress. Our previous work indicates that most acetylation occurs by a nonenzymatic mechanism. The role of SIRT3 and CobB in repairing nonenzymatic acetylation lesions is supported by studies showing that loss of these Sirtuins has a greater impact on biological function under metabolic conditions that promote nonenzymatic acetylation. The budding yeast sirtuin Sir2 is the founding member of the Sirtuin deacetylase family and perhaps one of the best studied Sirtuins. So far, the only known function of Sir2 is regulating chromatin silencing via histone deacetylation. Using our unpublished data we show that Sir2 is highly promiscuous and regulates acetylation levels at hundreds of sites, half of which occur on essential proteins. How do we interpret Sir2 promiscuity in light of its limited biological functions and nonessential phenotype? These data

show that a number of Sirtuin deacetylases in evolutionarily diverse organisms have a conserved function in suppressing acetylation at hundreds, if not thousands of sites. This activity may represent an ancient repair function which subsequently evolved specific regulatory functions by deacetylating enzyme-catalyzed acetylation, for example, of histones and transcription factors.

TUESDAY 3:00 – 4:20 PM
PROTEOGENOMICS, *Grand C*

TOE 3:25 pm - 3:50 pm: Insights into Dynamic Gene Expression Regulation from Integrative Analyses

Christine Vogel
New York University, New York, New York

The relative importance of different levels of gene expression regulation, i.e. transcription versus translation, has been subject to ongoing debate. We have moved beyond analysis of steady-state systems and asked how transcription and translation regulation compare in cells responding to a stimulus. Thanks to advance in transcriptomics and proteomics technologies, such time-series datasets of mRNA and matching protein concentrations have now become available. However, tools for their analysis are still rare. We developed such a tool, called PECA (Protein Expression Control Analysis), and applied it originally to yeast responding to rapamycin treatment, oxidative and osmotic stress [1]. PECA is the first such statistical tool that allows for systematic and robust comparisons of different levels of gene expression regulation, and it can extract not only genes that are significantly changing at either the mRNA or the protein level, but also the respective change points (time points). It deconvolutes dynamic gene expression regulation and provides summaries of gene-specific temporal behavior. We now applied PECA to datasets collected from mammalian cells, i.e. from a recently published study of cells responding to LPS treatment, and from our own experiments observing the cellular ER stress response over 30 hours. During ER stress, substantial proteomic rearrangements require both transcription and translation regulation, and PECA successfully identifies and quantifies these contributions. In contrast to other studies, we find that protein-level regulation is as important as mRNA-level regulation -- but the two levels show different temporal patterns. The dominant regulatory level, both in the LPS and the ER stress response, appears to show a switch-like behavior in which expression levels change once during the time-course and then establish a new steady-state. In contrast, non-dominant regulatory events appear in a more pulse-like fashion.

[1] Teo et al. Journal for Proteomics Research 2014

[2] Cheng et al. Molecular Systems Biology 2015

TOE 3:50 pm - 4:05 pm: Integration of PTM Knowledge Networks with Multi-Level Omics Data for Analysis of PTMs in Cancer

Karen E. Ross[1]; Cathy H. Wu[2]
[1]Georgetown University Medical Center, Washington, DC; [2]University of Delaware, Newark, DE

The quantity and quality of omics data is dramatically increasing, providing great opportunities for discovery. However, to take full advantage of this information, it should be analyzed in the context of our current biological knowledge. To address questions regarding the role of post-translational modifications (PTMs) in cancer, we have integrated information from our PTM knowledge resource, iPTMnet, with multi-level omics data from large-scale initiatives (e.g., The Cancer Genome Atlas (TCGA) and the Cancer Proteomic Tumor Analysis Consortium (CPTAC)) and other targeted studies.

iPTMnet (http://proteininformationresource.org/iPTMnet) supports network visualization and analysis of PTM information including PTM sites, enzymes, and functional impacts derived from literature-mining and curated databases and ontologies. We used iPTMnet to construct a PTM network for the mitochondrial enzyme pyruvate dehydrogenase (PDHA1), whose phosphorylation leads to metabolic abnormalities in cancer cells, and overlaid this network with mRNA, miRNA, protein and phosphoprotein expression/abundance data from the TCGA/CPTAC breast cancer datasets. We found evidence for three phosphorylated forms of PDHA1, including a doubly phosphorylated form, and examined the contributions of individual PDHA1 kinases to PDHA1 phosphorylation levels.

While phosphoproteomic studies provide a wealth of data on phosphorylation sites that are affected by disease or other perturbations, they do not directly provide information on the kinases responsible for these changes. To address this issue, we used iPTMnet to identify experimentally validated kinases for phosphorylation sites that were significantly up or down-regulated in a phosphoproteomic study of lung cancer cells treated with the EGFR inhibitor, erlotinib. About 20% of the sites had at least one associated kinase in iPTMnet. Many of these kinases belonged to key cancer-related signaling pathways that had connections to EGFR.

These studies demonstrate the advantages of combining omics data with information from a curated knowledge resource, such as iPTMnet, to gain insight into biological and translational research questions.

TOE 4:05 pm - 4:20 pm: Comprehensive Genomics and Proteomics Analyses Reveal Extensive Tumor Heterogeneity in Lung Adenocarcinoma

Xu Zhang; Shaojian Gao; Constance Cultraro; Romi Biswas; Tapan Maity; Udayan Guha
CCR, NCI, NIH, Bethesda,

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 comprehensive whole genome sequencing (WGS) and mass spectrometry-based proteomics analyses 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. WGS revealed unprecedented tumor heterogeneity between the lung and lymph node metastatic sites. Only 1% of somatic variants were common between the two sites. Interestingly, one novel somatic translocation, PLAG1-ACTA2 was identified in both sites resulting in overexpression of ACTA2 that might have been the driver of early metastasis in this patient. We further sought to correlate the genomic heterogeneity with alterations in the proteome and phosphoproteome using high-resolution mass spectrometry. We employed a "Super-SILAC" quantitative strategy to quantify the global proteome of the lung and two metastatic lymph nodes (LN1, LN2) acquired more than two years apart. Patient-specific databases were created using the WGS data to incorporate all somatic variants for protein identification. We identified around 5000 proteins and 3308 could be quantified. Significant heterogeneity was observed in global protein expression between the three sites. 669 proteins were differentially expressed with greater than 2-fold change between either two of the three sites. Expression of 104 proteins was greater in the lung compared to lymph nodes. These include proteins involved in ribonucleoprotein complexes, RNA binding, and ncRNA processing. Expression of 54 proteins was higher in LN2. Interestingly, expression of 46 proteins was lowest in LN1 and highest in LN2 indicating a temporal change in expression as the tumor evolved. Our comprehensive proteo-genomics analyses enabled us to correlate extensive tumor heterogeneity to targeted treatment response in this patient.

TUESDAY 4:30 – 5:50 PM
CANCER, *Grand A*

TOF 4:30 pm - 4:55 pm: Membrane Proteomics: Surface Markers & Horizontal Signaling

Thomas Kislinger
University of Toronto, Toronto, Canada

Solid tumors are complex tissues composed of different cell types and noncellular components such as extracellular matrix, soluble factors, pH, oxygen tension and interstitial pressure. While decades of research have identified key oncogenic signaling pathways and more recently through next-gen sequencing (epi)genetic alterations, current therapeutic strategies are still inefficient in curing most cancers. It has become evident that the highly complex crosstalk within the tumor microenvironment actively modulates tumor growth, metastasis and response to therapy. This intracellular crosstalk is mediated by cell-surface receptors, soluble ligands and extracellular vesicles. In-depth studies of membrane proteins have proven to be difficult because of

their low abundance and hydrophobicity. However, recent advances in proteomic technologies make it possible to investigate membrane proteins to an impressive depth. Here, I will present our recent work using in-depth proteomics to obtain novel insights into head and neck cancer. Cell-surface labeling strategies, such as silica-bead coating, have enabled us to interrogate the head and neck cancer surfaceome to an unprecedented depth. Integration of these results with publicly available genomics data enabled us to discover novel, previously unstudied surface markers that could serve as novel drugable targets. Functional data on some surface markers will be presented. Cancer-associated fibroblasts (CAFs) represent the most abundant cell type of the stroma and are key components involved in regulating carcinogenesis. Recently, CAF-secreted exosomes were shown to be important mediators of paracrine signals that promote motility and metastasis in breast cancer. To investigate stromal heterogeneity in OSCCs we isolated matched pairs of human primary fibroblasts from resected tumors (CAFs) and adjacent tissue (AFs) and characterized them according to established CAF markers. Comprehensive proteomics identified a signature of CAF-enriched exosomal proteins potentially involved in pathways mediating tumor-stromal crosstalk. Functional experiments in the context of migration, metabolic coupling and radiation response are in progress.

TOF 5:20 pm - 5:35 pm: Predicting Ovarian Cancer Patients' Clinical Response to Platinum-based Chemotherapy by their Tumor Proteomic Signatures

Kun-Hsing Yu[1]; Douglas Levine[2]; Hui Zhang[3]; Daniel Chan[3]; Zhen Zhang[3]; Michael Snyder[1]
[1]Stanford University, Stanford, CA; [2]Memorial Sloan Kettering Cancer Center, New York City, NY; [3]Johns Hopkins Medical Institutions, Baltimore, MD

Ovarian cancer is the deadliest gynecologic malignancy in the United States, with most patients diagnosed in the advanced stage of the disease. Platinum-based antineoplastic therapeutics is indispensable to treating advanced ovarian serous carcinoma. However, patients have heterogeneous response to platinum drugs, and it is difficult to predict these inter-individual differences before administering medication. In this study, we investigated the tumor proteomic profiles and clinical characteristics of 130 ovarian serous carcinoma patients analyzed by the Clinical Proteomic Tumor Analysis Consortium (CPTAC), predicted the platinum drug response using supervised machine learning methods, and evaluated our prediction models through a hold-out test set and leave-one-out cross-validation. Our best classifier predicted platinum response status (platinum sensitive or platinum resistant) with area under receiver operating characteristic curve (AUC) > 0.8. We also built a least absolute shrinkage and selection operator (LASSO)-Cox proportional hazards model that stratified patients into early relapse and late relapse groups (P=0.00011). The top proteomic features indicative of platinum response were involved in ATP synthesis pathways and Ran GTPase binding. Overall, we demonstrated that proteomic profiles of ovarian serous carcinoma patients predicted platinum drug responses as well as provided insights into the biological processes influencing the efficacy of platinum-based therapeutics. Our analytical approach is also extensible to predicting response to other anti-neoplastic agents or treatment modalities for both ovarian and other cancers.

TOF 5:35 pm - 5:50 pm: Affinity Proteomics Establishes a Bifurcated Signaling Cascade of NIMA-related Kinases that Regulates Cell Division

Sierra Cullati; Rufus Hards; Lilian Kabeche; Scott Gerber
Geisel School of Medicine at Dartmouth, Lebanon, New Hampshire

NIMA-related kinases (Neks) control several aspects of cell division downstream of master mitotic regulators such as Cdk1 and Plk1. Though depletion of Nek6, Nek7, or Nek9 results in cytokinesis failure, no molecular mechanism has been elucidated to explain this observation. Cytokinesis failure results in tetraploidy, which is often the precursor to aneuploidy and chromosome instability, hallmarks of cancer that correlate with poor prognosis and patient outcomes.

To investigate how Neks may interact with known mitotic proteins to affect the outcome of cytokinesis, we utilized AP-MS/MS to identify the binding partners of Nek9, which is upstream of Nek6 and Nek7 and contains several protein-protein interaction domains. We identified a variety of proteins known to be involved in cytokinesis, including two kinesins, Mklp2 and Kif14, which depend upon Nek9 for their localization to the central spindle in anaphase. Though both proteins retain midbody localization in telophase, prior residence at the central spindle was essential for successful completion of cytokinesis. We demonstrated that a Nek9, Nek6, and Mklp2 signaling module controls the timely localization and bundling activity of Mklp2 at the anaphase central spindle. We further showed that a separate Nek9, Nek7, and Kif14 signaling module is required for the recruitment of the Rho-dependent kinase citron to the anaphase midzone.

This bifurcation in the Nek6, Nek7, and Nek9 signaling cascade that is required for the localization and function of Mklp2 and Kif14 highlights the potential differences in substrate specificity between the related effector kinases Nek6 and Nek7. To further explore the distinction between Nek6 and Nek7, we investigated the downstream pathways regulated by these kinases by shRNA-mediated depletion followed by quantitative phosphoproteomics. Future work will determine direct kinase substrates utilizing the auxin-inducible degradation system. Overall, our work combines proteome-wide analysis with targeted cell biology to uncover the role of NIMA-related kinases in mitosis.

TUESDAY 4:30 – 5:50 PM
NEW DEVELOPMENTS IN PROTEOMICS, *Grand C*

TOG 4:30 pm - 4:55 pm: High Resolution/Accurate Mass Parallel Reaction Monitoring to Measure Stable Isotope Enrichment of Proteins in Kinetic Studies with Endogenous Labeling

Sasha Singh
Brigham and Women's Hospital, Boston, MA

Endogenous labeling with stable isotopes is used to study the metabolism of proteins in vivo. Multiple reaction monitoring is a common detection method to measure tracer enrichment in multiple proteins simultaneously, however, it is limited in the ability to measure precisely the low tracer enrichment in slowly turning over proteins such as those in HDL. Epidemiologic studies have consistently shown that low HDL, either measured by the levels of its cholesterol content (HDL-C) or its primary protein apolipoprotein A-I (apoA-I), is an independent risk factor for coronary heart disease.

We exploited the versatility of high resolution/accurate mass parallel reaction monitoring (HR/AM-PRM) on the quadrupole Orbitrap to measure bolus-administered D3-Leu tracer enrichment between 0.03 to 1.0 % in seven HDL apolipoproteins - apoA-I, apoA-II, apoA-IV, apoC-III, apoD, apoE, and apoM - across five HDL size fractions in humans. The D0-Leu and D3-Leu MS1 peaks were co-isolated and the fragment ions were scanned at R=140K permitting deconvolution of the low abundance 2H M3 ion from the natural M3 ion and surrounding, non-specific peaks. The resulting enrichment curve data (up to 15 time points over three days) were analyzed by multicompartmental modeling. Contrary to the classical view that posits small HDL/apoA-I particles are released into circulation and then are converted to larger HDL particles, our compartmental analysis revealed that these apolipoproteins in each HDL size mainly originate directly from their source compartments, presumably the liver and intestine. Flux of apolipoproteins from smaller to larger HDL contributed only slightly to apolipoprotein metabolism. These novel findings on HDL apolipoprotein metabolism demonstrate that HR/AM-PRM technology provides a foundation to design in vivo/clinical metabolism studies aimed to understand the metabolism of specific HDL apolipoproteins that have unique relations to risk of diseases such as diabetes and cardiovascular disease.

TOG 4:55 pm - 5:20 pm: Ion Mobility Spectrometry Coming of Age

Melvin Park
Bruker Daltonics, Billerica, MA

Ion mobility spectrometry (IMS) is an analytical method that separates ions based on their transport through an inert gas. IMS has existed as an analytical tool for at least a century[1] and has been broadly applied in the environmental and security fields. In recent decades, IMS was coupled with mass spectrometry (IMMS) in several labs for use in a wide range of biological, physical, and chemical applications. Importantly, for purposes of coupling, the timescale of an IMS separation (<1 s) is between that of LC and time-of-flight mass spectrometry. With commercialization, IMMS has become more

widespread; bringing to light some heretofore unrecognized opportunities. The increased interest in IMMS in recent years has thus spurred the development of new technology, bringing IMS to a level of maturity worthy of consideration in many analytical fields.

Trapped ion mobility spectrometry (TIMS) is one such new technology[2,3]. TIMS promises a simplicity, flexibility, and sensitivity unprecedented in the world of IMMS. Specifically, TIMS analyzers are small (~10 cm in length) and operate at low voltages (~200 V) as compared to conventional IMS analyzers, but can achieve a relatively high resolving power (max~400) and duty cycle (up to 90%). This presentation will introduce the basic construction, modes of operation, theory, and performance characteristics of TIMS as integrated into a prototype electrospray ionization time-of-flight mass spectrometer (ESI–TIMS–OTOF). The presentation aims to frame the question "How will the 'coming of age' of IMS influence the field of proteomics?"

1. J. Zeleny, *Philos. Mag.* **46**, 120(1898).
2. F. Fernandez-Lima, D.A. Kaplan, J. Suetering, and M.A. Park, *Int. J. Ion Mobil. Spec.* **14**, 93 (2011).
3. K. Michelmann, J.A. Silveira, M.E. Ridgeway, & M.A. Park, *J Am. Soc. Mass Spectrom.* **26**, 14(2015).

TOG 5:20 pm - 5:35 pm: N-Linked Glycoproteomic Analysis Using N-linked Glycans and Glycosite-containing (NGAG) Method

Shisheng Sun; Punit Shah; Shadi Toghi Eshghi; Hui Zhang
Johns Hopkins University, Baltimore, MD

Transmembrane proteins or proteins that are expressed on the extracellular surface are easily accessible to therapeutic drugs, antibodies, and ligands and constitute the interface between the cell interior and the outside of cells. These features make cell surface proteins a highly interesting class of proteins for clinical and biological research. A common feature shared by transmembrane proteins, cell surface proteins, proteins secreted to body fluids, and proteins on envelope of bacteria and viruses is that a large fraction of them is N-glycosylated glycoproteins.

Glycoproteins modified by N-linked oligosaccharides are complex with each glycoprotein may potentially containing multiple glycosylation sites and each glycosylation site consisting of heterogeneous glycoforms. Comprehensive characterization of protein glycosylation is critical to understanding the structures and functions of glycoproteins. However, due to the enormous complexity and heterogeneity of glycoprotein structures, the current glycoprotein analyses focus mainly on either released glycans or de-glycosylated glycosite-containing peptides.

In this study, we describe a novel chemoenzymatic method termed solid phase extraction of N-linked Glycans And Glycosite-containing peptides (NGAG) for the comprehensive characterization of glycoproteins by the simultaneous analysis of overall N-linked glycans, glycosites, glycoproteins, and site-specific glycans and glycosylation occupancies. We show that the NGAG method allowed us to identify 85 N-linked glycan compositions, 2,044 glycosite-containing peptides, 1, 242 glycoproteins and 1,562 intact glycopeptides from an ovarian cancer cell line (OVCAR-3). We also determined the glycosylation occupancies at 117 partially glycosylated sites, identified and validated two atypical glycosites from the same samples. Such a comprehensive and integrated characterization of protein glycosylation is complementary to genomic, transcriptomic and metabolomic data and is crucial for a systems biology-level understanding of the consequences of perturbed biological processes.

TOG 5:35 pm - 5:50 pm: SIMPLEX: A Combinatorial Multimolecular Omics Approach for Systems Biology

Cristina Coman; Fiorella Andrea Solari; Andreas Hentschel; Rene Peiman Zahedi; Albert Sickmann; Robert Ahrends
ISAS, Dortmund, Germany

Interconnected molecular networks are at the heart of signaling pathways that mediate adaptive plasticity of eukaryotic cells. To gain deeper insights into the underlying molecular mechanisms, a comprehensive and representative analysis demands a deep and parallel coverage of a broad spectrum of molecular species. Therefore, we introduce SIMPLEX (SImultaneous Metabolite, Protein, Lipid EXtraction procedure), a novel strategy for the quantitative investigation of lipids, metabolites and proteins. Compared to unimolecular workflows, SIMPLEX offers a fundamental turn in study design, since multiple molecular classes can be accessed in parallel from one sample with equal efficiency and reproducibility. Application of this method in mass spectrometry based workflows allowed the simultaneous quantification of 360 lipids, 75 metabolites and 3327 proteins from 10^6 cells. The versatility of this method is shown in a model system for adipogenesis - PPARG signaling in mesenchymal stem cells – where we explored with SIMPLEX cross-talk within and between all three molecular classes and identified novel potential molecular entry points for interventions, indicating that SIMPLEX provides a superior strategy compared to conventional workflows.

WEDNESDAY 9:50 – 11:10 AM
BIOMARKERS, *Grand A*

WOB 9:50 am - 10:15 am: Proteomics-based Biomarker Discovery: Mirage or Emerging Reality?

Steven A. Carr
Broad Institute of MIT and Harvard, Cambridge, MA

Better biomarkers are urgently needed to improve diagnosis, guide molecularly targeted therapy, and monitor activity and therapeutic response across a wide spectrum of disease. Proteomics methods based on mass spectrometry hold special promise for the discovery of novel biomarkers that might form the foundation for new clinical blood tests, but to date little has been delivered. Proteomics-based biomarker discovery has been severely hampered by inadequate number and quality of samples, poor study design, and technological approaches lacking sufficient sensitivity, quantitative precision, and capacity to analyze statistically relevant numbers of samples. Another key problem has been the lack of robust quantitative methods to credential candidate protein biomarkers in larger numbers of patient samples prior to clinical evaluation. This problem extends into biology where the lack of highly specific affinity reagents for novel candidate proteins and modified peptides with sufficient sensitivity, specificity, reproducibility and throughput has significantly hampered our ability to understand dynamic, protein-based biological processes. In our biomarker studies we are addressing both of these serious barriers. In the discovery phase, we are employing multiplexed, quantitative MS technologies that enable analysis of larger numbers of patient samples with improved precision leading to better-qualified candidates. For verification of candidate biomarkers, we are developing targeted mass spectrometry-based technologies to screen and quantify low abundance proteins and modified peptides in a variety of biological contexts including human tissue and plasma. I will present on a range of studies that are beginning to demonstrate that modern proteomic technologies when coherently integrated can yield novel, credentialed protein and peptide biomarker candidates of sufficient merit to warrant real clinical evaluation and to shed light on biological function.

WOB 10:40 am - 10:55 am: An Affinity Proteomics Strategy for Plasma Biomarker Validation

Claudia Fredolini; Sanna Byström; Elin Birgersson; Peter Nilsson; Mathias Uhlén; Jochen Schwenk
Science for Life Laboratory - KTH, Solna, Sweden

Utilizing multiplexed affinity proteomic assays in plasma allows for a systematic exploration of proteins in larger study sets. With access to reagents from the Human Protein Atlas, we have conducted targeted or discovery-driven projects using single-binder assays on a bead array platform. Such studies have been enabled by appropriately designed studies from well-categorized patient biobanks and revealed biomarker candidates across different diseases. Nevertheless, thorough validation is required both concerning the antibodies and the proposed biomarker candidates. Therefore, the bead-based assays offer several entry points into subsequent verification: straightforward assay replication at large scale; screening of additional plasma or proximal fluids; or profiling the target proteins by several antibodies. To investigate antibody susceptibility to off-target binding in body fluids, additional proteomic tools are applied, such as mapping antibody binding epitopes on high-density peptide or protein arrays. In order to identify the proteins, which were enriched by the antibodies from clinical plasma samples, appropriate validation assays apply the same conditions as chosen for the discovery. We recently developed a new multiplexed immunoassay based on dual antigen capture and

established a workflow for immuno-capture mass spectrometry analysis that both utilize antibody-coupled beads. Empowered by protein identification possibilities of LC-MS as well as the targeted SRM assays, antibody enrichment profiles are defined to serve as one important element towards developing sandwich assays. For the latter dual binder assays, an established routine is to pair capture and detection antibodies in multiplex on recombinant proteins and clinical samples. To then prepare the assay for translation, independent sample sets are studied to quantify the target and confirm the initial indications. Making use of the applicability of antibodies, the evaluation of indicative biomarkers can include supplementary cell or tissue-based assays to investigate protein expression at the location of the disease.

WOB 10:55 am - 11:10 am: Application and Optimization of MStern Blot for Translational Proteomics

Sebastian T. Berger[1, 2]; Saima Ahmed[1, 2]; Jan Muntel[1, 2]; Michaela Helmel[1, 2]; Richard Bachur[1]; Alex Kentsis[3]; Hanno Steen[1, 2]
[1]Boston Children's Hospital, Boston, MA; [2]Harvard Medical School, Boston, MA; [3]Sloan Kettering Institute / Cornell University, New York, NY

The discovery of disease specific biomarker is pivotal for development of 'precision' and/or personalized medicine. Body fluids such as blood/plasma/serum, urine or CSF are the preferred types of tissue for biomarker endeavors because they are relatively easily accessible. However, the development of proteomics sample preparation strategies with appropriate throughputs has been slow. A paradigm shift was the introduction of filtration membrane-based sample processing methods such as FASP. Recently, we developed a novel proteomic sample preparation strategy, called "MStern Blot". Our method employs large-pore hydrophobic polyvinylidene fluoride (PVDF) membranes to retain proteins by adsorption instead of small-pore ultrafiltration membranes that retain proteins because of their size. The pores in PVDF membranes are 100 times larger than the ones in ultrafiltration membranes, thereby allowing significantly faster liquid transfer in a matter of seconds/minutes even in the 96-well plate format. Furthermore, elution of tryptic peptides is achieved using acidified aqueous acetonitrile such that no further desalting steps are necessary. This new method allows the complete processing of 96 samples (or multiples thereof) in a single workday.

We used our newly developed method on a large urinary sample cohort (89 samples) to discover biomarker for different causes of abdominal pain in children. Our preliminary results provide insights into the pathophysiology of different diseases and identify diagnostic biomarker candidates.

In an effort to further optimize MStern blotting and to test its potential we implemented an easy fractionation procedure by step eluting the proteolytic peptides off the PVDF membrane, resulting in 30% more identified peptides. Similarly, peptide and protein identification rates could be increased by adding trifluoroethanol to the digestion buffer.

WEDNESDAY 9:50 – 11:10 AM
NEUROPROTEOMICS, *Grand C*

WOC 10:40 am - 10:55 am: Characterization of Depolarization-Dependent Signaling Pathways in the Active Zone In Isolated Nerve-Terminals

Martin R. Larsen[1]; Simone Sidoli[2]; Katarzyna Kulej[2]; Jing Xue[3]; Maria Ibanez Vea[1]; Mark Graham[3]; Phillip J. Robinson[3]
[1]University of Southern Denmark, Odense M, Denmark; [2]Perelman School of Medicine, University of Pennsyl, Philadelphia , PA; [3]Chrildren´s Medical Research Institute,, Wentworthville NSW 2145, Australia

The terminal part of neurons is termed synapses and synaptosomes are isolated synapses containing the intact pre-synaptic machinery, the active zone and part of the post-synapse. Synaptosomes are frequently used to study synaptic transmission, as they contain the machinery necessary for the uptake, storage, and release of neurotransmitters. Depolarization of synaptosomes lead to calcium influx, which trigger a number of events including exocytosis, endocytosis, synaptic vesicle (SV) filling and SV transportation. Many of these mechanisms are relying on phosphorylation dependent protein activation and protein-protein interactions. In the present study we have mapped the phosphoproteome of synaptosomes from rat brains, developed a method for enrichment of the active zone proteins from isolated synaptosomes and characterized the changes in the phosphoproteome of the enriched active zone after depolarization.

The synaptosome phosphoproteomics approach resulted in identification of 22137 phosphosites on 4786 proteins (4.6 phosphosites per protein). More than 50 proteins were identified with more than 30 phosphosites and the proteins Piccolo, Protein bassoon and Microtubule-associated protein 1B were identified with 147, 146 and 150 phosphosites, respectively. Using a fast protein extraction method we were able to enrich for the active zone of synaptosomes and characterize the alteration in protein phosphorylation after depolarization using KCl. Using this method we observed a 30% increase in the amount of protein in the enriched active zone after 10 sec depolarization illustrating a substantial protein translocation upon depolarization. More than 3000 phosphosites showed a significant regulation after depolarization in the enriched active zone. Pathway analysis revealed substantial and complex regulation of all major pathways in synaptosomes incl. endo- and exo-cytosis, SV modulation, mitochondrial function and modulation of several receptors, indicating a much higher complexity in the regulation of synaptic transmission than previously observed.

WOC 10:55 am - 11:10 am: Proteomic Analysis to Identify Molecular Regulators of Nerve Regeneration

Ajay Yekkirala[1, 2]; Hui Chen[1, 2]; Kristina Hempel[1, 2]; Judith Steen[1, 2]; Clifford Woolf[1, 2]
[1]Boston children's Hospital, Boston, MA; [2]Harvard Medical School, Boston, MA

Injuries in the central nervous system (CNS) are often debilitating with limited options for clinical treatment. Studies have shown that neurons in the adult CNS do not regenerate after injury as they remain in a growth-quiescent state. Significantly, neurons in the peripheral nervous system (PNS), such as neurons of the dorsal root ganglia (DRG), retain an intrinsic ability to regenerate and their central axons can grow into a lesion in the CNS when 'preconditioned' with a PNS injury. It is, therefore, important to delineate the master regulators responsible for such remarkable regenerative properties in DRG neurons as they have therapeutic potential in CNS repair. While genomic efforts have helped identify some of the major players involved in regeneration, to date a functional analysis at the whole protein level has not been conducted. To identify novel proteins involved in peripheral nerve regeneration we, for the first time, utilized a state of the art LCMS proteomic platform to analyze injured and non-injured mouse DRGs. A proteome of >9000 proteins was identified and quantified. To evaluate protein dynamics upon injury, we utilized a 10-plex TMT-labeling approach to analyze mouse DRG lysates at 0h, 6h, 24h, 3d and 5d after sciatic nerve transection injury (SNI). We then evaluated the regulation at the transcriptional and translation levels by cross referencing the data with RNASeq and microarray data. . This proteogenomics analysis yielded >200 unique proteins which are regulated at the protein level, but not at the genomic level. These results provide the first insights into novel proteome networks regulating the regenerative process in the PNS.

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Lightning Talks

There will be two rounds of Lightning Talks (Monday and Tuesday) highlighting 30 poster presentations. Each Lightning Talk session is a brief presentation (2 minutes max). Posters that will be featured in the Lightning Talk sessions are noted.

Biochemical Pathway Elucidation, 001

001 **Proteomic and Metabolomic Comparative Analyses of Plasma and Vasculature Tissue from TiO2 Nanoparticle Exposed Rats**; Megan M. Maurer[1]; Jinghai Yi[2]; Carroll McBride[2]; Timothy R. Nurkiewicz[2]; Stephen J. Valentine[1]; *[1]Department of Chemistry, West Virginia University, Morgantown, WV; [2]Dept of Physiology & Pharmacology,West Virginia U., Morgantown, WV*

Biochemical Pathway Elucidation, 002 - 014

002 **Optimized Sample Preparation of Human Tissue Proteome for LCMS-based Biomarker Discovery;** Anna Chen[1]; Ruiqi Jian[2]; Lihua Jiang[2]; Michael Snyder[2]; *[1]Mission San Jose High School, Fremont, CA; [2]Department of Genetics, Stanford University, Stanford, CA*

003 **Analysis of Selected Carboxylic and Amino Acids in Clinical Samples of Patients at Risk for Cardiovascular Disease**; Hassan Alamri; *Cleveland State University, Cleveland, OH*

004 **Heat Stabilization Preserves the Molecular Integrity of the Sample**; Charlotta Göransson; Camilla Sivertsson; Marcus Söderquist; Karl Sköld; Mats Borén; *Denator, Uppsala, Sweden*

005 **Quantitative Proteomic Approaches for Identifying Urinary Biomarkers in Lupus Nephritis**; Veronica Anania; *Genentech, Inc., South San Francisco, CA.* ***Featured in Lighting Talks – Round I, Monday at 11:10am.***

006 **Proteomic Analysis of Platelets of First Onset Psychosis Subjects: Oxidative Stress Pathway in the Search for Biomarkers;** Helena Joaquim[1]; José Matheus Bonatto[2]; Leda Talib[1]; Wagner Gattaz[1]; *[1]Laboratory of Neuroscience (LIM-27), São Paulo, Brazil; [2]BioMass, CEFAP-USP, São Paulo, Brazil*

007 **Mass-Spectrometry Discovery, Verification and Validation of Circulating Protein Biomarkers for the Early Detection of Breast Cancer**; Khadijeh Rajabi[1]; Matthew Rosenow[1]; Luisa Paris[2]; Victoria David[1]; Kristine Tsantilas[1]; Paul Russo[2]; Tony Tegeler[1]; Kristina Chapple[1]; Lance Liotta[2]; Emanuel Petricoin[2]; Patrick Pirrotte[1]; *[1]TGen, Phoenix, Arizona; [2]George Mason University, Manassas, VA*

008 **Glycan "Nodes" as Cancer Markers: Clinical Performance in Early Stage Lung Cancer**; Shadi Roshdiferdosi; Chad R. Borges; *Arizona State University, Tempe, Arizona*

009 **Application of Microfluidic/Tandem Quadrupole LC-MS/MS For MRM based Translational Research Analysis of Putative Heart Failure Peptide Biomarkers in Human Plasma**; Richard Mbasu[1, 2]; Liam Heaney[2]; Billy Molloy[3]; Chris Hughes[3]; Roy Martin[4]; Leong Ng[2]; Johannes Vissers[3]; James Langridge[3]; Don Jones[1, 2]; *[1]Department of Cancer Studies, RKCSB, University of, Leicester, UK; [2]Department of Cardiovascular Sciences and NIHR, Leicester, UK; [3]Waters Corp, Wilmslow, UK; [4]Waters, Beverly, Massachusetts*

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010 **Proteomic Characterisation of Renal Multilammelar Bodies Induced by Kidney Metabolic Injury**; Peter Ochodnicky[1]; Lee Gethings[2]; Roy Martin[3]; Johannes Vissers[2]; Joannes MFG Aerts[4]; Jaklien Leemans[1]; *[1]Dept of Pathology, Univ. of Amsterdam, Amsterdam, Netherlands; [2]Waters Corp, Manchester, United Kingdom; [3]Waters, Beverly, Massachusetts; [4]Faculty of Science,Leiden University, Leiden, Netherlands*

011 **Multiplexed Kinase Biosensor Technology to Detect Leukemia Signaling with Mass Spectrometry**; Tzu-Yi Yang; Laurie L. Parker; *University of Minnesota Twin Cities, Minneapolis, Minnesota*

012 **MRM Assays and Tools for Quantifying Multiplexed Panels of Proteins in 4 Mouse Tissues;** Andrew Percy[1]; Sarah Michaud[2]; Nicholas Sinclair[1]; Yassene Mohammed[3]; Christoph Borchers[1]; *[1]UVic-Genome BC Proteomics Centre, Victoria, Canada; [2]MRM Proteomics, Victoria, BC; [3]Leiden University Medical Center, Leiden, Netherlands*

013 **Multi-omics Analysis of Cytotrophoblasts from Second Trimester and Full-Term Primary Cultures**; Christie Hunter[1]; Katherine Williams[2]; Andrew Olsen[3]; Brigitte Simons[4]; *[1]SCIEX, Redwood City, CA; [2]UCSF, San Francisco, CA; [3]Advaita Biosciences, Plymouth, MI; [4]SCIEX, Concord, ON*

014 **Association of CDK10 with ETS2 during Human Corneal Wound Healing**; Shamim Mushtaq[1]; Meraj Zehra[2]; Nikhat Ahmed Siddiqui[1]; *[1]Ziauddin University, Karachi, Pakistan; [2]Karachi University, Karachi, Sindh*

Cancer Proteomics, 015 - 025

015 **A New Approach for Understanding Cancer and Chemoresistance: Metabolic Labeling and LC-MS to Measure Protein Turnover in Breast Cancer;** Monique Paré Speirs; Michael Porter; Bradley Naylor; John Price; *Brigham Young University, Provo, UT.* ***Featured in Lighting Talks – Round I, Monday at 11:10am.***

016 **Cell Death Resistance and Chemo-Resistance Due to SphK1 in Pancreatic Cancer**; Adam Swensen; *Brigham Young University, Springville, Utah*

017 **The Comparison of the Serum Proteome in Individuals with Cancers versus Those without Cancer, and Its Application to Wellness**; Haiyan Zheng[1]; Caifeng Zhao[1]; Swapan Roy[2]; Devjit Roy[3]; Amenah Soherwardy[2]; Ravish Amin[2]; Matthew Kuruc[2]; *[1]Rutgers Center for Integrative Proteomics, Piscataway, NJ; [2]Biotech Support Group LLC, Monmouth Junction, New Jersey; [3]Wyoming Medical Center, Casper, WY.* ***Featured in Lighting Talks – Round II, Tuesday at 11:10am.***

018 **Quantitative Analysis of AKT/mTOR Pathway using Multiplex-Immunoprecipitation and Targeted Mass Spectrometry;** Bhavin Patel[1]; Alex Behling[1]; Leigh Foster[1]; Ryan Bomgarden[1]; Carrie Clothier[1]; Kay Opperman[1]; Rosa Viner[2]; Andreas Huhmer[2]; John Rogers[1]; *[1]Thermo Fisher Scientific, Rockford, IL; [2]Thermo Fisher Scientific, San Jose, CA*

019 **Integrative Proteomic and Glycomic Profiling of Metastatic Signatures in Breast Tumor;** Le Meng; *Boston University, Boston, MA.* ***Featured in Lighting Talks – Round I, Monday at 11:10am.***

020 **Inter-grade Comparative Proteomic Analysis of Gliomas using Cerebrospinal Fluid**; Nikita Gahoi[1]; Darpan Malhotra[1]; Aliasgar Moiyadi[2]; Sanjeeva Srivastava*[1]; *[1]Indian Institute of Technology, Bombay, Mumbai, India; [2]Department of Neurosurgery, ACTREC, Mumbai , Maharastra.* ***Featured in Lighting Talks – Round II, Tuesday at 11:10am.***

021 **Dysregulation of HUWE1, An Essential E3 Ubiquitin Ligase, Reduces Chromosome Segregation Fidelity;** Katelyn Cassidy; Lilian Kabeche; Scott Gerber; *Dartmouth College, Hanover, NH*

022 **Src-Family Kinase Signaling Mediating Gemoitabine Resistance in Gallbladder Cancer Revealed by Quantitative Phosphoproteomics**; Patricia García[1]; Jun Zhong[2]; Carolina Bizama[1]; Jaime Espinoza[1]; Juan Carlos Roa[1]; Pamela Leal[3]; *[1]Pontificia Universidad Católica de Chile, Santiago, Chile; [2]Delta Omics Biotechnology, Rockville, MD; [3]Universidad de La Frontera, Temuco, Chile.* ***Featured in Lighting Talks – Round II, Tuesday at 11:10am.***

023 **Next Generation Signaling Pathway Characterization by IS-PRM;** Michael Blank[1]; Daniel Ayoub[2]; Sebastien Gallien[2]; Antoine Lesur[2]; Bruno Domon[2]; Julian Saba[3]; Yuriy Dunayevskiy[1]; Andreas Huhmer[1]; *[1]Thermo Fisher Scientific, San Jose, CA; [2]Luxembourg Clinical Proteomics Center, Strassen, Luxembourg; [3]Thermo Fisher Scientific, Mississauga, Canada*

024 **Understanding the Aggressive Nature of Glioblastoma Tumors Associated with the Subventricular Zone;** Kishore Gollapalli[1]; Saicharan Ghantasala[1]; Sachendra Kumar[1]; Rajneesh Srivastava[1]; Srikanth Rapole[2]; Aliasgar Moiyadi[3]; Epari Sridhar[3]; Sanjeeva Srivastava*[1]; *[1]Indian Institute of Technology Bombay, Mumbai, India; [2]National Centre for Cell Science, Pune, India; [3]TMC-ACTREC, Navi Mumbai, India*

025 **Accumulated Ion Monitoring (AIM) Improves Sensitivity and Quantitative Dynamic Range for Precision Functional Proteomics;** Paolo Cifani; Zheng Ser; Avantika Dhabaria; Alex Kentsis; *Sloan-Kettering Institute, New York, NY.* ***Featured in Lighting Talks – Round I, Monday at 11:10am.***

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Chemical Proteomics, 026 - 027

026 **Investigating the Cellular Interactions of BIRB796 Analogs Using a Novel Chloroalkane Capture Tag;** Michael Ford[1]; Richard Jones[1]; Rachel Friedman Ohana[2]; Thomas Kirkland[3]; Carolyn Woodroofe[2]; Paul Otto[2]; Danette Daniels[2]; Marjeta Urh[2]; Keith Wood[2]; *[1]MS Bioworks LLC, Ann Arbor, MICHIGAN; [2]Promega Corporation, Madison, WI; [3]Promega Biosciences, San Luis Obispo, CA*

027 **Development and Application of a Covalent-Labeling Strategy for the Large-Scale Thermodynamic Analysis of Protein Folding and Ligand Binding**; Yingrong Xu; Ryenne Ogburn; Michael C. Fitzgerald; *Duke University, Durham, NC.* ***Featured as a promoted talk in the Structural Proteomics session, Monday at 3:00 pm.***

Chromatin Dynamics, 028

028 **Proteomics Identifies Associated Factors of the Phosphorylated RNA Polymerase II C-Terminal Domain Linking Regulation of Chromatin Dynamics;** Christopher Ebmeier[1]; Benjamin Erickson[2]; Benjamin Allen[1]; William Old[1]; David Bentley[2]; Dylan Taatjes[1]; *[1]University of Colorado-Boulder, Boulder, Colorado; [2]University of Colorado-Denver, Denver, CO*

Clinical Proteomics, 029 - 035

029 **Circulating Peptide Signatures Derived from Enzymatic Activities for Tagging Human Immunodeficiency Virus-1 Elite Controllers;** Yaojun Li[1]; Zhengyu Ouyang[2]; Wei Zhang[1]; Zhen Zhao[3]; Jason Kimata[4]; Xu Yu[2]; Tony Hu[1]; *[1]Houston Methodist Research Inst., Houston, US-; [2]Ragon Institute of MGH, MIT and Harvard University, Boston, Massachusetts; [3]Department of Laboratory Medicine, Clinical Center, Bethesda, MD; [4]Department of Molecular Virology and Microbiology,, Houston, TX*

030 **Optimizing Global Proteomics Analysis for Clinical Biomarker Studies;** Monica Lane; Mahmud Hossain; Pavlina Wolf; Martha Stapels; Petra Oliva; Kate Zhang; *Sanofi Genzyme, Framingham, MA*

031 **PRM Coupled to an Intensity-based Quantification Strategy Accurately Measures D3-Leu Tracer of less than One Percent in apoA-I/HDL Clinical Samples;** Lang Ho Lee[1]; Brett Pieper[1]; Allison Andraski[2]; Frank Sacks[2]; Masanori Aikawa[1]; Sasha Singh[1]; *[1]Brigham and Women's Hospital, Boston, MA; [2]T.H. Chan Public Health Harvard University, Boston, MA.* ***Featured in Lighting Talks – Round I, Monday at 11:10am.***

032 **Getting a Grip on What Determines the Composition of Urinary Proteomes;** Jan Muntel[1, 2]; Sebastian T. Berger[1, 2]; Jennifer K. Cheng[1]; Sarah D. de Ferranti[1, 2]; Nirav K. Desai[1, 2]; Tracy K. Richmond[1, 2]; Kendrin R. Sonneville[3, 4]; Stavroula K. Osganian[1, 2]; Hanno Steen[1, 2]; *[1]Boston Children's Hospital, Boston, MA; [2]Harvard Medical School, Boston, MA; [3]Harvard T.H. Chan School of Public Health, Boston, MA; [4]University of Michigan School of Public Health, Ann Arbor, MI.* ***Featured in Lighting Talks – Round II, Tuesday at 11:10am.***

033 **The Development of Molecular Diagnostic Tool for Schizophrenia using Lymphoblastoid Cell Lines**; Akira Yoshimi[1, 2]; Shinnosuke Yamada[1, 2]; Shohko Kunimoto[1]; Branko Aleksic[1]; Akihiro Hirakawa[1]; Mitsuki Ohashi[2]; Yurie Matsumoto[1, 2]; Yuko Arioka[1]; Tomoko Oya-Ito[1]; Itaru Kushima[1]; Yukako Nakamura[1]; Tomoko Shiino[1]; Daisuke Mori[1]; Takuji Maeda[1]; Satoshi Tanaka[1]; Shuko Hamada[1]; Hiromi Noma[1]; Mami Yoshida[1]; Yukihiro Noda[1, 2]; Taku Nagai[1]; Kiyofumi Yamada[1]; Norio Ozaki[1]; *[1]Nagoya University, Nagoya, JAPAN; [2]Meijo University, Nagoya, JAPAN*

034 **Next-Generation Blood Biomarkers for Acute Liver Injury: *in silico* Discovery and Proteomics Quantification;** Virginie Brun; *CEA, Grenoble, France*

035 **A Cross-Sectional Study on Dietary Factors and their Correlation with Body Mass Index among School-going Teenagers in Karachi**; Arshma Zuberi; *Dow University of Health Sciences and Jinnah Unive, Karachi, Pakistan.* ***Featured in Lighting Talks – Round II, Tuesday at 11:10am.***

Complete Proteome Elucidation, 036 - 037

036 **Pushing the Limits of Bottom-Up Proteomics with State-of-the-Art Capillary UHPLC and Orbitrap Mass Spectrometry for Reproducible Quantitation of Proteomes;** Daniel Lopez-Ferrer[1]; Michael Blank[1]; Stephan Meding[2]; Aran Paulus[1]; Romain Huguet[1]; Remco Swart[2]; Julian Saba[3]; Susan Bird[1]; Andreas Huhmer[1]; *[1]Thermo Fisher Scientific, San Jose, California; [2]Thermo Fisher Scientific, Germering, Germany; [3]Thermo Fisher Scientific, Mississauga, ON, Canada*

037 **A Multiplexed Mass Spectrometry-based Strategy Quantifies Nicotine-Induced Protein Alterations across four Human Cell Lines**; Joao Paulo; Steven Gygi; *Harvard Medical School, Boston, MA*

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Computational Methods, 038 - 041

038 **A System Suitability Monitoring Method for LC MS/MS Proteomic Experiments;** Eralp DOGU; Olga Vitek; *Northeastern University, Boston, Massachusetts*

039 **A Novel and Robust Measure of Protein Co-localization for Super-Resolution Fluorescence Microscopy Images**; Sarah Keegan; Keria Bermudez; Yandong Yin; Dylan Reid; Eli Rothenberg; David Fenyo; *NYU Langone Medical Center, New York, New York*

040 **Parameterization of Averagine Composition Improved Feature Detection of Oligonucleotides**; Samuel Wein[1]; Ben Garcia[2]; *[1]University of Pennsylvani, Philadelphia, Pa; [2]University of Pennsylvania School of Medicine, Philadelphia, PA.* ***Featured in Lighting Talks – Round I, Monday at 11:10am.***

041 **Relative Protein Quantification in Mass Spectrometry-based Proteomics: A Split Plot Approach;** Meena Choi[1]; Lin-Yang Cheng[1]; Nick Shulman[2]; Maria Pavlou[3]; Cristina Chiva[4, 5]; Erik Verschueren[6]; Bernd Wollscheid[3]; Eduard Sabido[4, 5]; Brendan Maclean[2]; Olga Vitek[7]; *[1]Purdue University, West Lafayette, IN; [2]University of Washington Genome Science, Seattle, WA; [3]ETH Zurich, Zurich, Switzerland; [4]Proteomics Unit, Center for Genomics Regulation, Barcelona, Spain; [5]Proteomics Unit, Universitat Pompeu Fabra, Barcelona, Spain; [6]University of California, San Francisco, CA; [7]Northeastern University, Boston, MA*

Data Analysis / Bioinformatics / Algorithm Development, 042 - 050

042 **Decoding Histone Post-Translational Modifications by Bottom-Up Mass Spectrometry**; Zuo-Fei Yuan; Simone Sidoli; Shu Lin; Xiaoshi Wang; Natarajan V. Bhanu; Benjamin A. Garcia; *University of Pennsylvania, Philadelphia, PA*

043 **Species Identification using Bayesian Modeling and Mass Spectrometry;** Jennifer Teubl; *NYU Langone Medical Center, New York, NY.* ***Featured in Lighting Talks – Round II, Tuesday at 11:10am.***

044 **Top-Down Proteomics Data Analysis**; Christian Heckendorf; Roger Theberge; Catherine Costello; Mark Mccomb; *Boston University School of Medicine, Boston, MA*

045 **HTAPP: High-Throughput Autonomous Proteomic Pipeline for Automated Acquisition and Insightful Analysis of MS/MS Data ;** Judson Belmont[2]; Nagib Ahsan[1]; Bharat Ramratnam[1]; Arthur Salomon[1, 2]; *[1]CCRD Proteomics Facility, Rhode Island Hospital, Providence, RI; [2]MCB Department, Brown University, Providence, RI*

046 **Nonlinear Regression Avoids Overly Optimistic Assay Characterization**; Cyril Galitzine[1]; Jarrett Egertson[2]; Olga Vitek[1]; *[1]Northeastern University, Boston, MA; [2]Univ of Washington Genome Sci, Seattle, Washington*

047 **ProteoModlR for Quantitative Proteomics Pathway Modeling**; Paolo Cifani; Mojdeh Shakiba; Alex Kentsis; *Sloan-Kettering Institute, New York, NY*

048 **Comparative Proteomics of Time-Course Activation of Eosinophils with Cytokines, Applied Singly and in Pairs, Using Multiple Proteomic Platforms;** Kizhake Soman[1]; Susan Stafford[1]; Konrad Pazdrak[1]; Zheng Wu[1]; Xuemei Luo[1]; Wendy White[2]; John Wiktorowicz[1]; William Calhoun[1]; Alexander Kurosky[1]; *[1]University of Texas Medical Branch, Galveston, Texas; [2]MedImmune, LLC, Gaithersburg, MD*

049 **Protein-based PTM Quantitative Analysis with PEAKS Software**; Baozhen Shan; Lei Xin; *Bioinformatics Solutions Inc, Waterloo, Canada*

050 **The Scaling Complexity of Glycoproteomics Samples;** Joshua Klein; Kshitij Khatri; Joseph Zaia; *Boston University, Boston, MA*

Data Analysis / Bioinformatics / Algorithm Development, 051

051 **Integrative Systems Biology Approach to Identify Mechanisms of Action;** Akos Vertes[1]; Andrew Korte[1]; Camille Lombard-Banek[1]; Peter Nemes[1]; Lida Parvin[1]; Ziad Sahab[1]; Bindesh Shrestha[1]; Sylwia Stopka[1]; Wei Yuan[1]; Deborah Bunin[2]; Merrill Knapp[2]; Ian Mason[2]; Denise Nishita[2]; Andrew Poggio[2]; Carolyn Talcott[2]; Maneesh Yadav[2]; Brian Davis[3]; Adriana Larriera[3]; Christine Morton[3]; Christopher Sevinsky[3]; Maria Zavodszky[3]; Nicholas Morris[4]; Heather Anderson[4]; Matthew Powell[4]; Trust Razunguzwa[4]; *[1]George Washington University, Washington, DC; [2]SRI International, Menlo Park, CA; [3]GE Global Research, Niskayuna, NY; [4]Protea Biosciences Inc., Morgantown, WV.* ***Featured in Lighting Talks – Round I, Monday at 11:10am.***

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High-Resolution Mass Spectrometry, 052 - 055

052 **Differential Dynamics of the Mammalian mRNA and Protein Expression Response to Misfolding Stress;** Zhe Cheng[1]; Guoshou Teo[2]; Sabrina Krueger[3]; Tara Rock[1]; Hiromi Koh[2]; Hyungwon Choi[2]; Christine Vogel[1]; *[1]New York University, New York, NY; [2]National University of Singapore, Singapore, Singapore; [3]Max-Delbruck-Center, Berlin, Germany.* ***Featured in Lighting Talks – Round II, Tuesday at 11:10am.***

053 **Automated, High-throughput Hemoglobinopathies Profiling using Top-Down LC-MS Methods**; Scott M. Peterman[1]; David Sarracino[1]; Amol Prakash[2]; Shen Luan[1]; Mazi Mohiuddin[1]; *[1]Thermo Fisher Scientific, Cambridge, Massachusetts; [2]Optys Technologies Inc., Boston, MA*

054 **High Quantification Accuracy in Label-Free Proteomics;** Stephanie Kaspar-Schoenefeld[2]; Markus Lubeck[2]; Michael Andersen[1]; Pierre-Olivier Schmit[3]; *[1]Bruker Daltonics, Odense C, Denmark; [2]Bruker Daltonik GmbH, Bremen, Germany; [3]Bruker Daltonique, Wiissembourg, France*

055 **HDL Dysfunction in Patients with NASH is Related to Alteration of HDL Proteome Composition**; Arthur McCullough[2]; Jaividhya Dasarathy[3]; Belinda Willard[2]; Li Ling[2]; Jonathan Smith[2]; Srinivasan Dasarathy[2]; Takhar Kasumov[1, 2]; *[1]NEOMED, Rootstown, OH; [2]Cleveland Clinic Foundation, Cleveland, Oh 44195, Ohio; [3]Metro Health Hospitals, Cleveland, OH*

Neuroproteomics, 057 - 060

057 **Proteomic Profile of Dentate Gyrus of an Epilepsy Model Induced by Electrical Stimulation and Displaying Classical Hippocampal Sclerosis**; Amanda Morato Do Canto[1, 2]; Alexandre Hilario Berenguer Matos[1, 2]; Andre Schwambach Vieira[1, 2]; Rovilson Glioli[1, 3]; Iscia Lopes Cendes[1, 2]; *[1]State University of Campinas, Campinas, Brazil; [2]Department of Medical Genetics - BRAINN, Campinas, Brazil; [3]CEMIB - UNICAMP, Campinas, Brazil*

058 **Cerebral Organoid Proteomics to Study Central Nervous System Development in Down Syndrome**; Tristan Mcclure-Begley; Christopher Ebmeier; Michael Klymkowsky; Kerri Ball; William Old; *University of Colorado, Boulder, Boulder, CO.* ***Featured in Lighting Talks – Round II, Tuesday at 11:10am.***

059 **A High-resolution Anatomical Mouse Brain Proteome**; Sung Yun Jung; Jong Min Choi; Maxime William C. Rousseaux; Yi Wang; Huda Yahya Zoghbi; Jun Qin; *Baylor College of Medicine, Houston, TX*

060 **A Strategy to Study the Mitochondrial Proteome from Genetically Defined Cell Classes in the Nervous System**; Gulcin Pekkurnaz; Thomas L. Schwarz; *Boston Children's Hospital, Harvard Medical School, Boston, MA.* ***Featured in Lighting Talks – Round I, Monday at 11:10am.***

Pathogen Proteomics, 061 - 064

061 **Identifying Host Factors Associated with Replicating Viral DNA**; Emigdio D. Reyes; Katarzyna Kulej; Daphne C. Avgousti; Lisa Akhtar; Daniel Bricker; Neha Pancholi; Sarah Koniski; Benjamin A. Garcia; Matthew D. Weitzman; *University of Pennsylvania, PA.* ***Featured in Lighting Talks – Round II, Tuesday at 11:10am.***

062 **Identification of Missing MHC Class I HIV Epitopes**; Marijana Rucevic[1]; Renata Blatnik2[2]; Georgio Kourjian1[1]; Matthew J. Berberich[1]; Angelika B. Riemer[2]; Sylvie LeGall[1]; *[1]Ragon Institute of MGH, MIT and Harvard, Cambridge, MA; [2]German Cancer Research Center, DKFZ, Heidelberg, Germany.* ***Featured in Lighting Talks – Round I, Monday at 11:10am.***

063 **Plasmodium Digestomics: Endogenously Generated Peptides within the Infected Erythrocyte Link Hemoglobin Catabolism to Drug Resistance in the Malaria Parasite;** David Perlman[1, 2]; Travis Bingeman[2]; David Fidock[3]; Manuel Llinas[4]; Ian Lewis[2]; *[1]Princeton University, Princeton, NJ; [2]University of Calgary, Calgary, Canada; [3]Columbia University, New York, NY; [4]Penn State, University Park, PA*

064 **Trypanosome Chronic Infection: Combined Post-translational Analysis Suggests Causes for Chronic Infection**; John E. Wiktorowicz[1, 2]; Susan Stafford[1]; Kizhake Soman[1, 4]; Xuemei Luo[1, 2]; Sue-Ji Koo[1, 3]; Nisha Garg[1, 3]; Alexander Kurosky[1, 2]; *[1]Univ of Texas Medical Branch, Galveston, TX; [2]Department of Biochemistry and Molecular Biology, Galveston, TX; [3]Department of Microbiology and Immunology, Galveston, TX; [4]Sealy Center for Molecular Medicine, Galveston, TX*

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Post-Translational Modifications, 065 - 083

065 **Antioxidant and Anti-Inflammatory Properties of Sugarcane Fibre**; Daniel Bucio Noble[1]; Liisa Kautto[1]; Malcolm Ball[2]; Mark Molloy[1]; *[1]Macquarie University, Sydney, Australia; [2]Gratuk Pty Ltd, Sydney, Australia*

066 **Detecting Cysteine Modifications in Methanogen Methanosarcina Mazei Gö1**; Phuong Nguyen[1, 2]; Hong Hanh Nguyen[1]; Robert Gunsalus[1]; Joseph A Loo[1]; Rachel Loo[1]; *[1]University of California, Los Angeles, California; [2]University of Science, Ho Chi Minh City, Vietnam.* ***Featured in Lighting Talks – Round I, Monday at 11:10am.***

067 **Phosphoproteomic Analysis of in vivo Cdc14 Phosphatase Substrate Specificity by SWATH-MS;** Brendan Powers; Mark Hall; *Purdue University, West Lafayette, IN.* ***Featured in Lighting Talks – Round I, Monday at 11:10am.***

068 **A Novel Tandem Isobaric Tag (QUANTITY) for Quantitative Analysis of N-glycans;** Shuang Yang[1]; Meiyao Wang[2]; Lijun Chen[1]; Bojiao Yin[1]; Guoqiang Song[3]; Illarion V. Turko[2]; Karen W. Phinney[4]; Michael J. Betenbaugh[1]; Shuwei Li[2]; Hui Zhang[1]; *[1]Hopkins, Baltimore, Maryland; [2]University of Maryland, College Park, MD; [3]Changzhou University, Jiangsu, China; [4]NIST MML, Rockville, MD.* ***Featured in Lighting Talks – Round I, Monday at 11:10am.***

069 **Identification of Mutant FMS-like Tyrosine Kinase 3 Substrates using KALIP;** Minervo Perez[1, 2]; *[1]Purdue University, West Lafayette, IN; [2]University of Minnesota, Minneapolis, MN*

070 **Human Myogenesis Regulated Kinase Signaling-Associated Chromatin Proteins and Histone Modifications**; Natarajan Bhanu[1]; Simone Sidoli[1]; Zuo-fei Yuan[1]; Rosalynn Molden[2]; Benjamin A Garcia[1]; *[1]University of Pennsylvania School of Medicine, Philadelphia, US-; [2]Regeneron Pharmaceuticals, Tarrytown, NY*

071 **Phosphoproteomics-based Network Analysis Unravels High-Fat Diet-Induced Deregulated Signalling Pathways in Mouse White Adipose Tissues;** Asfa Alli Shaik[1]; Beiying Qiu[1]; Sheena Wee[1]; Hyungwon Choi[2]; Vinay Tergaonkar[1]; Jayantha Gunaratne[1]; *[1]Institute of Molecular & Cell Biology, Singapore, Singapore; [2]Saw Swee Hock School of Public Health, NUS, Singapore, Singapore*

072 **Efficient Enrichment of SUMOylated Peptides from Alpha-lytic Protease Digest Using K-Ɛ-GG Remnant Immuno-affinity Purification;** Hongbo Gu[1]; Xiaoying Jia[1]; Jianmin Ren[1]; Elizabeth Komives[2]; Matthew Stokes[1]; *[1]Cell Signaling Technology, Danvers, Massachusetts; [2]Department of Chemistry & Biochemistry, UCSD, La Jolla, CA*

073 **Ubiquitinated Proteins in MDSC Exosomes**; Catherine Fenselau[1]; Katherine R. Adams[1]; Meghan C. Burke[1]; Suzanne Ostrand-Rosenberg[2]; *[1]University of Maryland, College Park, Maryland; [2]UMBC, Baltimore, Maryland*

074 **When Can Glycopeptides Be Assigned Based Solely on Tandem Mass Spectrometry Data?;** Kshitij Khatri; Joshua Klein; Joseph Zaia; *Boston University, Boston, MA.* ***Featured in Lighting Talks – Round II, Tuesday at 11:10am.***

075 **Quantitative Phosphoproteomics Identifies a Role for PP6c in the Regulation of Chromosome Condensation;** Scott Rusin[1]; Kate Schlosser[2]; Mark Adamo[2]; Arminja N. Kettenbach[1, 2]; *[1]Dartmouth College, Hanover , NH; [2]Norris Cotton Cancer Center, Lebanon, NH*

076 **Identification of CDK1 Substrates in Mitosis**; Adam Petrone[1]; Arminja N. Kettenbach[1, 2]; *[1]Dartmouth College, Hanover, NH; [2]Norris Cotton Cancer Center, Lebanon, NH*

077 **Quantitative Profiling Tau Modifications with DIA for the Differentiation of Tauopathies;** Melissa Rotunno[1, 2]; Waltraud Mair[1, 2]; Hanno Steen[1, 2]; Judith Steen[1, 2]; *[1]Harvard Medical School, Boston, MA; [2]Boston Children's Hospital, Boston, Massachusetts.*

078 **Analysis of Vascular Endothelial Growth Factor Receptor 2 (VEGFR-2) N-Glycosylation Sites using PNGase F/18O Labeling and Tandem Mass Spectrometry**; Kevin Chandler; Deborah Leon; Rosana Meyer; Nader Rahimi; Catherine Costello; *Boston University School of Medicine, Boston, MA.* ***Featured in Lighting Talks – Round II, Tuesday at 11:10am.***

079 **OQ-STrap Technology for Processing of Large Protein Loads**; John Wilson[1]; Darryl Pappin[1]; Rosamonde Banks[2]; Alexandre Zougman[2]; *[1]Protifi, LLC, Huntington, NEW YORK; [2]University of Leeds, Leeds, Enbgland*

080 **Phosphoproteomic Comparison of Osteoblasts Stimulated with Forteo or Biased PTH1R Ligand as Determined via SILAC**; Grace Williams; *MUSC, Charleston, SC.* ***Featured in Lighting Talks – Round II, Tuesday at 11:10am.***

081 **Understanding DYRK1A Function through Phosphoproteomic Substrate Identification**; Zachary Poss; Christopher Ebmeier; William Old; *University of Colorado, Boulder, Colorado*

082 **Strategies for Global Phosphohistidinomics and the Analysis of other Labile Phosphospecies;** Rob Oslund; Jung-Min Kee; Tom Muir; David Perlman; *Princeton University, Princeton, NJ*

083 **Multiplexed Quantitative Analysis of Mammalian Cell and Tissue Ubiquitylomes using Isobaric Labels**; Christopher M. Rose[1]; Marta Isasa[1]; Miguel A. Prado[1]; Sean Beausoleil[2]; Mark P. Jedrychowski[1]; Daniel J. Finley[1]; Steven Gygi[1]; *[1]Harvard Medical School, Boston, MA; [2]Cell Signaling Technologies, Danvers, MA*

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Protein Quality Control, 084

084 **Quantitative Peptide Assay for Optimized and Reproducible Sample Preparations;** Xiaoyue Jiang[1]; Ryan Bomgarden[2]; Ramesh Ganapathy[2]; Sijian Hou[2]; Sergei Snovida[2]; Paul Haney[2]; John Rogers[2]; Julian Saba[3]; Rosa I Viner[1]; Andreas Huhmer[1]; *[1]Thermo Fisher Scientific, San Jose, CA; [2]Thermo Fisher Scientific, Rockford, Illinois; [3]Thermo Fisher Scientific, Mississauga, ON, Canada*

Proteogenomics, 085 - 087

085 **A Comprehensive Temporal Analysis of Differentiating Pancreatic β-Islet Cells from Human Embryonic Stem Cells Provides insights into Maturation;** A. Ertugrul Cansizoglu[1]; Quinn Peterson[3]; Shaojun Tang[1]; Douglas Melton[3]; Judith Steen[2]; *[1]Harvard Medical School / BCH, Boston, MA; [2]Boston Children's Hospital, Boston, MA; [3]Harvard Dep. of Stem Cell and Regenerative Biology, Cambridge, MA.* ***Featured in Lighting Talks – Round I, Monday at 11:10am.***

086 **A Proteogenomic Approach to Identify Surface Proteins in Acute Myeloid Leukemia**; Hossein Fazelinia[1]; Kian Huat Lim[1]; Tina Glisovic-Aplenc[1]; Lynn A. Spruce[1]; Ian R. Smith[1]; Sarah K. Tasian[1]; Saar Gill[2]; Richard Aplenc[1]; Steven H. Seeholzer[1]; *[1]The Children's Hospital of Philadelphia, Philadelphia, PA; [2]University of Pennsylvania School of Medicine, Philadelphia, PA.* ***Featured in Lighting Talks – Round I, Monday at 11:10am.***

087 **Monitoring Signal-Specific Changes in Ribosome Maintenance and Biosynthesis *in vivo***; Andrew Mathis; Bradley Naylor; John Price; *Brigham Young University, Provo, Utah.* ***Featured in Lighting Talks – Round II, Tuesday at 11:10am.***

Proteomics in Drug Development, 088 - 090

088 **The Signature Exosome Map for Bone Marrow Stromal Cells: The Development of an Optimal Sample Preparation Approach**; Qin (Stefanie) Liang; Michail A. Alterman; *FDA, Silver Spring, MD.* ***Featured in Lighting Talks – Round I, Monday at 11:10am.***

089 **Quantitative Proteomics & Network Analysis Identifies Retinoic Acid Therapy to Bypass Impaired Vitamin A Metabolism in Heart Failure;** Ni Yang[1]; TIng Liu[1]; Brian O'Rourke[1]; Maureen Kane[2]; D. Brian Foster[1]; *[1]Johns Hopkins School of Medicine, Baltimore, Maryland; [2]University of Maryland Medical Center, Baltimore, MD.* ***Featured in Lighting Talks – Round II, Tuesday at 11:10am.***

090 **RgpB - Arginine Specific Proteas with Applications in Proteomics**; Malin Mejare; Magdalena Widgren Sandberg; Stephan Bjork; Maria Nordgren; Frederick Olsson; *Genovis AB, Lund, Sweden*

Proteomics in Microbiology, 091 - 096

091 **A Biomimetic, Synthetic RNA platform for *in vivo*, Co-Translational Labeling of Proteins;** Randi Turner; Daniel Dwyer; *University of Maryland, College Park, MD.* ***Featured in Lighting Talks – Round II, Tuesday at 11:10am.***

092 **Rapid Changes in the Proteome of Gut Microbiota in Response to Short-Term Dietary Changes in Baboons**; Prahlad Rao; Kimberly Spradling-Reeves; Vicki Mattern; Laura Cox; Anthony Comuzzie; Michael Olivier; *Texas Biomed Res Inst., San Antonio, TX.* ***Featured in Lighting Talks – Round II, Tuesday at 11:10am.***

093 **Proteome and Secretome Profiling Reveals Temporal Differences in the Toll-like Receptor Responses to Different Ligands**; Marijke Koppenol-Raab; Virginie Sjoelund; Bhaskar Dutta; Zachary Benet; Iain Fraser; Aleksandra Nita-Lazar; *NIH/NIAID, Bethesda, MD.* ***Featured in Lighting Talks – Round I, Monday at 11:10am.***

094 **The Characterization of IFIX as an Anti-Viral Factor during Infection with DNA Viruses;** Marni S. Crow; Michelle-Ann R. Tan; Tuo Li; Benjamin A. Diner; Ileana M. Cristea; *Princeton Univeristy, Princeton, NJ.* ***Featured in Lighting Talks – Round I, Monday at 11:10am.***

095 **Identifying Organisms by MALDI Starting from Genomic Databases**; Kenneth Parker; *SimulTOF Systems, Marlborough, MA*

096 **Understanding the Network Signaling Capacity of HBx in HBV Host Infection**; Emanuela Milani; Charlotte Nicod; Bernd Wollscheid; *ETH Zurich, Zurich, Switzerland.* ***Featured in Lighting Talks – Round II, Tuesday at 11:10am.***

Proteomics of Aging, 097 - 100

097 **Proteomic Level Identification of Degradation Resistant Proteins, Complexes & Aggregates in Human Plasma**; Hannah Trasatti; Ke Xia; Wilfredo Colon; *RPI, Troy, NY.* ***Featured in Lighting Talks – Round I, Monday at 11:10am.***

098 **Analysis of the Effects of Dietary Signals on Protein Homeostasis;** Bradley Naylor; Richard Carson; Monique Speirs; John Price; *Brigham Young University, Provo, 0*

099 **High Purity Myonuclear Isolation from Skeletal Muscle**; Alicia Cutler; Grace Pavlath; *Emory University, Atlanta, Georgia*

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100 **Investigation into the Mechanism of AGE-Mediated Cancellation of Calorie Restriction Benefits;** Richard Carson; Bradley Naylor; John Price; *Brigham Young University, Provo, UT*

Structural Proteomics, 101

101 **Confident Identification of Chemical Crosslinks in Nonspecifically-Digested LC-MS/MS Samples by Locus-Centric Aggregate Scoring;** Mark Adamo[1]; Scott Gerber[2]; Andrew Grassetti[3]; *[1]Norris Cotton Cancer Center, Lebanon, New Hampshire; [2]Geisel School of Medicine at Dartmouth, Lebanon, New Hampshire; [3]Dartmouth College, Lebanon, NH*

Technology Development, 102 - 114

102 **Proteomic Visualization of Cellular Entry and Trafficking**; Linna Wang; Li Yang; Li Pan; W. Andy Tao; *Purdue University, West Lafayette, Indiana*

103 **SOMAmer® Reagents and the SOMAscan® Assay: Tools for Targeted Proteomic Measurements**; Sheri Wilcox; Stephan Kraemer; Dominic Zichi; Nebojsa Janjic; *SomaLogic, Inc., Boulder, Colorado*

104 **Offline Pentafluorophenyl (PFP)-RP Pre-fractionation for Comprehensive LC-MS/MS Proteomics and Phosphoproteomics;** Andrew Grassetti[1]; Rufus Hards[1]; Scott Gerber[2]; *[1]Dartmouth College, Lebanon, NH; [2]Geisel School of Medicine at Dartmouth, Lebanon, New Hampshire*

105 **Demystifying Proteolytic Digestion under High Pressure Cycling: Enzyme Specificity, Efficiency and Synergy of Pressure with Acid-Labile Detergents;** Vera Gross[1]; John Wilson[2]; Alexander Lazarev[1]; Darryl Pappin[2]; *[1]Pressure BioSciences, Inc, Medford, MA; [2]Protifi, LLC, Huntington, NY*

106 **Improving the HPLC Workflow with Vacuum Driven Samplicity Gen 2 Filtration System;** Jun Young Park; Vivek Josh; Chris Scott; *EMD Millipore, Danvers, Massachusetts*

107 **Novel Means for Coupleing Protein Separations with MALDI-TOF Mass Spectrometry for Top-Down Proteomics**; Kenneth Parker; Marvin Vestal; Stephen Hattan; *SimulTOF Systems, Marlborough, MA*

108 **Biosensor Development for Time-Resolved FRET Kinase Assay and Fluorescence Lifetime Imaging;** Wei Cui; Laurie L. Parker; *University of Minnesota Twin Cities, Minneapolis, Minnesota*

109 **Industrializing SWATH Proteomics with Microflow LC;** Christie Hunter[1]; Ken Hamill[2]; *[1]SCIEX, Redwood City, CA; [2]SCIEX, Framingham, MA*

110 **Rapid Protein Extraction and Digestion for Mass Spectrometric Analysis**; Brendan Redler; Rohan Varma; Natalie Hong; Jonathan Minden; *Carnegie Mellon University, Pittsburgh, PA*

111 **Rigorous MRM Quantitation of a Multiplexed Panel of Salivary Proteins for Biomarker Assessment Studies;** Andrew Percy[1]; Darryl Hardie[1]; Juncong Yang[1]; Armando Jardim[2]; Yassene Mohammed[3]; Christoph H. Borchers[1]; *[1]University of Victoria/Genome BC Proteomics Centre, Victoria, BC; [2]McGill University, Montreal, QB; [3]Leiden University Medical Center, Leiden, Netherlands.* ***Featured in Lighting Talks – Round II, Tuesday at 11:10am.***

112 **Protein Profiling Comparison of Modified DNA Aptamer Screening to Data Dependent Mass Spectrometry across Cancer Cell Lines;** Nancy Finkel[1]; Felipa Mapa[1]; Lori Jennings[1]; Jaison Jacob[1]; Joseph Loureiro[1]; Sahar Abubucker[1]; Stephan Kraemer[2]; Sherri Wilcox[2]; *[1]Novartis, Cambridge, MA; [2]SOMALogic, Boulder, CO*

113 **Two Product Extensions combine Albumin and Immunoglobulin Depletion in Consumable Formats – Called AlbuSorb™ PLUS and AlbuVoid™ PLUS**; Swapan Roy; Amenah Soherwardy; Ravish Amin; Matthew Kuruc; *Biotech Support Group LLC, Monmouth Junction, NJ*

114 **The Nuclear Proteome of a Vertebrate**; Martin Wühr[1]; Thomas Güttler[2]; Leonid Peshkin[2]; Graeme C. McAlister[2]; Matthew Sonnett[2]; Keisuke Ishihara[2]; Aaron C. Groen[2]; Marc Presler[2]; Brian K. Erickson[2]; Timothy J. Mitchison[2]; Marc Kirschner[2]; Steven P. Gygi[2]; *[1]Princeton University, Princeton, NJ; [2]Harvard Medical School, Boston, MA*

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Top-Down Proteomics, 115 - 119

115 **Quantitative Proteomic Profiling of PANDER Transgenic Mice Reveals Increased Lipogensis and Fatty Acid Synthesis Modulated by the Liver X Receptor**; Mark Athanason; Stanley Stevens; Brant Burkhardt; *University of South Florida, Tampa, FL.* ***Featured in Lighting Talks – Round II, Tuesday at 11:10am.***

116 **Characterization of Ubiquitin Trimers by Top-down Mass Spectrometry**; Catherine Fenselau; Amanda E. Lee; Lucia Geis-Asteggiante; Emma K. Dixon; Yeji Kim; Tanuja R. Kashyap; Yan Wang; David Fushman; *University of Maryland, College Park, MD.* ***Featured in Lighting Talks – Round II, Tuesday at 11:10am.***

117 **Use of a Digest-Free Profiling Approach for Neurological Disorder Biomarker Discovery Operations;** Jerome Vialaret[1]; Sylvain Lehrmann[1]; Audrey Gabelle[1, 2]; Pierre-Olivier Schmit[3]; Christophe Hirtz[1]; *[1]Laboratoire de Biochimie et Protéomique Clinique, Montpellier, France; [2]Centre Mémoire Ressources Recherche, Montpellier, France; [3]Bruker Daltonique, Wissembourg, France*

118 **IEF-SPLC-MS for Generalized High Resolution Intact Glyco-Proteoform Analysis and Top-Down Proteomics**; Steven Patrie; *UT Southwestern Medical Center, Dallas, TX.* ***Featured in Lighting Talks – Round I, Monday at 11:10am.***

119 **Investigation of N-terminal Sequence Heterogeneity and Comprehensive Glycosylation Modification from a Therapeutic Recombinant Enzyme**; Bao Quoc Tran; David R. Goodlett; Young Ah Goo; *University of Maryland, Baltimore, MD*

POSTER ABSTRACTS

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Poster 001: Proteomic and Metabolomic Comparative Analyses of Plasma and Vasculature Tissue from TiO2 Nanoparticle Exposed Rats

Megan M. Maurer[1]; Jinghai Yi[2]; Carroll McBride[2]; Timothy R. Nurkiewicz[2]; Stephen J. Valentine[1]

[1]Department of Chemistry, West Virginia University, Morgantown, WV; [2]Dept of Physiology & Pharmacology,West Virginia U., Morgantown, WV

Pulmonary TiO2 nanoparticle exposure has previously been shown to elicit immune and inflammatory responses and to increase biomarkers associated with oxidative and nitrosative stresses in the microcirculation. Molecular mechanisms relating pulmonary inflammation to microvasculature dysfunction have yet to have been fully elucidated. Our previous plasma proteomic analysis of rats exposed to TiO2 nanoparticles via inhalation implicated 13 canonical biological pathways as being significant ($p \leq 0.5$); however, none were found to be significantly up or down regulated ($z \geq |2|$) [Journal of proteomics 130 (2016): 85-93]. Continuing with this work, we have performed comparative proteomic and metabolomic analyses on plasma, aorta, and small vasculature in order to gain further insight as to the pathway activation mechanisms as well as to determine additional affected pathways.

Poster 002: Optimized Sample Preparation of Human Tissue Proteome for LCMS-based Biomarker Discovery

Anna Chen[1]; Ruiqi Jian[2]; Lihua Jiang[2]; Michael Snyder[2]

[1]Mission San Jose High School, Fremont, CA; [2]Department of Genetics, Stanford University, Stanford, CA

The identification of biomarkers is important for the diagnosis, prognosis and treatment of disease. Although the initial focus of biomarker discovery has been on serum and plasma samples, analysis of tissues would provide greater chance of discovering novel markers because the concentration of disease markers is likely the highest in tissues near the disease source. However, proteomic analysis of tissues faces challenges in sample preparation due to the difficulty in obtaining sufficient quantities of tissues and the poor solubility of membrane proteins.

We developed a tissue protein extraction and digestion procedure for subsequent liquid chromatography mass spectrometry (LCMS) analysis. Using 11 different types of PAXgene fixed human tissue samples, the procedure was optimized for the maximized protein or peptide recovery, as well as minimized contaminants that could potentially interfere with the LCMS analysis.

Three lysis buffers were compared for cell lysis and protein extraction from tissues. Each lysis buffer contains reagents for reduction and alkylation to prepare proteins for enzymatic digestion and LCMS analysis. However, it was found that several contaminant peaks suppressed the detection of biological signals. The incorporation of a protein precipitation step before enzymatic digestion reduced the contaminant signal at least 300-fold, allowing successful LCMS identification of co-eluted peptides. The two steps in the procedure, protein precipitation and peptide desalting, were optimized for recovery. Three protein precipitation protocols and 6 peptide desalting products were tested. The effect of initial protein concentration was also evaluated. An 80% protein recovery rate was consistently obtained from acetone precipitation and an 81-88% peptide recovery was achieved for most of the tissues types. The total recovery rate of protein/peptides from the tissue lysates is estimated to be 65-70% for most of the tissues studied. This study is one of the most systematic optimizations of protein extraction from human tissues for LCMS-based analysis.

Poster 003: Analysis of Selected Carboxylic and Amino Acids in Clinical Samples of Patients at Risk for Cardiovascular Disease

Hassan Alamri[1,4], Bruce Levison, Ph.D.[1,2], Tomas Cajka, Ph.D.[5], Oliver Fiehn, Ph.D.[5], Stanley L. Hazen, M.D., Ph.D.[2,3,4], Valentin Gogonea, Ph.D.[1,4]*

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Cardiovascular disease (CVD) is the leading cause of death worldwide. A novel biomarker for early identification of individuals at risk for future cardiovascular events is still critical to provide new ways to diagnose disease states and reveal new metabolic pathways connected to CVD pathogenesis. To identify novel biomarker, we performed non-targeted Gas Chromatography/Mass spectrometry-based (GC-MS) analysis on 1000 subjects at risk for CVD in a semi-quantitative fashion to screen for plasma small-molecule metabolites that predict risk for CVD. Among these metabolites, several mono-, di-, and tri-carboxylic acids presented a significant prediction for CVD risk. We are further developing a targeted Stable-isotope dilution high performance liquid chromatography-electrospray ionization-tandem mass spectrometry method (LC-ESI-MS/MS) for the quantitation of carboxylic acids and amino acids in order to demonstrate their clinical utility by analyzing them in a cohort study of 2000 subjects.

Poster 004: Heat Stabilization Preserves the Molecular Integrity of the Sample

Charlotta Göransson; Camilla Sivertsson; Marcus Söderquist; Karl Sköld; Mats Borén

Denator, Uppsala, Sweden

The enzymes in a sample remain active long after sampling and rapidly change the composition of biomolecules. Subsequent analytical results reflect a mix of the in vivo status and degradation products with increased inter-sample variation. Proteins, peptides and post translational modifications (PTM), of existing or potential biomarkers are affected. Effective enzyme inactivation and standardization of sample handling eliminate this problem.

A heat-stabilization system has been used to generate rapid, homogenous thermal denaturation of proteins, including enzymes, to stop enzymatic degradation in the sample. Comparisons were made to snap-freezing and inhibitors, and in time study manner, compared with different post-mortem intervals. Using mass spectrometry, Western blot, RPPA and activity assays, the protein, peptide, and PTMs, were examined.

The results show rapid changes in phospho-states on a variety of different proteins detected only minutes after excision whereas after heat-stabilization the phospho-levels remain unchanged during 2 hours in room temperature. In three minutes post-mortem both proteins and endogenous peptides/neuropeptides, including PTM's, are subjected to substantial degradation. Conversely, amounts and identities of the detected proteins/peptides in heat-stabilized samples show maintained integrity. Similarly, levels of pCREB, pGSK3β and pERK1/2 were unchanged for 2 hours, whereas snap-frozen samples showed a dramatic decrease in levels after 10 min in room temperature.

Post-mortem changes may distort our view of in vivo sample molecule composition. Adequate suppression of enzymatic activity is important for accurate proteome analyses. Heat-stabilization stops activity thereby enables sample analysis to reflect the in vivo status as closely as possible. This approach may be of great help in clinical research to differentiate true biomarkers from those potential degradation products found in any situation where cells are under stress.

POSTER ABSTRACTS

All posters will be attended by presenting authors on both Monday and Tuesday. All posters should be set up by 10:00 am on Monday morning and removed at 3:00 pm on Tuesday. **Odd**-numbered posters present **1:30 - 2:15 pm** and **even**-numbered posters **2:15 - 3:00 pm**.

Poster 005: Quantitative Proteomic Approaches for Identifying Urinary Biomarkers in Lupus Nephritis

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Lupus nephritis (LN) is a severe form of systemic lupus erythematosus (SLE) often associated with significant morbidity and mortality. Diagnosis and assessment of renal involvement in SLE requires a kidney biopsy; an invasive procedure with limited prognostic value. Despite years of research, there remains a need for non-invasive biomarkers to help inform treatment decisions and to monitor disease progression in clinical trials. A hallmark of LN is urinary proteinuria, however the composition of this proteome remains poorly characterized. Here we took a comprehensive approach to characterize the LN urinary proteome that included three complimentary discovery proteomic methods to identify urinary biomarkers. Using gel-fractionation, a chemical labeling approach, and a data-independent acquisition (DIA) method, >2500 proteins were identified, 220 of which are up-regulated >2-fold in LN samples compared to healthy controls. While the chemical labeling approach enabled identification of far more total proteins (2,350 with chemical labeling vs. 857 with DIA), the DIA approach outperformed the chemical labeling approach in identification of proteins up-regulated in LN samples (52 with chemical labeling vs. 199 with DIA). Furthermore, candidate biomarkers identified using the DIA method are easily adapted into a targeted, multiplexed mass spectrometry assay suitable for absolute quantitation of candidate biomarkers in a clinical trial. These results suggest that DIA-based approaches are less biased towards high abundance analytes and therefore potentially more suitable for proteomic profiling of biological matrices with a broad dynamic range like urine. Results from this study as well as insights from longitudinal and interventional studies focused on understanding the biological and clinical implications of these candidate biomarkers will be used to inform development of novel tools to evaluate disease progression and treatment efficacy of current and future LN therapeutics.

Poster 006: Proteomic Analysis of Platelets of First Onset Psychosis Subjects: Oxidative Stress Pathway in the Search for Biomarkers

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Despite popular assumptions, to date the molecular pathogenesis of psychosis is not fully understood. The first symptoms are frequently common to some neuropsychiatry disorders, making challenging the differentiation and diagnosis through clinical methods. Early symptoms recognition is important in the management of psychosis and the identification of molecular biomarkers is crucial to the diagnostic and therapeutic approach. The aim of this study was to determine the platelet proteome in first onset psychosis non-affective patients (n=11) and compared to first onset psychosis affective patients (psychiatry controls; n=8) and healthy controls (n=16). The proteins of each sample were separated by two-dimensional electrophoresis (2-DE). There were 34 spots overexpressed in healthy control samples. Those spots were analysed by nanoLC-LTQ-Orbitrap Velos method. 143 proteins were identified with at least 3 unique peptides some of them linked to oxidative stress and not associated to psychiatry disorders before. Oxidative stress from the mitochondria leads to DNA damage, altering gene expression and protein synthesis inducing apoptosis and cell death. This process can impair synaptic plasticity and thus neurotransmission. Such damage can begin at the stage of neurodevelopment, years before the first clinical signs. We identified heat shock proteins (HSP90AA1, HSP90AB1, HSP90B1, HSPA5, HSPA9, HSPD1, HSPA8) important to protein folding control; the gamma glutamyl transferase cycle (GGCT) which controls homeostasis of glutathione and the induction of apoptosis; the glutationa s-transferases associated with oxidation of cell (GSTO1, GSTP1) and PRDX6 responsible for reduction of peroxiredoxin hydrogen peroxide. These proteins must be validated in samples, but seem to be potential biomarkers for first onset psychosis.

Poster 007: Mass-spectrometry Discovery, Verification and Validation of Circulating Protein Biomarkers for the Early Detection of Breast Cancer

Khadijeh Rajabi[1]; Matthew Rosenow[1]; Luisa Paris[2]; Victoria David[1]; Kristine Tsantilas[1]; Paul Russo[2]; Tony Tegeler[1]; Kristina Chapple[1]; Lance Liotta[2]; Emanuel Petricoin[2]; Patrick Pirrotte[1]
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Early detection driving timely therapeutic intervention is the most determinant factor for surviving breast cancer. However, mammograms are particularly indiscriminate in screening early stages of the disease and often lead to false positives. In the case of breast cancer, 30% of patients with a suspicious mammogram are biopsied yielding less than 5% real cases. These high false positive rates may result in excessive treatments, such as repeated excisional biopsies or surgical resections, including partial or even complete mastectomies resulting in long-term emotional stress (e.g. anxiety, stress, feeling of loss) in patients. In addition, these time-intensive procedures are reflected as financial burdens to healthcare networks to the detriment of public health.

The following collaborative study aimed at identifying putative companion protein biomarkers by liquid chromatography mass-spectrometry (LC-MS) to supplement mammograms and reduce false-positive screening rates. Sera of 20 cases (biopsy confirmed suspicion) and 20 controls (rejected suspicion) were enriched for low-abundance low-molecular weight proteins using N-isopropylacrylamide (NIPAm) core shell hydrogel particles functionalized with Cibacron blue F3GA and vinylsulfonic acid and processed for unbiased mass-spectrometry analysis. In this discovery phase, 47 differentially expressed proteins were identified. These putative markers were validated using a highly sensitive and quantitative Multiple Reaction Monitoring assay (LC-MRM) in two independent cohorts of 60/49 and 30/40 of case/control. To ensure consistent generation of high quality data, heavy-labeled peptide standards were spiked at known concentrations across all sera. Out of 47 candidate biomarkers, several were significantly differentially expressed. The validated biomarkers were further divided into two subcategories: breast cancer specific biomarkers and those that are not breast cancer specific. The identified biomarkers will be further evaluated for clinical qualification as companion screening markers to current diagnostic imaging modalities for early detection of breast cancer (2D and 3D mammography).

Poster 008: Glycan "Nodes" as Cancer Markers: Clinical Performance in Early Stage Lung Cancer

Shadi Roshdiferdosi; Chad R. Borges
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Lung cancer is the most deadly cancer for both men and women. Due to a lack of early stage symptoms, approximately 33% of individuals diagnosed with lung cancer are already at stage IIIB and another ~40% are at stage IV, which causes a 5-year survival rate of 4%. However, scholarly estimates suggest that approximately 70% of lung cancer deaths could be avoided if diagnosed at an early stage.

Glycans represent a highly promising but marginally accessed source of clinically useful lung cancer markers. Any single glycan structure is only one of numerous intact glycan structures that arise due to do glycosyltransferase (GT) dysregulation in cancer—resulting in unavoidable signal dilution from this upstream cause of abnormal glycosylation. We have developed a glycan analysis technique in which we have adapted a global glycan methylation analysis procedure to whole blood plasma/serum. The technology allows us to quantify in a single assay over two dozen monosaccharide-and-linkage-specific, pre-defined types of glycan polymer chain links—"glycan nodes"—many of which serve as direct 1:1 molecular surrogates for GT activity. As part of the process, interesting glycan features such as "6-sialylation", "core fucosylation", "bisecting GlcNAc", and "beta-1,6-branching" are condensed from across dozens of unique intact glycan structures into single analytical signals via GC/MS—an analytical modality that is already well established in clinical medicine.

Our initial studies have proven the utility of this approach toward the detection of lung cancer, including stage IA lung adenocarcinoma relative to high risk age/gender/smoking status-matched controls with a clinical sensitivity of 42% at a specificity of 97%. Results will be

presented from an expanded case-control study of 170 Stage I lung adenocarcinoma patients and 170 age/gender/smoking-status matched controls to validate glycan node analysis as a tool for early detection of lung adenocarcinoma.

Poster 009: Application of Microfluidic/Tandem Quadrupole LC-MS/MS for MRM Based Translational Research Analysis of Putative Heart Failure Peptide Biomarkers inHuman Plasma

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The application of tandem quadrupole MS with microfluidic chromatography for the analysis of proteolytic peptides in human plasma is compared and contrasted with nanoscale LC and high-resolution oa-ToF MS configurations. A tandem quadrupole platform was ultimately considered for its performance in terms of sensitivity, selectivity, precision, and linearity. Microfluidic chromatography was selected as it afforded the optimum balance of sensitivity and throughput, whilst minimizing isobaric coelution, thereby providing an ideal LC-MS configuration for the application to large sample cohorts in translational studies. This LC-MS configuration was also utilized to demonstrate that proteolytically digested, non-depleted plasma samples from heart failure patients could be classified with good discriminative power using a subset of proteins previously suggested as candidate biomarkers for cardiovascular diseases. Heart failure (HF) represents a clinical population for which validated biomarkers are sparse. The etiology of the disease also means that the phenotypic changes are likely to be multifactorial. As a research proof of principle and to test the potential sensitivity to classify this disease, twenty healthy controls, twenty HF patients with preserved ejection fraction (HFPEF) and twenty HF patients with reduced ejection fraction (HFREF) were analyzed. Multivariate analysis showed that samples could be classified using OPLS-DA. Near complete separation of healthy controls and HF (combined HFPEF and HFREF) patients can be observed. The discriminating proteins primarily contributing to the separation were ApoA1, CRP and plasma protease C1 inhibitor. Univariate analysis of these three proteins showed significant changes in levels between the groups. Good discriminating power was obtained by combining these protein surrogate peptides, with an AUC of 0.937 obtained for ROC curve analysis ($p < 0.001$). Separation between all three groups, control, HFPEF and HFREF, was not obtained with the selected subset of peptides, however, a partial separation model was developed for HFREF/HFPEF.

Poster 010: Proteomic Characterisation of Renal Multilammelar Bodies Induced by Kidney Metabolic Injury

Peter Ochodnicky[1]; Lee Gethings[2]; Roy Martin[3]; Johannes Vissers[2]; Joannes MFG Aerts[4]; Jaklien Leemans[1]
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Ectopic fat accumulation in organs other than adipose tissue, including the kidney, has been proposed to reflect the severity of obesity-induced end-organ damage. We have recently found extensive accumulation of free cholesterol and polar complex lipids in large multilamellar bodies (MLBs) within proximal tubular epithelium in mice fed with Western-type of diet, an animal model of obesity-induced kidney injury. Here we aim to characterize the protein composition of the multilamellar structures in order to investigate novel potential biomarkers of metabolic renal injury. MLBs were isolated by discontinuous sucrose gradient centrifugation from kidney tissue homogenates and urine of mice fed with Western-type or control of diet for 16 weeks. Composition of the MLB fraction was confirmed by transmission electron microscopy. The proteome complement of MLB fractions was characterized using ion mobility separation enabled, data independent label-free LC-MS. Relative within sample abundances were expressed by normalization to the total estimated amount affording comparative proteomes analysis of tissue and body-fluid samples. Proteins of mitochondrial and (endo) lysosomal origin were strongly overrepresented among the most abundant proteins of MLB fractions. The proteins largely overlapped between tissue and urinary fractions, suggesting MLBs are excreted in urine. Lysosomal membrane proteins, including LIMP-2, LAMP-1, LAMP-2, CD63 and subunits of lysosomal proton pump were detected in both tissue and urinary MLB-fractions from Western-diet fed animals, but largely absent in corresponding fractions from control animals. Overexpression of several lysosomal markers, LIMP-2, LAMP-1, LAMP-2 and CD63 in kidneys and their localization in the MLBs were confirmed by immunohistochemistry. MLBs formed in the kidney upon metabolic/obesity-induced renal injury are most likely of lysosomal origin. MLB-derived lysosomal membrane markers, such as LIMP-2 might potentially serve as tissue or urinary markers of obesity-associated renal injury.

Poster 011: Multiplexed Kinase Biosensor Technology to Detect Leukemia Signaling with Mass Spectrometry

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The development of tyrosine kinase inhibitors (TKIs) and the advances in precision medicine have revolutionized strategies for treating cancer patients. TKIs have benefited numerous cancer patients; however, problems including drug resistance and residual disease have emerged over time. Preliminary evidence suggests that individual variability in response to drugs is due to distinct pharmacodynamics among patients. The goal of this project is to develop a technique for measuring patients' response to TKI treatments by investigating kinase signaling profiles in their tumor cells. In treating chronic myelogenous leukemia (CML), it has been shown that that decreased Bcr-Abl substrate phosphorylation in mononuclear cells may be an indicator of initial TKI response and could even predict longer-term prognostics. Such 'real-time' monitoring of drug response during treatment is not widely performed, because technical hurdles present a challenge to efficient analysis of patient material.

This project attempts to use multiple reaction monitoring (MRM) mass spectrometry to measure intracellular kinase activity through a peptide 'biosensor' developed in our group. Our goal has been to develop a sensitive, multiplexed kinase assay for leukemia-related signaling in patient material. The biosensors are comprised of peptide sequences that serve as surrogate kinase substrates and a cell penetrating sequence that drives the biosensors into live cells. This technique is antibody independent, and can be used to detect real time kinase activity. Because patient samples are often precious and only available in low quantities, targeted mass spectrometry is exploited for its high sensitivity and specificity in peptide detection, as well as its excellent multiplexability and quantitative capabilities.

To date we have designed and begun biological characterization of peptide biosensors for several kinases important in leukemia signaling processes. We anticipate that measurement of several kinase activities using the additional biosensors will reveal a more comprehensive picture of cellular processes in responding TKI treatment.

Poster 012: MRM Assays and Tools for Quantifying Multiplexed Panels of Proteins in 4 Mouse Tissues

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The laboratory mouse is the most commonly used mammalian organism in biological research due to a variety of factors (e.g., availability, size, and low cost). Because its genome and proteome are also well annotated and share considerable sequence homology with humans, mice are frequently used as surrogate disease models in biomedical research. Although the number and diversity of mouse models is increasing rapidly through the advancement of new genetic engineering strategies, detailed characterization of these new models is still challenging because most phenotypic information is derived from time-consuming histological and biochemical analyses. To expedite molecular phenotyping, we aimed to develop an inventory of reliable MRM-based assays with our well-characterized internal standards (i.e.,

isotopically labeled peptides) for quantifying candidate protein biomarkers in four different mouse tissues – plasma, heart, lung, and brain. Development involved target selection, standard peptide production, panel optimization, assay evaluation (by 1D and 2D LC-MRM/MS), quantitative determination (via linear regression and standard addition), and panel verification/validation. To date, we have developed a series of MRM assays for quantifying >610 disease-related proteins (from >920 interference-free peptides) in various mouse tissues (plasma, heart, lung, and brain), with concentrations that span approximately 5 orders of magnitude. An additional aim of this research was to establish kits which could be widely used by biologists and biochemists for the molecular phenotyping of new mouse models of disease. The developed BAK-81, for instance, contains the necessary materials and tools for the rapid phenotyping of 81 proteins in undepleted and non-enriched mouse plasma. Effort is underway to expand the kit plasma panel and extend the kits to include other mouse tissues. To be presented here is an overview of the assay developments, the assay metrics, and the quantitation kits for quality control and biomarker assessment of mouse proteins in these tissues.

Poster 013: Multi-omics Analysis of Cytotrophoblasts from Second Trimester and Full-Term Primary Cultures

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During human pregnancy, a subset of placental cytotrophoblasts (CTBs) differentiates into cells that aggressively invade the uterus and its vasculature, anchoring the progeny and rerouting maternal blood to the placenta. Defects in this process are the hallmark of the pregnancy complication preeclampsia. While disease-associated genes or transcripts may serve as useful biomarkers, they are not necessarily predictive of disease mechanisms. Thus, we performed global proteomic and transcriptional profiling to measure expression patterns of CTBs from second trimester and term normal placentas to gain further understanding of CTB differentiation in healthy pregnancy.

Primary CTBs were isolated using collagenase and trypsin digestion and Percoll gradient centrifugation. For proteomics analysis, cells were lysed and digested with trypsin. Variable window SWATH MS data were acquired over a 180 min. gradient using a nanoLC 425 cHiPLC-TripleTOF 6600 System interfaced (Sciex). SWATH data were processed using OneOmics applications in BaseSpace (Illumina). For transcriptomics, a second set of CTBs were analyzed using RNAseq (Kundaje, Nature 2015). iPathwayGuide (Advaita) was used to compare protein and RNA levels for pathway and gene ontology analyses.

Approximately 3000 proteins were quantified and ~400 showed differential expression in second trimester CTBs vs. term. Proteins known to function in CTB differentiation processes, e.g. angiogenesis and hypoxia response, as well as previously uncharacterized processes, e.g., NF-kappaB signaling, metal ion transport and muscle contraction, were altered. Integration with RNAseq data showed variations at the molecular level but concordance among pathways and processes. Multi-omics data sets comprised of SWATH MS protein quantification and RNAseq expression results showed gestation age differences in healthy CTB populations corresponding to known and novel processes. Using these methods to study CTBs from patients with preeclampsia is likely to identify aberrations that could contribute to disease and/or serve as diagnostic markers.

Poster 014: Association of CDK10 with ETS2 during Human Corneal Wound Healing

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Introduction and Objectives: Corneal related complications are major health concerns worldwide because its progression is associated with significant impaired vision. Therefore, there is an urgent need to develop reliable understanding of the underlying mechanism of corneal epithelial wound healing to apply therapeutic options. We aimed to investigate the alterations in protein expression during corneal epithelial migration. To demonstrate the networks of the total identified proteins with potential dual functions. Methods: In this study, human corneal epithelial cells lines (HCEC) have been used for wound healing model. Mechanical wound was made in HCEC lines and healing was monitored at 24, 48 and 72 hours of post wounding. Epithelium was scrapped at 24, 48 and 72 hours, followed by protein quantification using BCA kit. The wounded and unwounded cells were subjected to SDS-PAGE and two dimensional electrophoresis

(2DE). Mass Spectrometry (MALDI TOF) was done to identify the proteins through protein database searches. The identified protein were further analyzed and validated by western blot analysis. A further insight into the links among the identified proteins and their functional roles were analysed by STRING 8.3, KEGG and REACTOME pathway databases.

Results and Discussion: A significant finding of the present study is the identification of Cdk10, EFNB3, RAB 34, RRAS, HSP22 and HSP90 in healing Corneal epithelium at active phase of migration. The results were further validated using Cdk10 antibody by western blot. Interaction association network analysis further confirms the close interacting relationship among identified proteins.

Conclusion: The present communication initially provides new evidence for the potential role of identified proteins in migrating epithelial cells. We assume that these findings are one step forward in identifying the mechanism of wound repair or re-epithelialization. This study may also increase the understanding of normal and abnormal corneal function with likely relevance to corneal disease and transplants.

Poster 015: A New Approach for Understanding Cancer and Chemoresistance: Metabolic Labeling and LC-MS to Measure Protein Turnover in Breast Cancer

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Metabolic remodeling is an emerging hallmark of cancer and may play a role in chemoresistance. Recent studies show that as part of a metabolic stress response, cancer cells increase protein turnover through an alternative “self-eating” pathway called autophagy, somehow promoting cell fitness and chemoresistance. Increased autophagy may enhance cell fitness by reallocating resources such as amino acids. Efforts to improve cancer therapy through the manipulation of tumor metabolism and autophagy are exciting because they target cancer in an entirely new way. However, research has been hindered by the difficulty in measuring autophagy in vivo.

Triple-negative is the most aggressive form of breast cancer (TNBC) and tumors have a high tendency for developing chemoresistance. Here, we use metabolic labeling and mass spectrometry to directly measure the kinetics of protein and amino acid turnover in chemosensitive and chemoresistant TNBC cell culture. We have identified key differences in cell growth and amino acid turnover between sensitive and resistant TNBC, providing evidence that amino acid metabolism is altered in chemoresistance. The apparent differences between resistant and sensitive cells suggest a global metabolic shift that collectively promotes chemoresistance, perhaps though mechanisms of selective autophagy. This is supported by oxygen utilization measurement, which are significantly changed relative to the growth rate in resistant TNBC. This may indicate that the autophagy-directed survival response is specific to protein components required for aerobic metabolism such as mitochondrial complexes (mitophagy). To verify this hypothesis, we are measuring autophagy-specific signaling and comparing changes in distinct turnover rates across the proteome. We anticipate that specific degradation of the mitochondria plays a significant role in chemoresistance and thus may be exploited as a new therapeutic target.

Poster 016: Cell Death Resistance and Chemo-Resistance Due to SphK1 in Pancreatic Cancer

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All cells and organelles contain a complex, highly regulated, dynamic set of lipid molecules that are vital to cellular signaling, structure, shape,

and function. Changes in lipidome composition and regulation can cause dramatic and drastic changes to cell morphology and pathway regulation. Over one thousand bioactive lipid species have been discovered to play vital roles in cellular regulation. Mass spectrometry based lipidomic analysis of human ductal pancreatic cancer has revealed that Sphingosine Kinase 1 (SphK1) activity is abnormally up-regulated when compared to non-cancerous pancreatic cells. SphK1 is a kinase enzyme in the sphingomyelin metabolic pathway that is responsible for the phosphorylation of sphingosine created from C-16 ceramide –which act as vital second-messengers in the apoptotic cell signaling cascade—into sphingosine-1-phosphate (S1P) molecules. The generation of the product lipid, S1P, regulates proliferation, angiogenesis, metastasis, and cell survival both intracellularly and extracellularly. This conversion of signaling lipids removes the pro-apoptotic second-messenger C-16 ceramide molecules required for mitochondrial mediated apoptosis, effectively eliminating the ability of these cells to die. Traditional chemotherapeutics often rely on the highly regulated pathways of mitochondrial mediated apoptosis in order to function and kill the cancerous cells. When SphK1 is over active this avenue for self-elimination through apoptosis becomes difficult if not impossible for these cells thereby explaining partially the chemotherapy resistant nature of pancreatic cancer. Even more alarming is the fact that these pro-apoptotic molecules are being converted into anti-apoptotic signals that impart some of the characteristic proliferative and metastatic character of pancreatic cancer. This 'rheostat' imbalance due to SphK1 activity may prove to be one of the primary reasons why pancreatic cancers harbor such a poor prognosis and why they are so difficult to treat.

Poster 017: The Comparison of the Serum Proteome in Individuals with Cancers versus Those without Cancer, and its Application to Wellness

Haiyan Zheng[1]; Caifeng Zhao[1]; Swapan Roy[2]; Devjit Roy[3]; Amenah Soherwardy[2]; Ravish Amin[2]; Matthew Kuruc[2]

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For many diseases, pancreatic cancer for example, long term survival is critically dependent upon early detection. So various strategies for early detectable markers are being investigated. One such strategy is to identify a singular biomarker, derived from genomic analysis, and then determine its derivative protein concentration in blood. This has been challenging as many differentially regulated genes do not generate a differentially regulated protein. An alternative biomarker strategy is to consider panels of various proteins that may be up and/or down regulated proteins, or proteins with altered post-translational modifications, which differ in diseased and normal states. In this study we adopt a new product which combines Albumin depletion and on-bead digestion of the depleted serum in a seamless process, called AlbuVoid™ LC-MS On-Bead. From these methods, we were able to compare labeled quantification of proteins from normal and disease state sera – for this case, breast, lung and pancreatic cancer. The methods spectrally quantify over 200 total proteins, in a cost effective and reproducible manner. No offline peptide level fractionation prior to LC-MS was employed, lowering the LC-MS acquisition time 5-10x compared to common serum proteomic workflows. We describe these workflow advantages applied towards a "wellness" proteome strategy whereby knowledge and data surrounding individual normal and healthy proteomes can be annotated, compared and contrasted to those with a clinically definable disease.

Poster 018: Quantitative Analysis of AKT/mTOR Pathway using Multiplex-Immunoprecipitation and Targeted Mass Spectrometry

Bhavin Patel[1]; Alex Behling[1]; Leigh Foster[1]; Ryan Bomgarden[1]; Carrie Clothier[1]; Kay Opperman[1]; Rosa Viner[2]; Andreas Huhmer[2]; John Rogers[1]

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Background: A major bottleneck in the quantitation of signaling pathway proteins is the lack of rigorously validated methods/reagents and a reliance on semi-quantitative results from current immunoassay technologies (Western blot, ELISA and Luminex). Mass Spectrometry (MS) is increasingly becoming the detection methodology of choice for proteins and their post-translational modifications (PTMs). Immunoprecipitation (IP) is commonly used upstream of MS as an enrichment tool for low-abundant proteins. The objective of this study was to determine the efficacy of multiplex IP to targeted MS (mIP-tMS) technique for measurement of the total and phosphorylated AKT/mTOR pathway targets and to evaluate whether mIP-tMS assays are as effective as the current singleplex immunoassay (WB and ELISA) and multiplex Luminex assays.

Methods: Serum starved HCT116, MCF7 and A549 cells were stimulated with IGF-1. mIP-tMS assays were developed and validated for absolute quantitation of eleven total and ten phosphorylated AKT/mTOR pathway targets. Validated mIP-tMS assays were benchmarked against currently available WB, ELISA and multiplex Luminex immunoassays across three unstimulated and IGF-1 stimulated cell lysates.

Results: We validated multiple antibodies for eleven total and ten phosphorylated AKT/mTOR pathway targets using the optimized IP-MS workflow. mIP-tMS assays allowed absolute quantitation for all eleven total and ten phosphorylated targets in low to sub nanogram concentrations across all cell lines. The benchmarking of mIP-tMS assays showed high correlation for quantitation of total target relative abundance compared to WB, ELISA and Luminex assays. However, for some phosphorylated targets, mIP-tMS assays had low concordance to the other immunoassays possibly due to differences in the specificity of anti-phospho antibodies used for each assay.

Conclusion: The mIP-tMS assay can be used for quantification of AKT/mTOR pathway proteins in cancer cell lines or tissue samples. Major advantages of this assay are high confidence in target identity coupled with simultaneous quantitation of multiple targets and their PTMs.

Poster 019: Integrative Proteomic and Glycomic Profiling of Metastatic Signatures in Breast Tumor

Le Meng

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Metastatic mammary malignancies that cause massive mortality are closely associated with dysregulated cellular signaling and tumor microenvironment. Here we report a workflow to comprehensively investigate proteomic and glycomic profiles from a panel of breast cancer cell lines. Proteomic data with label-free quantification showed distinct functional clustering across different sub-types of breast cancer, while many protein expression patterns correlated with their genotypes. N-glycan and glycosaminoglycan profiles showed complicated and wider variability, indicating glycoforms undergo more dynamic changes, thus allowing these cells to possess altered signaling networks and to respond to stimuli differentially. Next we utilized this platform to explore metastatic traits of spontaneous mammary tumors from two groups of mice that showing distinct metastasis capability. Clustering of these profiles provided comprehensive "oncomic" hallmarks with pro-metastatic potential. These data identified a set of molecular signatures as regulators of metastasis, including proteoglycans, ECM molecules, DNA damage response, immune responses, cytokines, EMT molecules, kinases/phosphatases.

Poster 020: Inter-grade Comparative Proteomic Analysis of Gliomas using Cerebrospinal Fluid

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Gliomas, originating from glial cells, are the most prevalent form of brain tumors categorized into four different grades depending upon their aggressive nature. Several genomic markers are implemented for classifying these tumors; however, accessibility of tumor tissue for early diagnosis and prognosis remains a clinical challenge. Cerebrospinal fluid (CSF) being a proximal fluid of central nervous system (CNS) can reflect the alterations arising in the brain during disease progression. Therefore, analyzing the alterations in the CSF may lead to the

identification of putative biomarker candidates. In this study, we intended to identify the inter-grade proteomic alterations in glioma patients using two complementary approaches; 2D-DIGE in combination with MALDI-TOF/TOF MS, and iTRAQ in combination with ESI-Q-TOF LC-MS/MS.Deferentially expressed proteins identified in quantitative proteomics profiling were subjected to bioinformatic analysis. Intriguingly, several signaling and metabolic pathways including integrin signaling pathway, cytoskeletal regulation, gluconeogenesis, and glycolysis were found to be altered in gliomas. Further, autoantibody (AAbs) screening using CSF was performed on the human proteome arrays containing ~17000 recombinant human proteins. AAbs against SMC1A, PIP5K2B, ADRBK1, FGFR1OP and SMARCC2 were found to be present in the CSF of grade III tumors. Some of the differentially regulated proteins such as Vimentin, Profilin, Macrophage-capping protein, Selenium-binding protein, Protein disulfide-isomerase, Peptidyl-prolyl cis-trans isomerase, etc. were found to be increased with the increase in tumor grades, while Vitronectin, Apolipoprotein C-III, Synapsin-1, Superoxide dismutase (Cu-Zn) etc. showed a negative correlation with the increase in malignancy of gliomas. These proteins could serve as potential predictive markers and may provide some novel mechanistic insights into glioma pathogenesis. Furthermore, a panel of deferentially expressed proteins may act as potential biomarkers for early diagnosis of gliomas. However, there is a need for further validation of the identified targets on a larger cohort of glioma patients before anticipating their diagnostic impact.

Poster 021: Dysregulation of HUWE1, An Essential E3 Ubiquitin Ligase, Reduces Chromosome Segregation Fidelity

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Huwe1 (Mule, UREB1 or ARF-BP1) is a HECT- E3 ubiquitin ligase that regulates the stability of essential protein effectors, such as the anti-apoptotic protein Mcl1 or the tumor suppressor p53. Recent research has implicated Huwe1 in the regulation of diverse cellular processes such as base excision repair, neural development and cell proliferation. We are interested in studying Huwe1 to assess its relevance to cellular transformation and cancer.

Huwe1 expression is often deregulated in cancer cells. Overexpression of Huwe1 is commonly seen in breast, colon and lung tumors; however Huwe1 is also downregulated, as is seen in gliomas. Curiously, perturbation of total Huwe1 levels is poorly tolerated in many transformed cell lines. In order to understand how this is regulated, we studied overexpression of Huwe1 in a bone osteosarcoma cell line (U2OS) and found a correlation between Huwe1 levels and chromosome segregation fidelity: overexpression of Huwe1 results in an increase in the number of anaphase bridges and acentric fragments. Additionally, in a subsequent cell cycle, we see additional increases in cells with combined segregation defects than control. We also observe an increase in binucleation and micronucleation, which could be a direct outcome of chromosome missegregation mediated by Huwe1 overexpression. Thus we have discovered a putative role for Huwe1 in safeguarding DNA segregation fidelity. Future work will rely on quantitative proteomics and AP-MS to assess how Huwe1 is regulated within the cell cycle, as well as to determine the effectors responsible for the phenotype that we report here.

Methods: Huwe1 overexpression is effected by a GFP-tagged baculovirus with a mammalian promoter. Chromosome segregation was assessed by indirect immunofluoresence. Endogenous tagging of Huwe1 was accomplished with CRISPR/Cas9 editing technology for use in interactome and substratome studies. Quantitative proteomics experiments will utilize an isobaric labeling and Orbitrap Fusion-based workflow.

Poster 022: Src-Family Kinase Signaling Mediating Gemoitabine Resistance in Gallbladder Cancer Revealed by Quantitative Phosphoproteomics

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Gallbladder cancer is the second leading cause of cancer-related death in women in Chile. It often arises in the setting of chronic inflammation. Patients with advanced gallbladder often develop the resistance to current chemotherapy. However, the underlying mechanisms for drug resistance remain uninvestigated. Here, by combining SILAC-based quantitation with anti-pTyr antibody-based phosphopeptide enrichment method, we quantified the difference of tyrosine phosphoproteomes between a parental gallbladder cancer cell line and the corresponding gemoitabine-resistant cell line. Interestingly, our data revealed that growth factor signaling pathways are more active in parental gallbladder cancer cells while Src-family kinase signaling pathways are more active in gemoitabine-resistant gallbladder cancer cells, which is consistent with our observation that parental gallbladder cancer cells grow more quickly than corresponding drug-resistant cells. Furthermore, a kinase inhibitor screen revealed several potential therapeutic targets for inhibiting the growth of drug-resistant gallbladder cancer cells. Our study is the first phosphoproteomic analysis of abnormal signaling pathway in drug-resistant gallbladder cancer that greatly expands our understanding of the underlying mechanisms of drug-resistance in gallbladder cancer and provides novel targeted therapy for gallbladder cancer patients.

Poster 023: Next Generation Signaling Pathway Characterization by IS-PRM

Michael Blank[1]; Daniel Ayoub[2]; Sebastien Gallien[2]; Antoine Lesur[2]; Bruno Domon[2]; Julian Saba[3]; Yuriy Dunayevskiy[1]; Andreas Huhmer[1]

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Reproducible and accurate quantitation on low abundance, biologically-significant proteins and post translational modifications of interest, especially within important signaling pathways, is one of the most formidable challenges in modern proteomics. Data-independent methods provide high content screening while targeted methods on focused set of analytes provide the sensitivity required to accurately quantify proteins across a large dynamic range (>10E5) and at the lowest extremes of natural abundance1. This work demonstrates the application of a next generation sensitive and reproducible targeted method for large-scale quantitation using high resolution mass spectrometry. The method intelligently screens for targeted endogenous peptides and manages system resources by only utilizing higher resolution and increased ion injection times when the MS confirms peptide by validation against an onboard spectral library. This allows for monitoring a substantially larger number of targets while overcoming the issue of missing data and dramatically enhancing sensitivity.

Two non-small cell lung cancer cell lines, one with an apparent drug resistance, were selected for proteomics analysis. An IS-PRM method was performed targeting many noteworthy protein/peptide targets belonging to the MAPK, WnT, and mTOR signaling pathways.

Nearly 300 endogenous peptides were quantified with normalized CVs ranging from 1 to 10% IS-PRM results were highly consistent with those from DIA but depth and comprehensiveness of coverage for these pathways was substantially increased using the IS-PRM. Detection and reproducible quantitation of additional membrane receptors (such as ERBb2) and many more transcription factors such as c-FOS which showed a substantial change in protein level expression between the cell lines. Normalized nominal protein abundance could be estimated thanks to internal standards for each peptide, which also served as positive controls. Finally, IS-PRM can be expanded to routinely and sensitively quantify a larger number of targets, up to 1500 per hour, many more than would be possible by conventional PRM methods.

All posters will be attended by presenting authors on both Monday and Tuesday. All posters should be set up by 10:00 am on Monday morning and removed at 3:00 pm on Tuesday. **Odd**-numbered posters present **1:30 - 2:15 pm** and **even**-numbered posters **2:15 - 3:00 pm**.

Poster 024: Understanding the Aggressive Nature of Glioblastoma Tumors Associated with the Subventricular Zone

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Glioblastomas are most aggressive among all four grades of gliomas. Recent reports revealed a significant decrease in survival period of GBM patients with tumors located closely to the subventricular zone (SVZ) than those with tumors away from the SVZ region. Subventricular zone (SVZ) is rich in neural stem cells and the tumors associated with this region (SVZ+) are more aggressive than the tumors which are away from the subventricular zone. To gain an insight into the molecular features responsible for the increased aggressiveness of SVZ+ GBM tumors over SVZ- GBM tumors, we performed a global proteomic analysis using 2D-DIGE and iTRAQ approaches. Serum proteomic analysis of SVZ- & SVZ+ GBM patients showed significant alteration of lipid binding proteins like apolipoproteins. Tissue proteomic analysis revealed increased expression of various proteins like thymosin beta 4 like protein 3, alpha-1-antitrypsin, cytoskeletal proteins in SVZ+ GBM tumors over SVZ- GBM tumors thereby providing a plausible explanation to the increased aggressiveness of SVZ+ GBM tumors. Further understanding of these complex subtypes of GBM tumors can prove beneficial towards development of prognostic and therapeutic targets.

Poster 025: Accumulated ion Monitoring (AIM) Improves Sensitivity and Quantitative Dynamic Range for Precision Functional Proteomics

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Mass spectrometric characterization of rare proteins is hindered by their low physiological copy-number and the sub-stoichiometric occupancy of their post-translational chemical modification sites. Selected reaction monitoring benefits from excellent sensitivity and broad dynamic range but is limited in accuracy by low resolution mass analyzers. Using external ion storage for high-resolution Penning and Orbitraps allows their coupling to continuous ion sources, thereby enabling high mass-accuracy measurements. Here we hypothesized that extensive accumulation and enrichment of ions for selected ion monitoring (SIM) prior to Orbitrap detection and analysis will enhance sensitivity and quantitation dynamic range. The phosphorylation-activated human transcription factor MEF2C was studied as an archetypical low abundance phospho-protein. We used an Orbitrap Fusion mass spectrometer coupled via nanoelectrospray ion source to a nanocapillary liquid chromatograph. A panel of synthetic peptides diluted in neat solvent and delivered by continuous infusion was used to determine the absolute limits of detection and quantitation with maximum multipole ion accumulation time varying in the 10-5000 ms range. Under these conditions we measured absolute limits of detection at 0.95-2.7 ymol/ms, corresponding to approximately 150 molecules/scan, and 7-8 orders of magnitude of linear dynamic range. Nanoelectrospray ionization efficiencies of up 20% were achieved using in-house fabricated 2 µm emitter tips. When analyzed in the context of chromatographically resolved human acute myeloid leukemia proteome without any enrichment, this method enabled the quantitation of MEF2C phosphorylation at the level of 10,000 molecules/cell from 1 µg of total whole-cell extract, representing 3 orders of magnitude improvement in practical sensitivity. Increasing the chromatographic resolution by online multi-dimensional chromatography is expected to reduce co-accumulation of target and contaminant ions, thereby enabling robust quantitative functional proteomics using AIM in complex proteomes, such as the Quantitative Cell Proteomics Atlas (http://alexkentsis.net/qcpa/).

Poster 026: Investigating the Cellular Interactions of BIRB796 Analogs using a Novel Chloroalkane Capture Tag

Michael Ford[1]; Richard Jones[1]; Rachel Friedman Ohana[2]; Thomas Kirkland[3]; Carolyn Woodroofe[2]; Paul Otto[2]; Danette Daniels[2]; Marjeta Urh[2]; Keith Wood[2]

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Identifying the targets of bioactive compounds is often the rate limiting step toward understanding the molecular mechanism of drug action. We have developed a method based on a novel chloroalkane capture tag which can be chemically attached to small molecules to isolate their respective protein partners. The chloroalkane tag minimally affects compound potency and cell permeability allowing verification of the pharmacological activity of the modified compound, thus increasing the confidence in the biological relevance of captured proteins. In addition, by allowing the chloroalkane-modified compound to bind the targets within living cells, interactions occur under the conditions that the unaltered compound would normally engage these targets.

Following binding with the tagged compound inside living cells, the cells were lysed and the chloroalkylated compound, together with the bound targets, was rapidly captured onto immobilized HaloTag protein. Unmodified compound was used to competitively elute putative interacting proteins. The eluted proteins were analyzed using nanoscale LC-MS/MS. The putative targets identified by mass spectrometry were validated for direct binding relationship with the bioactive compound by bioluminescence energy transfer (BRET).

We tested this target capture/target-validation workflow using the interaction of MAP kinases (MAPK) with two allosteric kinase inhibitors, BIRB796 and a BIRB analog. Using the two BIRB-chloroalkane derivatives to selectively enrich for targets from HEPG2 cells, we identified and validated multiple relevant MAPK as well as additional off-targets. Interestingly, all the discovered off-targets bind purines. Kinase inhibitors such as BIRB796 which acts by binding to the kinase ATP binding site can interact in a similar manner with other purine binding proteins. Using bioluminescence energy transfer we interrogated the affinity and residence time of the two BIRB compounds to multiple MAPK. Our results indicates that the BIRB analog exhibits 30-1000 fold reduced affinity to multiple MAPK as well as a significant shorter residence time compared to BIRB796.

Poster 027: Development and Application of a Covalent-Labeling Strategy for the Large-Scale Thermodynamic Analysis of Protein Folding and Ligand Binding

Yingrong Xu; Ryenne Ogburn; Michael C. Fitzgerald; *Duke University, Durham, NC*

Thermodynamic measurements on proteins and protein-ligand complexes can offer insights not only into the fundamental properties of protein folding reactions and protein functions, but also into the development of protein-directed therapeutic agents to combat disease. Conventional calorimetric or spectroscopic approaches for measuring protein stability typically require large amounts of purified protein. This requirement has precluded their use in proteomic applications. Here we report on a mass spectrometry-based protocol for making thermodynamic measurements of protein folding and ligand binding reactions on the proteomic scale. The protocol, which can be combined with quantitative, bottom-up, shotgun proteomics technologies, enables the evaluation of protein folding free energies using the denaturant dependence of the rate at which globally protected tryptophan and methionine residues are modified with dimethyl (2-hydroxyl-5-nitrobenzyl) sulfonium bromide and hydrogen peroxide, respectively.

Presented here will be the results of proteome-wide experiments, in which the above tryptophan and methionine labeling strategies were simultaneously used to evaluate the thermodynamic stability of proteins in lysates derived from yeast, human (MCF-7) and dust mite (D. Farinae) cells. The described protocol enabled the thermodynamic stability of ~1000 proteins in each cell lysate to be evaluated using ~2000 different peptide probes. The dual labeling strategy increased the proteomic coverage by 50%-100% compared to the coverage observed using the methionine modification strategy alone. Also reported will be results obtained using the described protocol to detect and quantify the binding of geldanamycin to Hsp90 in cell lysates. To date, we have successfully detected and quantified the binding of geldanamycin to one of its known protein targets, Hsp90, in MCF-7 cell lysate. The measured K_d, 0.62 µM, is in the range of literature values (0.08-0.6 µM) obtained using purified Hsp90. To our knowledge, this is

the first K_d value measurement of the human Hsp90-geldanamycin complex in a cell lysate.

Poster 028: Proteomics Identifies Associated Factors of the Phosphorylated RNA Polymerase II C-Terminal Domain Linking Regulation of Chromatin Dynamics

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RNA Polymerase II (Pol II) is the central enzyme at the core of a multimeric megadalton protein complex required for all gene expression. The unique C-terminal domain (CTD) of Pol II in humans is 52-repeats of the concensus sequence YSPTSPS, with minor exceptions mostly in the distal half. The Pol II CTD is phosphorylated throughout the transcription cycle by several kinases, two of which are the Cdk7 subunit of the general transcription factor TFIIH and Cdk9 of the dimeric positive transcription elongation factor b (P-TEFb). We have purified to near homogeneity two CTD kinases, the 10-subunit endogenous human TFIIH and recombinant P-TEFb. These were used to phosphorylate a GST-tagged Pol II CTD as affinity ligands to interrogate the interactome of both the unphosphorylated and hyperphosphorylated full length Pol II CTD in HeLa nuclear extracts. To further dissect the function of the Pol II CTD, we also included affinity ligands of the proximal CTD, or the first 26 repeats, and the distal CTD, or the last C-terminal 26 repeats. Each affinity purification was analyzed by mass spectrometry. Cofactors known to associate with the human Pol II CTD, such as Mediator, Integrator, PAF and the mRNA capping enzyme were identified validating the approach. Factors identified exclusively with hyperphosphorylated CTD included all of the subunits of the chromatin modifying histone H3K4me3 methyltransferases SET1 (TFIIH and P-TEFb) and histone H3K36me3 SETD2 (P-TEFb only with a preference for the proximal CTD). Furthermore, ChIP-Seq analyses after inhibition of TFIIH in analog-sensitive CDK7-AS cells showed modified localization of the H3K4me3 and the H3K36me3 marks throughout the body of many genes.

Poster 029: Circulating Peptide Signatures Derived from Enzymatic Activities for Tagging Human Immunodeficiency Virus-1 elite Controllers

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The mechanism of spontaneous HIV-1 suppression in elite controllers (EC) remains poorly understood despite several immunological, genetic, and proteomics studies. Many cytokines, complement factors, and endogenous peptides, as parts of the innate immune system, are believed to form the first barriers against HIV infection. Complement factor I (CFI) is a critical regulator of the complement cascade; however, its role in HIV-specific immune responses has been rarely studied. Here, we report a novel peptidomics study to explore HIV-blocking enzymatic pathways and their proteolytic peptide products in the circulation to investigate the mechanisms of HIV suppression in EC.

Using NanoTrap-MALDI platform, we found that three CFI substrate peptides (1626.88, 1739.94, and 1896.04 m/z) were significantly up-regulated in the blood of EC (n=25), compared with HT (highly active antiretroviral therapy recipients, n=25) and HN (HIV-1 negative controls, n=20). IL-6-induced elevated levels of CFI were observed in EC. Interestingly, we found that levels of CFI, CFI substrate peptides, and IL-6 in the plasma were present in the same order of highest level: EC > VC > HT = HN. However, R5 infectivity luciferase assay did not show the selected peptides to have functions against HIV entry. Receiver operating characteristic analysis showed that EC was distinguished from HN with high sensitivity (87.0%, 95% confidence interval: 0.679–0.955) and high specificity (77.3%, 95% confidence interval: 0.566–0.899) using optimal cutoff intensities of the three peptide signatures in this cohort.

Conclusion: There was a strong association of CFI substrate peptides, CFI, and IL-6 with HIV-1 virus control. Circulating peptide signatures of EC might potentially help clinicians and researchers screen a large number of EC via routine blood tests. Further functional studies on the selected peptides and regulation mechanism studies on CFI and IL-6 are needed.

Poster 030: Optimizing Global Proteomics Analysis for Clinical Biomarker Studies

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Increased demand for biomarker discovery studies including large cohorts of clinical samples with wide dynamic range requires high-throughput LCMS solutions for efficient and reproducible profiling of biological samples, such as plasma and urine. Targeting the most comprehensive experiments (i.e. enrichment and fractionation), toward the complex and information-rich matrix of plasma, and shorter, more robust experiments toward matrices with less dynamic range is one strategy to improve efficiency and quality for studies of >100 samples. To address these challenges, we optimized a platform to provide comprehensive nanoscale profiling of the less abundant proteins of depleted plasma, introduced parallel sample preparative techniques where possible to improve efficiency, and applied faster, analytical scale chromatography to reproducibly quantitate the abundant proteins in clinical samples. We monitored the abundant proteins of non-depleted plasma in a high-throughput UPLC method with data independent (DIA) mass spectrometry. A platform using abundant protein depletion and offline plate fractionation prior to nanoscale LC with data dependent (DDA) mass spectrometry was used for profiling the lower abundant plasma proteins. For urine clinical samples, we again evaluated nanoLC-DDA-MS, but omitted fractionation, to reproducibly profile proteins in a sample type often limited by low protein concentration. In this study, healthy human plasma or urine standard was used to evaluate the sensitivity and reproducibility of each platform with label-free quantification. The data-independent UPLC-MS method was most efficient and reproducible, profiling abundant proteins in non-depleted plasma at ~30 minutes/sample. The 2D analysis of batch-depleted plasma resulted in a reproducible and automated method for comprehensive identification of ~300 proteins in plasma (3 hours/sample). Spin-columns allowed for reproducible and high throughput sample depletion. Similar levels of proteins were consistently detected in the analysis of normal human urine without fractionation (90 minutes/sample).

Poster 031: PRM Coupled to an Intensity-Based Quantification Strategy Accurately Measures D3-Leu Tracer of less than One Percent in apoA-I/HDL Clinical Samples

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Background Better understanding of apolipoprotein metabolism requires exact quantification methods. Using parallel reaction monitoring (PRM, Q Exactive, Thermo) as a readout, we performed D3-Leu tracer enrichment kinetics studies in humans enrolled in a apoA-I/HDL metabolism clinical study. D3-Leu enrichment in apoA-I did not exceed 0.6% as verified by manual quantification of the extracted ion chromatograms (XICs) of the 2H M3 (tracer) and M0 ions (tracee). Moreover, deuterium labeling incurs a shift in peptide retention time resulting in reduced quantification accuracy when automated XIC-based quantification was used.

Methods and Results We implemented an intensity-based approach that takes advantage of high resolution/accurate mass (HR/AM)-PRM scans to confidently identify the 2H M3 ion from surrounding non-specific peaks. Our workflow includes 5 modules for extracting the targeted PRM peak intensities (XPIs): 1) Peak centroiding, 2) noise removal, 3) fragment ion matching using delta-*m/z* windows, 4) quantification based on ion intensities in eight ways, and 5) validation and visualization outputs. We optimized the XPI workflow using *in vitro* synthesized D0-Leu- and D3-Leu-apoA-I standards (mixing-ratio range, 1:1 to 5,000:1 (D0:D3)), and apoA-I/HDL clinical data (15 time

points over 3 days). We tested XPI using different PRM resolution settings, 35K, 70K and 140K and determined that R=140K measurements were the least variable and could be used for kinetic parameter calculations using SAAMII (TEG, Charlottesville, VA). We then implemented the workflow to study the metabolism of apoA-I/HDL in three subjects that participated in a low fat versus high fat crossover study. Five HDL size fractions (large to small HDL) were analyzed per diet sample per subject. XPI+SAAMII analysis revealed that dietary unsaturated fat intake alters the metabolism of several HDL proteins across the HDL size fractions.

Conclusions Our PRM intensity-based quantification workflow will facilitate endogenous labeling using D3-Leu in clinical kinetics studies of HDL or other molecules.

Poster 032: Getting a Grip on what Determines the Composition of Urinary Proteomes

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Urine is the "waste fluid" of the body and contains several molecules, including some proteins. Proteins in urine have two major origins: firstly the kidney and urinary tract organ system, which is in direct contact with the urine into which cells are shed and proteins are secreted; secondly, blood which is filtered in the glomeruli of the kidney. It is thought that this filtration process is controlled by the size of the proteins and their charge (since the basement membrane is negatively charged). While there is no clear positive and/or negative correlation between blood and urine protein levels, it seems to be a valid assumption that altered blood proteomes will translate into altered urinary proteomes. Thus, given that urine is analytically much less challenging than blood-derived fluid specimens, one actually might argue that looking for biomarkers in urine might be tractable compared with blood.

To obtain a better understanding of the determinants of the urinary proteome composition, we analyzed 93 pediatric urine samples, which were exquisitely annotated with information about BMI, percent body fat, systolic and diastolic blood pressure, and CRP, insulin, LDL, HDL, and triglyceride levels. These samples were processed using our recently developed MStern blotting technique and analyzed using data independent acquisition (DIA) routines. More than 1700 proteins were identified and quantified. Subsequently, the analyzed data show that certain proteins clearly correlate with specific measurements linking the organismal state with the urinary proteome composition.

Poster 033: The Development of Molecular Diagnostic Tool for Schizophrenia using Lymphoblastoid Cell Lines

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Schizophrenia (SCZ) is a chronic and disabling mental disorder with a lifetime prevalence of approximately 1% worldwide. The behavioral diagnoses represent nonspecific expressions of factors that affect brain development and function. Their diagnostic criteria are not designed for patients with developmental disabilities and so may lead to the reporting of erroneously high rates of these phenotypes. Additional complications may arise owing to the association with SCZ. For example, diagnoses of autism spectrum disorders may reflect misdiagnosis of social impairments associated with premorbidity to SCZ. Therapeutic optimization based on pathophysiology should be performed as early as possible to improve functional outcomes and prognosis. Therefore the identification of biomarkers for SCZ is necessary to provide timely diagnosis and effective therapy. Although there is growing evidence for a widespread role of copy-number variants (CNVs), which include chromosomal microdeletions and microduplications, in determining susceptibility to cognitive disorders and SCZ, protein profiles between SCZ and healthy control are not clear. Therefore, the proteome analyses were performed on Epstein-Barr virus (EBV)-transformed lymphoblastoid cell lines (LCLs) derived from SCZ patients and age- and sex- matched healthy control (CON). EBV-transformed LCLs have been used extensively in the detection of human genetic diseases. For the identification of proteins, we employed florescence two-dimensional differential gel electrophoresis (2D-DIGE). Twenty protein spots were differentially expressed between SCZ and CON in 2D-DIGE analysis, and 22 unique proteins were identified by liquid chromatography tandem-mass spectrometry. Differential expression of 8 proteins in these 22 proteins was confirmed by Western blotting. Among the 8 candidate proteins (HSPA4L, MX1, GLRX3, UROD, MAPRE1, TBCB, IGHM, and GART), we successfully constructed logistic regression models comprised of 4- and 6-markers with good discriminative ability between SCZ and CON. These findings might provide insight into the pathophysiology of SCZ and potentially provide diagnostic and prognostic biomarkers.

Poster 034: Next-Generation Blood Biomarkers for Acute Liver Injury: *in silico* Discovery and Proteomics Quantification

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Acute liver injury (ALI) is a severe disorder resulting from excessive hepatocyte cell death and frequently caused by acetaminophen intoxication. ALI can rapidly progress to acute liver failure (ALF), a systemic and life-threatening condition. The paucity of blood biomarkers of ALI progression makes its clinical management difficult. The goal of this study was to explore the huge amount of information generated by large-scale biology to discover new mechanistic biomarkers for ALI. Bioinformatics databases were explored to select proteins with a liver-predominant expression and a high detectability in the blood. Then, we developed an innovative proteomics pipeline based on targeted mass spectrometry in SRM mode (Selected Reaction Monitoring) to assay six of these proteins in serum or plasma samples. For reliable quantification of the selected proteins, we used PSAQ (Protein Standard Absolute Quantification) standards which are isotopically labelled versions of the target proteins. Finally, the six selected proteins were assessed as potential biomarkers of hepatocyte cell death in serum samples from patients with ALI or ALF of different aetiologies. In patients with acetaminophen-induced ALI/ALF, the serum concentration of alcohol dehydrogenase 1 (ADH1), alcohol dehydrogenase 4 (ADH4) and betaine-homocysteine S-methyltransferase (BHMT) markedly increased during the acute phase of the disease and dropped to undetectable levels during the recovery period. This time-progression was closely correlated with coagulation parameters and cytokeratin-18 serum levels. In patients with non-acetaminophen-induced ALI/ALF, variable but significant increases in serum ADH1, ADH4 and BHMT concentrations were also observed, except for the autoimmune aetiology. ADH1, ADH4 and BHMT emerged as novel candidate biomarkers to detect drug-induced liver injury and evaluate the severity and progression of ALI.

Poster 035: A Cross-Sectional Study on Dietary Factors and their Correlation with Body Mass Index among School-Going Teenagers in Karachi

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The purpose of this research is to assess the nutritional dietary habits/life-style among the School-going teenagers of Karachi and their co-relation with the current Asian-standard guidelines of BMI.A survey-based cross-sectional study among teenagers from Grade-8th to Grade-10th in 5 government and 5 private schools of Karachi. Data was collected using pre-tested questionnaire from 500-teenagers after taking informed consent. Height-and-weight of the students was measured and BMI was calculated on calibrated-scale. SPSS-software was used for data evaluation.43.4% of the teenagers were underweight, 34.4% had normal weight-and-height, Overweight and obese were 12.2%. And 10% respectively.52% of underweight teenagers' belonged to lower SES (socio-economic-status), 39% from

middle-class while 9% from high SES. Improper nutrition, skipping meals mainly breakfast and dieting were main factors respectively. Out of all overweight respondents, 34% frequently consumed junk-food, 22% had high caloric-beverage intake, 18% were due to eating several times a day, 14% were lacking physical-activity, 10% due to unknown-reason and 2% due to lack of sleep, stress or may be genetically.

Most students did not meet the recommended dietary habits. Under nutrition is comparatively higher but obesity and under-nutrition both co-exist in teenagers and are directly related to high and low socio-economic status ,respectively. Dietary habits of teenagers were found to be unhealthy and significantly associated with BMI. Study revealed that both under and over nutrition co-exist among teenagers because of Socio-economic factors and unhealthy life-style. Balanced-diet and regular physical activity would be an effective recommendation for both

Poster 036: Pushing the Limits of Bottom-Up Proteomics with State-ofyThe-Art Capillary UHPLC and Orbitrap Mass Spectrometry for Reproducible Quantitation of Proteomes

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Since its inception, bottom up proteomics has aimed to identify and quantify the complete proteome from a cell, tissue, or whole organism. Although many advances have been made in the last 15 years, there are still several challenges to overcome, such as identifying all the expressed proteins in a given time, being able to work with samples of limited amount like clinical biopsies, and to achieve sufficient analytical throughput. To further complicate matters; being able to discriminate the most important proteins constituting a given cellular state requires accurate peptide measurement across several orders of magnitude.

In this work, we outline the cooperative use of an EASY-nL 1200 with an Orbitrap Fusion Lumos mass spectrometer, to separate a HeLa cell lysate in a 75 cm long 75
□m, ID Acclaim PepMap capillary column
using both 2 and 4 hour gradients, and compare the results with those obtained under the same conditions with a 50 cm column, a contemporary high performance commercial column for routine bottom up proteomics. As expected, the length increase results in the separation and detection of 10% more unique peptides, and 7% more protein identification in a 4 hour gradient, with protein identifications exceeding 5700 proteins for a single injection of mammalian cell lysate. More importantly, longer columns showed better reproducibility as seen by increased correlation among technical replicates, higher numbers of quantifiable peptides, and a smaller coefficient of variance (CV), resulting in improved protein quantification for complex lysates by HRAM LC-MS. These results represent a new standard in the proteomics paradigm and rival quantitation results derived from DIA methods in terms of reproducibility and depth of analysis.

Poster 037: A Multiplexed Mass Spectrometry-based Strategy Quantifies Nicotine-Induced Protein Alterations across Four Human Cell Lines

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Mass spectrometry-based proteomic strategies can identify thousands of proteins and allow for the quantification of relative protein abundances in response to external stimuli. Nicotine can affect diverse cellular pathways, however, nicotine-induced alterations on the global proteome across human cell lines have not been fully elucidated. We used a multiplexed Tandem Mass Tag (TMT10plex)-based approach to study the proteomic alterations resulting from the addition of 1μM nicotine for 24h in four common cell lines: HEK, HeLa, PaSC, and SH-SY5Y. The four cell types (with PaSC in duplicate) were propagated and designated cultures were mock treated or nicotine treated. Proteins were extracted via methanol-chloroform precipitation and digested with LysC and trypsin. The resulting peptides were labeled with TMT, pooled, and fractionated via basic pH reversed-phase high performance liquid chromatography (BPRP-HPLC) prior to SPS-MS3 analysis on an OrbitrapFusion mass spectrometer. In total, we quantified 8545 proteins across all 4 cell lines. A total of 435 non-redundant proteins demonstrated a fold change in relative abundance of 1.5 fold or greater upon treatment with nicotine. Of these, nicotine treatment resulted in 31 proteins having a 1.5-fold or greater increase in abundance in all cell lines. Considering proteins with altered abundance in at least 3 of the 4 cell lines, 64 were increased, while 2 were decreased. Gene ontology analysis revealed that ~40% of these proteins were membrane-bound, and the majority of functional categories included those with roles in transmembrane signaling and receptor activity. We highlighted proteins, including APP, APLP2, LAPTM4B, and NCOA4, which were altered by nicotine in all four cell lines investigated and may have implications in downstream signaling pathways, particularly autophagy. Using the outlined methodology, studies in other cell lines will provide further evidence that alterations in the abundance of these proteins are a general response to nicotine treatment and merit further investigation.

Poster 038: A System Suitability Monitoring Method for LC MS/MS Proteomic Experiments

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Statistical process control (SPC) is a well-established method of quality control (QC) which is applied to monitor and improve the quality of a process. Our method uses SPC tools to monitor LC-MS/MS process performance by tracking system suitability metrics including peak area, retention time, full width at half maximum (FWHM) and full width at base (FWB). This method considers monitoring the mean and dispersion simultaneously for each suitability metric and significantly improves the ability to detect special causes of variation earlier, therefore, reduces cost of control and cost of failure. Variation in LC-MS/MS process performance can occur in various types such as large shifts or slow drifts in process mean and variation. Our approach introduces alternative methods of monitoring such as time weighted control charts to ensure that various types of process disturbances are detected effectively. Simultaneous control charts used in this framework can be classified into two groups: individual-moving range (XmR) control charts and mean and dispersion cumulative sum (CUSUM) control charts. Experiment specific control limits are provided with the control charts to distinguish between random noise and systematic error. These control charts are illustrated in several case studies: case of isolated outlier QC measurements, sustained step shifts of process mean and variation and slow linear drifts of process mean. Case study results include comparisons for the widely used Levey-Jennings plots and indicate that our approach significantly improves the detection performance. The method introduced here can be applied in a diverse range of QC metrics for system suitability analysis. Proposed monitoring method is implemented in open source software.

Poster 039: A Novel and Robust Measure of Protein Co-Localization for Super-Resolution Fluorescence Microscopy Images

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The development of super-resolution microscopy techniques has enabled the study of biological samples at the sub-diffraction level, opening up the possibility to observe proteins and their interactions as never before. An important area of interest is protein co-localization, since the presence of co-localization between clusters of two or more proteins in an image can indicate possible interaction between these proteins. However, there exist few methods that focus on measuring and quantifying co-localization of protein clusters, especially methods specifically designed for super-resolution images. Furthermore, many of these methods lack the ability to determine whether the measured co-localization is merely due to random placement of clusters and fail to distinguish between varying degrees of co-localization measured in comparable images, for example, for different conditions in an experiment.

We have developed a novel algorithm which makes use of Monte Carlo Simulations to predict the “attraction factor” of an image of potentially interacting proteins. As input the algorithm will take a segmented image or images and run simulations based on the identified protein

clusters. Not only will the algorithm compare the image to a random distribution, but it will also simulate distributions with increasing "attraction" between the proteins. We use these results to produce a measure, termed an "attraction factor", which represents the amount of co-localization found in an image. This measure can be compared among images, allowing for a non-biased quantification of potential protein interaction. The algorithm has been validated with both simulated and experimental data, and a detailed analysis of images with different levels of cluster density has been performed.

Both an ImageJ plugin and python scripts have been developed based on this method and will be made freely available for public use.

Poster 040: Parameterization of Averagine Composition Improved Feature Detection of Oligonucleotides

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During the pursuit of a computational platform to streamline the discovery of modification on oligonucleotides we discovered that the feature selection algorithm present in OpenMS does not perform well on nucleotides. After some research we determined that the cause of these issues was assumptions made about the isotopic distribution of the molecules based on a standard averagine model. In determining the theoretical isotopic distribution of a molecule at a given mass feature dectection algorithms typically calculate an the atomic composition of the molecule based on the atomic formula of the average amino acid or "averagine". We wrote code allowing the user to specify the atomic composition of the averagine, which corrected the expected isotopic distribution and substantially improved feature identification.

Feature detection is an important step toward the identification of molecules of interest, as it decreases the amount of data that later detection and assignment steps must incorporate in order to provide the user with proper identification. This decreases false positives and decreases the time required for analysis.

We see the improvements that we made as being of benefit not only for work on nucleotides, but for feature selection data from any experiment where the isotopic distribution of the experimental material differs from the standard averagine model, such as for histone samples, which contain a statistically different amino acid frequency than the whole proteome.

Poster 041: Relative Protein Quantification in Mass Spectrometry-based Proteomics: A Split Plot Approach

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As the proteomics field grows, quantitative mass spectrometry (MS)-based proteomics workflows are becoming more complex and diverse. The accuracy and the throughput of the MS measurements and of the signal processing tools dramatically increased. However, many existing statistical tools and workflows have not followed the technological development. Therefore, there is a need for flexible statistical tools, which reflect diverse and complex workflows, are computationally efficient for large datasets, and maximize the reproducibility of the results.

We propose a general statistical analysis framework with a family of linear mixed effects models, and a split-plot view of the experimental design, that represent measurements from quantitative mass spectrometry-based proteomics. The whole plot part of the design reflects the structure of the biological variation of the experiment, such as case-control design, paired design, or time-course design. The subplot part of the design reflects the structure of the technological variation, such as fragmentation patterns, labeling strategy, and presence of multiple peptides per protein. We propose an estimation procedure that separately estimates the parameters of the subplot considering the censored peak intensities and outliers and the whole plot parts of the design, to maximize the flexibility of the model, increase the speed of the analysis, and facilitate the interpretation. The proposed modeling framework was validated using 10 controlled mixtures and 10 experimental datasets from targeted selected reaction monitoring (SRM), Data-Dependent Acquisition (DDA or shotgun), and Data-Independent Acquisition (DIA or SWATH-MS), where signals were extracted with multiple signal processing tools. We implemented the proposed method in the software package MSstats v3.0 or later. It is interoperable with other existing computational tools such as MaxQuant and Skyline.

Poster 042: Decoding Histone Post-Translational Modifications by Bottom-Up Mass Spectrometry

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

In this study, we developed a program named EpiProfile to quantify all histone PTMs. The decoding workflow contains sample preparation, nanoflow LC-MS/MS, and data analysis. Sample organism includes human and mouse. Sample preparation includes histone extraction, the first propionylation, trypsin digestion, the second propionylation, and stable isotope labeling (e.g. SILAC, C13 glucose, or N15) if needed. Nanoflow LC-MS/MS can use different instruments (e.g. high-resolution or low-resolution MS), different fragmentation (e.g. collision-induced dissociation (CID), Higher-energy C-trap dissociation (HCD), or electron-transfer dissociation (ETD)), and different data acquisition (e.g. data-dependent acquisition (DDA) or data-independent acquisition (DIA)). Data analysis includes discriminating the mixture of isobaric peptides and determining the retention time of modified peptides for all histones H3, H4, H1, H2A, and H2B.

In the decoding workflow, we can use different MS and bioinformatics (peptide identification, label-free or labeling quantification, isobaric peptides discrimination). In conclusion, we developed EpiProfile to decode histone PTMs, including all PTMs and different types of MS.

Poster 043: Species Identification Using Bayesian Modeling and Mass Spectrometry

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Criminal investigations often hinge on the positive identification of remains. In many cases this can been done by a pathologist, or by using genetic techniques like short tandem repeat (STR) analysis. However, under certain circumstances, these methods are unviable. When samples are degraded either over time or chemically DNA may be irretrievable. Additionally, when there are too many samples STR analysis becomes financially unreasonable. We believe proteomic analysis using mass spectrometry can aide in moving these roadblocks to identification. We propose an algorithm that uses mass spectrometry data from an unknown sample and returns its likely taxonomic branch.

We are using NCBI's non-redundant database, which we have filtered to include only vertebrate proteins. The X! Tandem search algorithm is used to map the mass spectra to possible peptides and their proteins. Identified peptides are then counted toward species they may belong to. For each possible species a Bayesian model is built differentiating

peptides that map to the species and peptides that do not. We use the sum intensity to score to quantify each peptide. This process is repeated moving up the taxonomic tree from species to phylum. Each step up the tree incorporates more peptides, thus improving the likelihood or correct identification.

In conjunction with the New York City Office of the Chief Medical Examiner we have examined 84 samples of blood (10), bone (64), and tissue (10) originating from over 20 species. The samples were not obtained for experimental design and thus range in age and handling methods. Because of this the number of identified peptides per samples ranges from 65 to 473 for bone, 52 to 504 for blood, and 93 to 950 for tissue. The likelihood scores are dependent on the number of peptides identified, so similarly range with the quality of the sample.

Poster 044: Top-Down Proteomics Data Analysis

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Top-down mass spectrometry is maturing into an excellent method to analyze proteins for identification, sequencing, variant determination and characterization of post-translational modifications. However, the adoption of the technique has been hindered by the limited availability of readily accessible data interpretation tools. Here we describe the continued development of a web-based open-access search engine for top-down proteomics: BUPID Top-Down (Boston University Protein Identifier Top-Down). The software is designed for use as an automated pipeline to analyze spectra obtained with different top-down fragmentation methods including CID, ECD and ETD. The BUPID Top-Down software suite consists of several tools, each responsible for a different stage of data analysis, and has been integrated into a pipeline capable of using each tool individually or multiple tools without additional user intervention. By submitting spectra as raw profile mode mzML or deconvoluted peak lists, the data can be analyzed using the appropriate tools and the results will be returned. The results can either be viewed through the web interface or imported into R using a custom package designed to simplify further processing of the results. An overview of the software and representative results will be presented. The development of an open-access top-down data interpretation tool via a web interface will facilitate the penetration of top-down techniques in a greater number of mass spectrometry laboratories.

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Poster 045: HTAPP: High-Throughput Autonomous Proteomic Pipeline for Automated Acquisition and Insightful Analysis of MS/MS Data

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The development of a lab-based proteomic platform for the automated MS/MS data collection, database search, quantitative analysis, storage, and visualization of expansive datasets is critically important for the proteomics community. We have developed a flexible high-throughput proteomic platform for automated acquisition and insightful analysis of MS/MS data by integrating many bioinformatics tools: LC/MS acquisition control tool, MS/MS database search, peptide spectral validation, peptide quantitation, data exploration tool within a relational database, cached public protein information databases, GO ontology and protein network exploration tool. The LC/MS control tool integrates LIMS to provide unmonitored multi-dimensional sample analysis, as well as capture meta-data during analysis and associate them with sample preparation protocols and experiment results in a relational database. Instrument acquired raw data are streamlined to a customized interface for database searching followed by peptide validation. The logistic spectral score we developed for high-throughput statistical validation out performs both XCorr and the X!Tandem E-Value at a 1% false discovery rate estimated by decoy database approach. Peptide identifications, along with data-dependent calculation results are directed into a relational FileMaker/MySQL database for organization of expansive proteomic data sets, collation of proteomic data with available protein information resources, and visual comparison of multiple quantitative proteomic experiments. In addition, HTAPP offers to detect and quantify all kinds of peptide modifications. With some particular aspects, this platform provides flexible adaptation to diverse workflows for the unique requirements of the individual proteomics lab, enabling proteomic end-user to modify the presentation of the proteomic data, implement extra data-dependent analysis task, process additional input formats and control new types of instrument. The ultimate purpose of this system is to allow users focusing on extraction of biological meaning from vast data sets instead of routine data manipulation tasks.

Poster 046: Nonlinear Regression Avoids Overly Optimistic Assay Characterization

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The need for assay characterization is ubiquitous in quantitative mass spectrometry-based proteomics. While many assay characteristics can be defined and measured, the limit of detection (LoD) and limit of quantification (LoQ) are particularly useful figures of merit. In practice, these are determined by repeatedly measuring the intensity of samples with known analyte concentrations so as to build an intensity-concentration response (measured intensity vs spiked concentration). They, for instance, allow the comparison of competing quantitative mass spectrometric workflows, or the quantification of the sensitivity and reproducibility of a clinical research assay. As such, as of early 2016, over 1,300 articles indexed on PubMed report LOD/LOQ values. Many different methods have been proposed although methods based on linear regression are currently the most commonly used to calculate the LoD and LoQ. Their first step is to fit a linear regression to the intensity-concentration response (i.e. measured intensity vs known concentration) for every peptide. LoD and LoQ are then calculated based on the fit. Linear methods do not, however, provide accurate LoD/LoQ values when a noise threshold is present at low concentrations, which is a very common situation. In this poster, we thus illustrate the problems with current methods and then propose a new non-linear assay characterization method which correctly captures this threshold thus resulting in more accurate LoD/LoQ values. The performance of the proposed method is evaluated for an SRM and DIA dataset. Whenever substantial noise thresholds are present, large accuracy improvements (~20-40%) are observed when the new method is used. The latter furthermore corrects the consistent overestimation of the LoD/LoQ characteristic of the standard method.

Poster 047: ProteoModlR for Quantitative Proteomics Pathway Modeling

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High-accuracy mass spectrometry now enables near-comprehensive measurements of cellular proteomes. Existing computational proteomics methods provide advanced tools for mass spectral analysis and statistical inference, but lack integrated functions for quantitative downstream analysis of post-translationally modified proteins. Here, we present ProteoModlR, a program for quantitative analysis of relative and absolute abundance and stoichiometries of post-translational chemical modifications across temporal and steady-state biological states. ProteoModlR supports the analysis of labeled and label-free datasets, acquired in both data-dependent and data-independent modes. In particular, ProteoModlR deconvolves the contribution of chemical modifications of peptides to their mass spectrometry signals, thereby calculating both stoichiometries of post-translational modifications and protein abundances. Its modular design and interchangeable format are optimally suited for integration with existing tools, such as MaxQuant, Skyline, MSstats, and NetworKIN. We anticipate that ProteoModlR's computational framework to be useful for a wide variety of quantitative mass spectrometry studies, including the comprehensive investigation of cellular signaling (http://github.com/kentsisresearchgroup/ProteoModlR/).

POSTER ABSTRACTS

All posters will be attended by presenting authors on both Monday and Tuesday. All posters should be set up by 10:00 am on Monday morning and removed at 3:00 pm on Tuesday. **Odd**-numbered posters present **1:30 - 2:15 pm** and **even**-numbered posters **2:15 - 3:00 pm**.

Poster 048: Comparative Proteomics of Time-Course Activation of Eosinophils with Cytokines, Applied Singly and in Pairs, using Multiple Proteomic Platforms

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Activated eosinophils contribute to airway dysfunction and tissue remodeling in asthma and are considered an important factor in asthma pathology. We have previously reported the time-course proteomic changes accompanying eosinophil activation with these eight cytokines singly: IL-5, GM-CSF, IL-3, eotaxin-1, eotaxin-2, IL-4, IL-13, and RANTES. The results allowed us to delineate and compare the proteomic and phosphoproteomic profiles associated with each stimulus. To better mimic the physiologic situation where multiple cytokines simultaneously participate in eosinophil activation, we extended our studies to stimulations with pairs of cytokines, and compared the results to our earlier observations. We stimulated peripheral blood eosinophils from normal (non-asthmatic) subjects with IL-5+Eotaxin2 and GM-CSF+IL-3, and acquired time-course protein expression data using 2D-gel (2DE) and label-free LC-MS/MS platforms. In parallel, we performed stimulation with the above stimulants and monitored eosinophil activation by flow cytometry, using CD69 as the activation marker. Time-course phosphoproteomic changes were profiled by 2DE employing a phospho-specific dye. In all experiments, protein differential expression/phosphorylation was calculated by comparison with quiescent (unstimulated) eosinophils as the control. In the 2DE experiments, the differential spots were identified by MALDI-TOF/TOF MS. On comparing activation by the pairs to that by single cytokines, we observed some proteomic differences and a few pattern similarities: (1) There were 38 identified proteins with notable protein abundance/ phosphorylation fold-change differences between single and paired cytokines, (2) Hierarchical clustering analysis showed that paired cytokine data cluster separately from their single cytokine counterparts, both in the case of protein abundance and phosphorylation. The duration of stimulation was also seen to have a significant impact on clustering patterns, (3) However, the pathway analysis program IPA yielded substantially similar functional classifications and canonical pathways in the paired and single cytokine activations.

Poster 049: Protein-based PTM Quantitative Analysis with PEAKS Software

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Quantification of modifications on an interest of protein is one of the major concerns in the biopharmaceutical industry. For example, oxidation of methionine alters the physicochemical and functional properties of a monoclonal antibody (mAB). LC/MS technology is a powerful and sensitive technique for the identification of modifications on proteins. To facilitate downstream analysis of LC-MS data, in this work, we demonstrate how to use PEAKS software to quantitatively analyze the oxidation and deamidation sites of a mAB treated with hydrogen peroxide to simulate potential oxidative modifications.

Immunoglobulin G (IgG) was obtained from ProMab Biotechnologies, Inc. The monoclonal antibody samples were incubated with different concentrations of hydrogen peroxide. The treated samples were dried and subjected to trypsin digestion. The samples were analyzed by LC/MS/MS on AB Sciex 5600.

The LC-MS/MS data was analyzed using PEAKS database search with carbamidomethyl cysteine as a fixed modification, methionine oxidation, deamidation and pyro-glu as variable. Highly confident de novo tags were then further analyzed using PEAKS PTM. Annotated chromatogram was obtained with 1% of false discovery rate at peptide level. Relative percentage of oxidation for each site was estimated by the ratio of the sum of extracted ion chromatograms of Met-oxidized peptides to the total chromatogram of all Met-containing peptides. The study inferred that there were differences in susceptibility to oxidation for different peptides containing Met residues. DTLMISR (Met 252) was found to be more susceptible to oxidation than the peptide WQQGNVFSCSVMHEALHNHYTQK (Met 428). Relative percentage of oxidation on Met 252 increased from 2% to 28% after treatment with hydrogen peroxide, whereas remained same (10%) on Met 428. PTM search also reveals the elimination of CH3SOH (dethiomethyl) from the oxidized methionine of the peptide DTLMISR.

Quantification of deamidations of the interest of proteins showed little changes between treated and control samples.

Poster 050: The Scaling Complexity of Glycoproteomics Samples

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The glycoproteome, proteins and the combinations of glycans that are attached to them, are substantially more complex than the proteome with the addition of small mass post-translational modifications (PTMs). As a glycoproteome becomes more complex, more information is necessary to accurately identify each observed glycopeptide. We studied the effect of increasing glycoproteome complexity on our ability to accurately recover glycopeptide identifications.

We used as an example human α1-acid glycoprotein (AGP). AGP contains 5 N-glycosylation sequons and two protein isoform variants. As such, it represents a moderately complex glycoprotein. We acquired data dependent LC-tandem mass spectrometric data on tryptic digests of AGP and assigned site specific glycopeptide glycosylation assignments for AGP using an assumption that the protein was pure. We the acquire data dependent proteomics data that showed that the supposedly pure AGP standard contained contaminating serum glycoproteins including haptoglobin. The assumption that the AGP protein was pure therefore resulted in inaccurate interpretation of the glycopeptide tandem mass spectrometric data. We then constructed a series of samples of increasing complexity by mixing AGP with a set of purified serum glycoproteins. In order to evaluate a case of extreme sample complexity, we added AGP to a blood serum sample. Compared the ability to assign AGP site specific glycosylation for the set of samples using (1) an assumption that AGP was the only glycoprotein present, (2) an assumption that all glycoproteins detected in proteomics data on the samples were present, and (3) an assumption that only peptide backbones detected in deglycosylated proteomics data were present. Our data demonstrate that the ability to accurately characterize AGP in complex samples is best when using prior knowledge of the sample proteome. The greatest informatics power is achieved when the peptide backbones present in the glycopeptide sample have been determined from de-glycosylated proteomics data.

Poster 051: Integrative Systems Biology Approach to Identify Mechanisms of Action

Akos Vertes[1]; Andrew Korte[1]; Camille Lombard-Banek[1]; Peter Nemes[1]; Lida Parvin[1]; Ziad Sahab[1]; Bindesh Shrestha[1]; Sylwia Stopka[1]; Wei Yuan[1]; Deborah Bunin[2]; Merrill Knapp[2]; Ian Mason[2]; Denise Nishita[2]; Andrew Poggio[2]; Carolyn Talcott[2]; Maneesh Yadav[2]; Brian Davis[3]; Adriana Larriera[3]; Christine Morton[3]; Christopher Sevinsky[3]; Maria Zavodszky[3]; Nicholas Morris[4]; Heather Anderson[4]; Matthew Powell[4]; Trust Razunguzwa[4]

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Pandemics caused by toxins and emerging/re-emerging pathogens can have catastrophic global health consequences. The objective reconstruction of the mechanism of action (MoA) for an unknown toxin requires abandoning targeted analysis and minimizing inferences that might give rise to subjective data collection and interpretation. Furthermore, the exponential progression of harm caused by pandemics requires the timely characterization of the threat agent at its earliest onset. This challenge is best addressed by developing high-throughput comparative analytical methodologies to capture the system-wide changes caused by exposure to the substance. Here we describe the acquisition and monitoring of molecular changes in HepG2-C3A hepatocytes treated with forskolin using high-throughput proteomics, micro-array based transcriptomics, and untargeted metabolomics. The comprehensive analysis compared the quantitative levels of 67,528 transcripts and 3,531 protein groups at several time-

point between 2 min and 48 h following forskolin exposure. Furthermore, untargeted metabolomics analysis was performed by laser ablation electrospray ionization (LAESI) mass spectrometry (MS) in combination with ion mobility separation, and high resolution MS using laser desorption ionization from nanopost arrays (NAPA). Antibody-based assays and qRT-PCR were implemented to confirm the proteomics and transcriptomics data respectively. Statistically-significant changes in quantified transcripts, proteins, and metabolites were combined and utilized to perform knowledge-based pathway analysis to reconstruct the MoA of forskolin known for its stimulation of the cAMP signaling pathway. This analysis identified forskolin as the upstream regulator and cAMP-mediated signaling was identified among the top canonical pathways. Events downstream from the cAMP-mediated signaling were discovered by de novo Bayesian network inference. Finally, the MoA was validated by conventional phenotypic and intracellular signaling assays. The entire process was completed in less than 30 days.

Poster 052: Differential Dynamics of the Mammalian mRNA and Protein Expression Response to Misfolding Stress

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The relative importance of regulation at the mRNA versus protein level is subject to ongoing debate. To address this question in a dynamic system, we mapped the proteomics and transcriptomics changes in mammalian cells responding to stress induced by dithiothreitol over 30 hours. Specifically, we estimated the kinetic parameters for synthesis and degradation of RNA and proteins, and deconvoluted response patterns common and unique to each regulatory level using a new statistical tool. Overall, both regulatory levels were equally important, but differed in their impact on molecule concentrations. Both mRNA and protein changes peaked between two and eight hours, but mRNA expression fold changes were much smaller than those of the proteins. Further, mRNA concentrations were regulated in a transient, spike-like pattern and returned to values close to pre-treatment levels by the end of the experiment. In contrast, protein concentrations switched only once and established a new steady state, consistent with the dominant role of protein regulation during misfolding stress. Finally, we generated hypotheses on specific regulatory modes for example groups of genes.

Poster 053: Automated, High-throughput Hemoglobinopathies Profiling using Top-Down LC-MS Methods

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Hemoglobinopathies consists of profiling hemoglobin chains for sequence determination and in some cases, relative quantification. Accurate profiling requires intact protein analysis to identify variants as well as localize point mutation. In addition, the analysis must be automated and high-throughput to meet the typical sample demand. Thus we have developed a routine incorporating sample preparation, MS and MS/MS acquisition, and automated data processing to determine the molecular weight (MW) and sequencing for all possible chains. Molecular weight filtering is performed using a 96-well cation exchange plate and each sample is directly loaded onto a size exclusion column (SEC) for a 2 minute data acquisition and 6 minute total injection cycle method. Both MS and tandem MS data is acquired using HR/AM on an Orbitrap-based mass spectrometer (Fusion and Q Exactive Focus) and the effects of resolution are compared for accurate precursor and product ion sequence determination. To increase sequence coverage, various DIA strategies are used (all ion fragmentation vs. standard DIA) and evaluated on diluted whole blood as well as human blood spiked with various amounts of bovine hemoglobin. Presented results show accurate determination of all hemoglobin chains as well as minor forms resulting from truncation and modification over a 20-fold spiked range. Product ion sequence coverage is shown to increase through extending the precursor m/z range sampled during the interspersed DIA routine. Automated data processing utilizes high resolution to determine MW profiles based on isotopic spacing and accurate mass analysis as compared against known sequence libraries and compared to processing strategies which first deconvolute and then match against known sequences. Reproducibility and robustness was evaluated using 25 biological replicates for 7 different samples covering the spiked range of neat to 20:1 between human and bovine hemoglobin samples.

Poster 054: High Quantification Accuracy in Label-Free Proteomics

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In order to understand the dynamic of the proteome it is important to not only list number of proteins identified, but to decipher differences between samples using quantitative proteomics.

Complexity and concentration range, however, pose a great challenge to the MS instrumentation in terms of sensitivity, resolution and dynamic range. This can be handled by up-to-date mass spectrometer in combination with sophisticated software solutions.

We present here the excellent capabilities of a modern Q-TOF instrument (impact II, Bruker Daltonics) for quantitative proteomics, focusing on label-free quantitation.

Different complex tryptic reference digests, which are commercially available (UPS, Yeast, human cell line) were analyzed with nano-flow UHPLC and a CaptiveSpray ion source connected to the impact II.

For data processing the MaxQuant software package was used (Nature Biotechnology 26, 1367 - 1372 (2008)). Obtained data clearly shows very accurate quantitation over four orders of magnitude of UPS2 proteins spiked in complex yeast matrix, detecting a ratio of 0.49 (± 0.06), which is extremely close to the theoretical ratio of 0.5.

Furthermore results show high reproducibility between replicates, being a prerequisite for accurate quantitation. Additional data underlying the quantitative benefits of Q-TOF instruments originating from the high sequencing speed and from the fact that resolution is independent of the scan speed, will be shown for samples consisting of mixture of two proteomes.

Poster 055: HDL Dysfunction in Patients with NASH is Related to Alteration of HDL Proteome Composition

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Oxidative stress and inflammation play central role in the pathogenesis of atherosclerosis and NASH. HDL protects against CVD through reverse cholesterol transport, anti-oxidant and anti-inflammatory functions. Inflammation and oxidative stress may cause HDL dysfunction through alteration of HDL proteome composition. We hypothesize *that oxidative modification of key HDL proteins and pro-inflammatory alteration of HDL composition leads to HDL dysfunction in NASH.*

HDL from patients NASH and healthy controls (n=9/group) was isolated and HDL proteome composition was analyzed by mass spectrometer. The relative post-translational modification was quantified in the MRM mode. The MPO activity and HDL's inflammatory index were quantified. Patients with NASH had higher BMI, HOMA-IR, plasma AST, ALT, triglycerides, and impaired cholesterol efflux capacity. NASH patients had increased MPO activity and that HDL_{NASH} is pro-inflammatory. We identified 72 HDL proteins and determined that proteins involved in the acute phase response (serum amyloid amylase, hemopexin), the complement factor B and C5) were increased, while the anti-oxidant proteins (apoE, PON1) were decreased in NASH. HDL_{NASH} was significantly enriched with Tyr-18 and Tyr-29 nitrated apoAI, the major protein of HDL. MPO activity was positively associated with the pro-inflammatory index of HDL (r=0.52,

p=0.004) and nitration of apoAI (r=0.54, p=0.003). In contrast, cholesterol efflux capacity of HDL was inversely correlated with the MPO activity (r=-0.37, p=0.01) and nitration of apoAI (r=-0.41, p=0.01). Our *in vivo* HDL proteome dynamics study demonstrated that the changes in the HDL proteins were associated with their altered turnover rates.

Thus, HDL dysfunction in NASH is related to the alteration of HDL composition, including MPO-induced modification of apoAI that leads to increased degradation of this protein. Pro-inflammatory-shift in HDL proteome composition may contrubute to CVD related moratlity in NAFLD.

Poster 057: Proteomic Profile of Dentate Gyrus of an Epilepsy Model Induced by Electrical Stimulation and Displaying Classical Hippocampal Sclerosis

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Purpose. Proteomic analysis is a promising tool for the identification of key biological processes leading to epilepsy. However, the power of such "omic" approach is dependent on the preparation of homogeneous cell populations. In this context, laser-capture microdissection presents the ability to select specific cell populations that would give the most informative data in proteomic studies.

The aim of this study is to identify differentially expressed proteins in the dorsal and ventral Dentate Gyrus (dDG and vDG) from epileptic rats. In these animals epilepsy was induced by a perforant pathway stimulation protocol which leads to classical hippocampal sclerosis, similar to what is seem in patients with mesial temporal epilepsy.

Methods. Rats were induced as described by Norwood et al., 2010. Frozen sections were prepared and the dDG and vDG were laser microdissected (Zeiss PALM). Total proteins were obtained from using 8M urea and analyzed by LC-MS/MS using an LQT-Orbitrap (Waters) and the quantitative data were obtained using the software Scaffold 4.0, using both Average TIC and Spectral Counting methods.

Results. We have identified a total of 1271 proteins in samples of dDG and vDG combined. Of these, 60 proteins were identified as differentially expressed in dDG and 62 in vDG. Although there was some overlap between proteins that were differentially expressed in dDG and vDG, we have found that approximately 75% of proteins differentially expressed in dDG and 80% in vDG were unique to these sub-fields. Most of the differentially expressed proteins are involved in neuronal pathways such as neurofilaments remodeling, and in the oxidative phosphorylation, as indicated by gene ontology analysis employing the Metacore® software (Thomson Reuters).

Conclusion. The proteins identified in the present study can indicate new pathways involved in epileptogenesis. Furthermore, we have found that additional molecular complexities could be elicited as hippocampal subfields were analyzed separately.

Poster 058: Cerebral Organoid Proteomics to Study Central Nervous System Development in Down Syndrome

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To better understand the cellular and molecular processes associated with Down syndrome (DS) in the human central nervous system (CNS), we generated a model of early neuronal development using human induced pluripotent stem cells (IPSc) as a starting template. We obtained IPSc from an individual with Down syndrome and a line from an unrelated euploid individual. With some modifications to the method first described by Lancaster et al (Nature, 2013), we successfully generated human cerebral organoids from both cell lines and used them for imaging experiments with whole-mount immunostaining and deep proteome profiling with label-free quantitation of over 8,500 proteins in each sample. Our imaging analysis shows neurons populating the outer edges of the tissue, with neuronal progenitors restricted to inner regions of the tissue; a cell type distribution of radial migration and differentiation similar to human cortex development. Our proteomics analysis shows many proteins changing in significant abundance due to Trisomy 21, with alterations in members of Wnt and Notch signaling pathways, catecholamine metabolism, axon guidance, and cell adhesion. A following experiment collected samples from each stage in organoid development: a.) IPSc growing in 2-dimensional standard maintenance culture, b.) embryoid bodies grown in suspension, 3.) neurospheres with fate-restricted neural progenitor populations and radial neuroectoderm, and 4.) organoids grown following embedding in extracellular matrix, cultured in suspension for 21 days. Analysis of confocal images taken from representative samples and sequential deep proteome profiles allows us to identify changes in protein abundances and functional pathway enrichments over an in vitro developmental trajectory that both supports previous molecular findings relevant to Down syndrome neurobiology as well as provides new directions for investigating potential therapeutic interventions. These data are the first to interrogate cerebral organoids with proteomic approaches in the study of complex genetic conditions with a spectrum of neurological phenotypes.

Poster 059: A High-resolution Anatomical Mouse Brain Proteome

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Brain atlases like the Allen Brain Atlas, which provides mRNA expression information on murine brain anatomy at the single cell level, have increased our understanding of the brain's architecture. However, further proteomic validation of mRNA expression is necessary for deeper insight into cellular function. Here we describe a mouse brain protein atlas that covers 17 surgically distinctive neuroanatomical regions of the adult mouse brain. The protein distribution is provided for 5,000 to 7,000 gene protein products from each region and over 12,000 gene products for the entire brain, documenting the physiological repertoire of mouse brain proteins in an anatomically comprehensive manner. We explored the utility of our protein atlas in a mouse model of Parkinson's disease (PD). We compared the proteome from the vulnerable region (Substantia nigra pars compacta, SNc) of wild type and Parkinsonian mice and found ~90 proteins with significantly altered abundance, revealing potential new pathways for future studies in PD. This protein-based atlas is a valuable resource and offers a practical framework for investigating the molecular intricacies of normal brain function as well as regional vulnerability in neurological diseases.

Poster 060: A Strategy to Study the Mitochondrial Proteome from Genetically Defined Cell Classes in the Nervous System

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Mitochondrial proteomes isolated from different tissues can vary with profound functional consequences. Tissues can be further deconstructed into their cellular components, but mitochondria have not been systematically studied in a cell-specific fashion. In the context of the brain, where cellular diversity is extremely complex, this raises many important questions. As a relatively simple example, how do mitochondria differ between glia and neurons? Going deeper, how do mitochondria vary with the function of specific classes of neurons based on their unique metabolic needs? Here, we describe a method to immunopurify mitochondria from specific cell types. This method allows us to study mitochondrial proteome *in vivo* from genetically defined cell classes in the nervous system.

Poster 061: Identifying Host Factors Associated with Replicating Viral DNA

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As obligate intracellular parasites, viruses must promote a permissive cellular environment by manipulating host factors that may facilitate or hamper their replication. Most DNA viruses, including Adenovirus

serotype-5 (Ad5) and Herpes Simplex Virus type-1 (HSV-1), replicate in the nucleus of infected cells, where their genomes associate with host factors that function in different nuclear processes. Early viral proteins redirect these host factors to facilitate viral replication and evade innate cellular defenses. Poxviruses, such as Vaccinia virus (VACV), are unique among DNA viruses as they establish viral factories in the cytoplasm, suggesting that their genome may interact with a different set of host proteins. Here, we profiled the proteome associated with nascent viral DNA of Ad5, HSV-1 and VACV using Isolation of Proteins on Nascent DNA (iPOND) coupled to Mass Spectrometry (MS). Using different bioinformatics tools, we first compared protein profiles from each virus to that of the host, and identified host factors similarly or differentially enriched between each virus and host nascent genomes. We then compared these differentially enriched host factors across viruses, and identified commonalities and differences in the cellular proteins associated with replicating viral genomes in nuclear or cytoplasmic viral domains. Using fluorescence microscopy, we confirmed association of host factors with viral replication compartments during active infection. Interestingly, we found nuclear proteins enriched on the replicating genomes of all three viruses, including the cytoplasmic-replicating VACV. However, a large group of nucleolar proteins were strongly enriched on Ad5 and HSV-1, but depleted from VACV nascent DNA. We hypothesize that nuclear factors play a key role in the infectious process for all DNA viruses, including those replicating in the cytoplasm. Uncovering cellular factors associated with nascent viral DNA sheds light on the mechanism of virus replication and facilitates our understanding of host-pathogens interactions.

Poster 062: Identification of Missing MHC Class I HIV Epitopes

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The MCH class-I presentation of HIV peptides is the first required step essential for immune recognition and killing of HIV infected cells by cytotoxic T cells (CTL). However, our knowledge of HIV HLA class I-bound peptides naturally presented by HIV-infected cells remains limited. Our recent work lead to unbiased MS-based identification of HIV peptides sequenced directly from the surface of human primary live cells. Identified HIV peptides were derived from expected and distinctive areas of primarily HIV-1 Gag and Pol and were of characteristic and atypical length compared to HIV-1 LANL epitopes identified by peptide screen of CTL responses in HIV+ patients' PBMC. Importantly, 75% previously unreported HIV-1 epitopes were found efficiently presented by HLA molecules and exhibited substantial immunoreactivity in HIV-1 infected donors thus, revealing novel T cell responses. However, known immunodominant HIV-1 epitopes expected to bind to specific HLA class-I molecules expressed by infected primary cells were not identified. Thus we set out to identify "missing" low-abundance HIV epitopes presented specifically by HLA-A02 after transfection of HIV in 293T cells. HLA-A02/epitope complexes were isolated from the membrane of HIV-transfected 293T cells by immunoprecipitation and peptides were purified by acid elution. Eluted HLA-A02 HIV peptides were analyzed by targeted MS2 and MS3 strategy pre-optimized using selected reaction monitoring for identification of "missing" low-abundance epitopes. Well-described immunodominant HIV-1 Nef-derived VL10-VLEWRFDSRL and Gag p15-derived FK10-FLGKIWPSHK HLA-A*02 epitopes were successfully identified. Moreover, targeted approach confirmed HLA-A*02 presentation of novel HIV-1 Gag p24-derived epitope EY-11-EPFRDYVDRFY and non-canonical Gag p15-derived KF13-KIWPSHKGRPGNF epitope revealed by the untargeted approach. In summary, parallel targeted and non-targeted MS-based strategies to identify the MHC-bound peptidome holds great promise to reveal the full repertoire of HIV peptides presented by HIV infected cells, which could lead to the identification of additional novel T cell responses of particular interest for immunogen design.

Poster 063: Plasmodium Digestomics: Endogenously Generated Peptides within the Infected Erythrocyte Link Hemoglobin Catabolism To Drug Resistance in the Malaria Parasite

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We recently reported an unexpected connection between chloroquine resistance in the malaria parasite, Plasmodium falciparum, and the accumulation of small peptides within the parasite. These seemingly unrelated phenomena occur when parasites carry drug resistant forms of the vacuolar chloroquine resistance transporter (PfCRT). Despite intensive research, little is known about the native function of PfCRT or why mutations in this protein would affect the levels of endogenous peptides.

We employed high-resolution peptidomics to analyze a comprehensive panel of transgenic parasites carrying genetically modified PfCRT proteins. All endogenous peptides found in parasite extracts were mapped to the human and malaria proteomes and the qualitative and quantitative changes in the global peptide profiles were associated with genetic changes in the parasite.

We identified over 500 naturally-occurring peptides ranging in size from 2- to 32-mers. Most of these peptides mapped to α and β hemoglobin in clusters of overlapping sequences. Breaks in these sequences corresponded to established protease (plasmepsin and falcipain) cleavage sites and the sub-cleavage of the peptides was consistent with the action of known peptidases. We interpret these data as a comprehensive phenotype of the complete hemoglobin digestion pathway and mapped qualitative and quantitative perturbations in this pathway to genetic changes in PfCRT. Using this strategy, we showed that drug resistance, impaired hemoglobin metabolism, and parasite fitness are closely related phenomena and that several mutations in the PfCRT sequence can have a significant impact on this metabolic pathway.

These data provide a molecular explanation for the resistance-associated accumulation of peptides and show that PfCRT plays a direct role in vacuolar metabolism. The fitness costs of disrupted hemoglobin metabolism may explain why drug resistant parasites disappear from wild populations following the cessation of chloroquine treatment.

Poster 064: Trypanosome Chronic Infection: Combined Post-translational Analysis Suggests Causes for Chronic Infection

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Trypanosome cruzi (Tc) has emerged as the third most prevalent tropical disease world-wide, with > 10 million infected individuals and more than 300,000 patients in the US. Therapy against the disease is most effective early in the infection, but is difficult and can be toxic subsequently. Moreover, the disease can become chronic, with decades of infection that has a high probability of resulting in severe cardiomyopathy. Both parasite- and host-specific factors lead to the cardiopathology, with the parasite evading immune clearance, and the host response contributing to the cardiopathology as a result of chronic infection of heart tissue. In the latter case, although displaying some degree of activation, macrophage molecular responses to parasitic infection, including the generation of reactive nitrogen species, seem unable to clear the pathogen. To dissect the molecular events that lead to chronic infection, we focused on protein modification: through redox signal pathways involving protein cysteinyl-S-nitrosylation (SNO), and through phosphorylation signal pathways. While in some notable examples these two post-translational modifications display an inverse relationship, this may not be the case in pathogen clearance-host response pathways. To investigate this further, we combined our SNO by fluorescence technology with a phospho-specific dye in a mouse infection model to globally and specifically quantify protein and PTM changes in Tc infected, M1 (proinflammatory), and M2 (immunomodulatory) macrophages, and normal and infected

cardiomyocyte mitochondria. In this approach we are able to gauge the global cellular response in terms of protein SNO and phosphorylation, as well as specific protein PTM status within the same experimental analysis. We describe the major pathways affected and propose possible mechanisms that may lead to chronic trypanosome infection. The overarching aim is to discover potential therapeutic targets that may relieve the parasite burden in Chagasic patients and prevent the ensuing life-threatening cardiomyopathy.

Poster 065: Antioxidant and Anti-Inflammatory Properties of Sugarcane Fibre

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Chronic inflammation involves the dysregulation in the synthesis of pro-inflammatory mediators which are associated with several diseases including autoimmune diseases, diabetes and cancer. Some natural plant products are known to possess anti-inflammatory properties based on their high content of phytochemicals. In this study, we demonstrate that sugarcane dietary fibre (SCF) is a potent source of phytochemicals presenting more than two-fold polyphenols, flavonoids and antioxidants compared with raisins and cranberry juice. In vitro studies performed in a cellular model of intestinal inflammation using LPS-stimulated SW480 and HepG2 cells show that extracts from SCF supresses the phosphorylation of transcription factor NF-kB, and the protein kinase Akt as has been shown for the well-known polyphenol, resveratrol. Mass spectrometry based phosphoproteomic analysis is being used to uncover other modes of action to explain the anti-inflammatory events associated with SCF extracts. Preliminary data shows profound changes in the phosphorylation of proteins including the inhibition of protein kinase C and sirtuin 1. Our findings suggest that sugarcane fibre is a valuable source of antioxidants with potential to impart health benefits associated with inflammatory-related conditions.

Poster 066: Detecting Cysteine Modifications in Methanogen Methanosarcina Mazei Gö1

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Archaea in genus Methanosarcina are distributed broadly from marine to fresh water environments. They produce methane from a wide range of substrates including acetate, methylamines, and methanol and account for a large percentage of global methane emission. In methanogenesis, several important steps rely on thiol intermediates; e.g., methyl transfer from tetrahydrosarcinopterin (H4SPT) to coenzyme M (mercaptoethanesulfonate), methane release by oxidation of coenzyme M and coenzyme B to form a heterodimer, and recycling of coenzymes M and B after reduction by heterodisulfide reductase. The importance of thiols to methanogenesis encouraged us to explore cysteine modifications in Methanosarcina mazei.

Tryptic peptides were generated with and without reduction/alkylation from cell lysates of Methanosarcina cultivated on methanol and on other carbon substrates. Peptides were analyzed by LC-MS/MS to identify proteins and to inventory post-translational modifications. Among the most abundant modifications observed was cysteinylation (Cys+119), identified on over 40 of proteins. Protein cysteinylation was observed not only from cultures maintaining reducing conditions with Na2S/cysteine addition, but also from those supplementing with Na2S only. Other modifications detected included Cys+30 (trisulfide in multi-cysteine peptides), Cys+140, Cys+151, and Cys+152. Modified cysteines appeared in active sites of some metabolic enzymes. The significance of these modifications is being explored.

Poster 067: Phosphoproteomic Analysis of *in vivo* Cdc14 Phosphatase Substrate Specificity by SWATH-MS

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Accurate transmission of genetic information during cell division is achieved by a core set of basic cellular machinery that coordinates the ordered series of events known as the cell cycle. Central to the eukaryotic cell cycle are the periodic phosphorylation of proteins by cyclin-dependent kinases (Cdks) to initiate mitosis and the inactivation of Cdk to complete mitosis. In addition to inactivation, Cdk substrates must be dephosphorylated in a regulated pattern to coordinate late mitotic events like chromosome segregation and cytokinesis. In budding yeast, the highly conserved protein phosphatase Cdc14 is required for inactivation of Cdk and is thought to directly dephosphorylate Cdk substrates. But how Cdc14 coordinates the order of mitotic exit events remains poorly understood. In vitro, Cdc14 has a strong preference for a subset of Cdk-type sites containing the consensus sequence pSer-Pro-x-Lys, suggesting that its intrinsic specificity could contribute to the order of Cdk substrate dephosphorylation. However, the physiological significance of this enzymatic specificity remains unclear. Therefore, a label-free SWATH-MS approach was used to characterize the specificity of Cdc14 in vivo and test if it is capable of effecting ordered dephosphorylation of Cdk substrates. The phosphoproteome of mitotically arrested cultures was quantitatively monitored over time following CDC14 induction. 2312 phosphopeptides were identified and quantified. Of these, only 171 phosphosites on 114 proteins (containing 8 previously characterized Cdc14 substrates) were rapidly dephosphorylated in response to Cdc14 expression compared to the uninduced control. The sequences around the rapidly dephosphorylated sites were strikingly consistent with optimal Cdc14 substrate sites defined previously in vitro. We have established SWATH-MS as an effective tool to globally profile phosphatase or kinase specificities in vivo and provided evidence that the intrinsic specificity of the cell cycle phosphatase Cdc14 contributes to the ordered dephosphorylation of Cdk substrates that is a fundamental requirement for completion of eukaryotic mitosis.

Poster 068: A Novel Tandem Isobaric Tag (QUANTITY) for Quantitative Analysis of N-Glycans

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Glycan is an important class of macromolecules that play numerous biological roles in biological processes and their abnormality could be associated with various diseases. Understanding glycan functions is of great significance in academic research, pharmaceutical industry and healthcare. However, quantitative glycomics - analysis of glycans at global level is far behind genomics and proteomics owing to technical challenges associated with their chemical properties and structural complexity. As a result, technologies that can facilitate global glycan analysis are highly sought after.

To assist structural analysis, glycans are usually derivatized for quantitative analysis on various analytical platforms, e.g., fluorescent detection, chromatography, and mass spectrometry (MS). MS has become one of the most popular tools for glycan analysis mainly because of its ability to determine glycan compositions, as well as relative abundance via isobaric tag. As of today, there are very few successes on development of isobaric tags for glycan quantification. The existing isobaric tags were based on a tertiary amine structure that was originally designed for peptide quantification and fragments less favorably than glycosidic bonds in MS/MS. They are unable to generate reporter ions strong enough for accurate quantification of labeled glycans, not to mention characterization on high molecular weight glycans.

Here, we present **QUANTITY** (**Qu**aternary **A**mi**n**e Con**t**aining **I**sobaric **T**ag for Gl**y**can), A quantitative approach that can not only enhance detection of glycans by mass spectrometry, but also allow high-throughput glycomic analysis from multiple biological samples. This robust tool enabled us for the first time to accomplish glycomic survey of bioengineered Chinese Hamster Ovary (CHO) cells with knock-in/out enzymes involved in protein glycosylation. We further applied the tags for analysis of N-glycosylation in glycol-engineered Erythropoietin (EPO) that is expressed in CHO cell lines. Our results demonstrated QUANTITY is an invaluable technique for N-glycan analysis, bioengineering and glycosylation design in pharmaceutically used glycoproteins.

All posters will be attended by presenting authors on both Monday and Tuesday. All posters should be set up by 10:00 am on Monday morning and removed at 3:00 pm on Tuesday. **Odd**-numbered posters present **1:30 - 2:15 pm** and **even**-numbered posters **2:15 - 3:00 pm**.

Poster 069: Identification of Mutant FMS-like Tyrosine Kinase 3 Substrates using KALIP

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FMS-like tyrosine kinase 3 (FLT3) is a class 3 type of membrane bound receptor which plays an integral role haematopoiesis, and alterations to this cohesive signaling machinery lead to haematopoietic malignancies. Point mutations occurring in the activation loop of the FLT3 tyrosine kinase domain and have been shown to confer resistance to type II FLT3 inhibitors. Computational modeling suggest that D835Y/V/I/F mutations alter the structure of the activation loop, leading to decreased binding of type II FLT3 inhibitor potency. Currently, few FLT3 substrates and their phosphorylation sites are known, which limits our insight of how FLT3 interacts with signaling pathways under disease conditions. The objective of this study is to identify and generate optimal FLT3-specific peptide substrates in a high-throughput manner. The "kinase assay linked with phosphoproteomics" (KALIP) technique allows the identification of proteins and sites that are phosphorylated by a kinase of interest. We will use KALIP to compare the phosphoproteomic profile of wild type and mutated FLT3-D85Y, in order to determine if and how mutations alter FLT3 substrate selectivity.

Poster 070: Human Myogenesis Regulated Kinase Signaling-Associated Chromatin Proteins and Histone Modifications

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Human myoblasts capable of differentiation into myotubes are powerful tools to study myogenesis. In this study, we performed a phosphoproteomics analysis of proliferating myoblasts versus differentiated myotubes using Stable Isotope Labeling by Aminoacids in Cell culture (SILAC) and mass spectrometry. We quantified regulated phosphopeptides and extracted significant phosphorylation motifs for the following kinases: ERK1/2, CDK1/2/4/5, Cdc2 and GSK3a/3b and used inhibitors to perturb differentiation: GW8510 affecting CDK1, 2 and 4 and Roscovitine targeting Cdc2, CDK2 and 5, PD325901 inhibiting MAPK and CHIR targeting GSK3. Myoblasts switched to low serum medium prematurely differentiated by day 2 in both PD325901 and GW8510, while differentiation was arrested in roscovitine and CHIR99021, as scored by morphology and MHC expression. We identified 7204 proteins of which 4618 were annotated in relation to muscle, revealing a considerable number of proteins that have not been studied in context of muscle. Interestingly, drugs accelerating differentiation (PD325901 and GW8510) had more downregulated proteins, drugs that arrested differentiation (roscovitine and CHIR99021) seemed to have more upregulated proteins. Using STRING, we demonstrate the links between signaling pathways and significant regulated kinase targets, histones and chromatin related proteins. Next, we performed histone PTM analysis by label-free quantitative mass spectrometry for drug-treated and control differentiating cells. Several activating and repressive marks were variously modulated when there is abnormal differentiation, established by dysregulated methyl transferases. We chose H3K4me3 and H4K20me3 to do ChIP-qPCR for myogenic genes MyoD1, MyoG and myf5 and correlated with their mRNA expression. These marks are completely complementary to gene activity. Taken together, this study demonstrates that key kinases in modulates chromatin remodeling proteins and epigenome in myoblasts, initiating differentiation program in human myogenesis.

Poster 071: Phosphoproteomics-based Network Analysis Unravels High-Fat Diet-Induced Deregulated Signalling Pathways in Mouse White Adipose Tissues

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Obesity is a complex metabolic disorder and its underlying molecular mechanisms remain enigmatic. To obtain an in-depth molecular perspective of the events that are altered upon obesity, we performed in vivo phosphoproteome profiling of white adipose tissues from mice fed a high-fat diet (HFD) or low-fat diet (LFD) using label-free quantitative mass spectrometry. We quantified 7696 phosphopeptides, of which 282 phosphosites on 191 proteins were differentially regulated in response to HFD. This data set uncovered an array of phosphorylation changes on several key enzymes involved in lipid and glucose homeostasis, implying that these events may have important functional consequences in adipocyte reprogramming during obesity or insulin resistance. Kinase predictions of altered phosphosites and in-depth network analysis of corresponding phosphoproteins revealed possibilities of deregulation of lipogenesis and lipolysis pathways, and transcriptional rewiring in HFD. Functional validations of selected phosphosites derived from key metabolic enzymes involved in those deregulated pathways are in progress. Altogether, our study provides a comprehensive map of adipose tissue phosphoproteome in mouse obesity models, and reveals several previously unknown phosphoproteins attributed to aberrant molecular pathways in obesity.

Poster 072: Efficient Enrichment of SUMOylated Peptides from Alpha-lytic Protease Digest Using K-Ɛ-GG Remnant Immuno-affinity Purification

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Small Ubiquitin-like Modifier (SUMO) proteins are a family of small proteins that are covalently attached to the lysine residue of their target proteins and modify their function. SUMOylation has been demonstrated being involved in various cellular processes including nuclear-cytosolic transportation, transcriptional regulation, apoptosis, protein stability, response to stress, and progression through cell cycles.

Compared to ubiquitination, much fewer SUMOylation sites were identified so far largely due to the lack of efficient enrichment method for sumoylation. Although SUMO proteins are similar in amino acid sequence as ubiquitin, the third amino acid in SUMO is threonine instead of arginine in ubiquitin when linked to the lysine residue of target protein, which prevents trypsin digestion to obtain a short remnant to be enriched and interpreted by LC-MS/MS analysis.

We have developed a robust enrichment method for SUMOylated peptides from whole cell lysate digested by alpha-lytic protease (WaLP) that preferentially cut at the C-terminus of T, S, A and V resulting a GlyGly remnant left on the lysine residue previously carrying SUMO-1 and SUMO-2/3. Digested peptides were subject to immuno-affinity purification using K-Ɛ-GG remnant antibody and LC-MS/MS analysis for identification and quantification of SUMOylation sites.

To verify the KGG sites identified by our method originated from SUMOylation, we performed in-vitro sumoylation removal from heat-shocked Hela cell lysate by specific sumo-proteases, which efficiently removed SUMO-1 and SUMO-2/3 while did not affect ubiquitination level. We then conducted a quantitative proteomic comparison between KGG sites between untreated and sumo-proteases treated lysates digested by WaLP and trypsin, respectively. Consistent with western-blot results, 612 out of 724 KGG sites identified in WaLP digest showed significant lower intensities in SUMO-proteases treated samples; while only 16 out of 9,031 KGG sites identified in trypsin digest showed significant intensity changes, which demonstrated the specificity and efficiency of our enrichment method.

Poster 073: Ubiquitinated Proteins in MDSC Exosomes

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Myeloid-derived suppressor cells (MDSC) accumulate in individuals with cancer where they facilitate tumor progression. These cells are immune-suppressive through several mechanisms including polarization of macrophages toward a tumor-promoting phenotype and inhibition of T cell activation. MDSC release exosomes, 30-100 nm extracellular vesicles, which we have shown to have chemotactic

activity towards MDSC and to polarize macrophages towards a tumor-promoting phenotype. The protein cargos of exosomes have received considerable attention because of their potential bioactivities, because they may reveal more about formation of exosomes and the endosome pathway, and because disposal of unwanted proteins is still considered a possible exosome function. MDSC exosomes have previously been shown by our research group to carry proteins with ubiquitination as a major post-translational modification (Burke et al, J. Prot. Res. 2014). In the continuing study reported here, ubiquitinated proteins have been targeted for identification using immunoprecipitation with two different antibodies against ubiquitin, in one case supplemented by immunoprecipitation of GG-peptides. This report considers nearly 150 ubiquitinated proteins identified in MDSC exosomes and compares their chemical and biological characteristics to those of the entire protein cohort identified in exosome lysate to look for biased distributions that suggest the role of ubiquitination in exosome cargo. LC-MS/MS analyses were made with an orbitrap fusion lumos mass spectrometer. PIR GO Slim cellular component annotations were used to evaluate functional assignments and cell locations referenced to parental MDSC. Comments will be offered on a possible role of ubiquitination of exosome protein cargo.

Poster 074: When Can Glycopeptides Be Assigned Based Solely on Tandem Mass Spectrometry Data?

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Effective glycoprotein analysis depends heavily on superior analytical and informatics power. The primary challenge in analyzing glycoproteins is the heterogeneity of this modification. With increase in the number of glycosylation sites, the sample and resulting data become increasingly complex. Advances in analytical methods, have enabled acquisition of good quality glycopeptidomics data. However, the tools to mine these data are still not mature.

Proteomics software allow error-tolerant and de novo searches to be performed which allow data mining beyond the expert user's ability and identification of unexpected modifications, mutations and artefacts from sample handling. These approaches can't be applied readily to glycoproteomics data due to the already complex search space resulting from glycan heterogeneity. In order to best explain glycoproteomics data, we integrated data from proteomics and glycomics and built an exhaustive search-space against which the glycoproteomics data were searched.

We used this strategy to analyze serum glycoproteins ranging in complexity, generated by mixing together purified standard glycoproteins from human serum. The samples were subjected to proteomics after tryptic digestion and deglycosylation using PNGaseF. The released glycans were analyzed by HILIC-MS. These data were used to construct the glycopeptide search database.

Our strategy for glycopeptide analysis allowed us to maximize depth of analysis in a complex mixture, demonstrating that tandem MS performed on glycopeptides alone is not sufficient to get the best information on site-specific glycosylation. This is why proteomics and glycomics information must be integrated with glycopeptidomics. At the same time, we were able to gauge the power of our analytical methods, whereby we were able to determine how complex a sample can be handled using existing analytical methods. This information helps design better methods for analysis and guides the experimentalist on how to improve the sample preparation, separation and fractionation methods prior to mass spectrometric analysis of glycoproteins.

Poster 075: Quantitative Phosphoproteomics Identifies a Role for PP6c in the Regulation of Chromosome Condensation

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Faithful chromosome segregation in mitosis is crucial to the formation of two viable daughter cells. This is in part controlled by post translational modifications, including phosphorylation events regulated by kinases and phosphatases to control the timing and fidelity of mitotic progression. Protein phosphatase 6 (PP6c) is a conserved and essential regulator of chromosome segregation from yeast to humans. Depletion of PP6c in human cells causes spindle defects and chromosome missegregation; however, only two mitotic PP6c substrates have been identified, which are not sufficient for a mechanistic understanding of how PP6 participates in signaling networks essential for regulating chromosome segregation. Furthermore, PP6c has been found mutated in melanoma and overexpressed in glioblastoma, making the enzyme a potential target for cancer chemotherapy. Here we develop a baculovirus-mediated shRNA delivery approach to efficiently deplete PP6c in HeLa cells. Using reductive dimethyl-labeling and TiO2 microsphere-based phosphopeptide enrichment, we quantitatively determine changes in phosphorylation in mitotic HeLa cells upon PP6c depletion. In addition, we identify changes in the protein abundance to account for differences in phosphorylation due to protein changes. Novel identified PP6c substrates were validated by in vitro phosphatase assays. Finally, we use a combination of cell biological and biochemical approaches, as well as immunofluorescence microscopy, to identify the biological significance of novel PP6c substrates.

Poster 076: Identification of CDK1 Substrates in Mitosis

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Cyclin-dependent kinase 1 (Cdk1) is an essential regulator of phosphorylation signaling during mitotic progression. Phosphorylation networks controlled by Cdk1 regulate the dramatic changes in cellular structure and organization undergone by cells during division. Specifically, Cdk1 plays important roles in chromosome condensation, nuclear envelope breakdown, disassembly of the endoplasmic reticulum, and formation of the mitotic spindle. Deregulation of these processes can produce daughter cells that are aneuploid which is a hallmark of human cancer, as well as the underlying cause of many birth defects. However, our understanding of Cdk1-dependent phosphorylation pathways regulating mitotic progression remains incomplete. Furthermore, recent studies have suggested that additional Cdk1 substrates exist that do not fit the canonical 'SP' motif. In order to expand our understanding of Cdk1-dependent phosphorylation pathways in mitosis, it is necessary to comprehensively identify Cdk1 substrates. Here, we performed a quantitative phosphoproteomic analysis utilizing two small molecule molecule inhibitors of Cdk1, Flavopiridol and RO-3306, in order to uncover additional components of the Cdk1-mitotic signaling network. In these analyses, we eere able to identify 802 phosphopeptides on 420 proteins that decrease significantly in both treatment cases. We performed bioinformatic analyses using tools such as Gene Ontology analysis (GO), STRING protein association networks, and the CORUM protein complex database to characterize candidate substrates. Lastly, we were able to validate eight potential Cdk1 substrates using in vitro kinase assays. These data provide additional novel insight into the complex Cdk1 mitotic signaling network and identify candidate substrates for future study.

Poster 077: Quantitative Profiling Tau Modifications with DIA for the Differentiation of Tauopathies

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Tau is a microtubule stabilizing protein that has been implicated, through its enhanced aggregation and increased abundance, in a subset of neurodegenerative diseases termed tauopathies. This group of tauopathies includes Alzheimer's disease (AD), the most common neurodegenerative disease. To develop optimum therapeutics and companion diagnostics for specific tauopathies, understanding differences and commonalities between these diseases is vital. Modern mass spectrometry techniques allow us to exploit the intricate landscape of post-translational modifications (PTMs) found on Tau to differentiate amongst tauopathies. Using data independent acquisition (DIA), we identified and quantified these differences in post-mortem

brain tissue in specific tauopathies including, AD, corticobasal degeneration (CBD), progressive supranuclear palsy (PSP), and Pick disease (PiD). The next step is to build on this assay to develop a novel diagnostic and prognostic tool utilizing cerebrospinal fluid (CSF) of living patients for accurate *in vivo* tauopathy classification.

Poster 078: Analysis of Vascular Endothelial Growth Factor Receptor 2 (VEGFR-2) N-Glycosylation Sites using PNGase F/18O Labeling and Tandem Mass Spectrometry

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Vascular endothelial growth factor receptor-2 (VEGFR-2) is one of the most important receptor tyrosine kinases among the VEGF receptor subfamily; activation of VEGFR-2 is essential for tumor angiogenesis. The extracellular domain of VEGFR-2 contains seven immunoglobulin-like domains, each with multiple potential N-glycosylation sites. N-glycosylation is thought to play a central role in receptor stability, ligand binding and trafficking. However, the N-glycosylation sites and their putative role(s) in VEGFR-2 function remain largely unstudied. Our objective was characterization of VEGFR-2 N-glycosylation sites via (1) enzymatic incorporation of 18O into formerly N-glycosylated sites, followed by tandem mass spectrometry (MS/MS) analysis and (2) MS/MS analysis of VEGFR-2 glycopeptides to assess N-glycosylation site occupancy.

The experimental workflow involved immunoprecipitation of VEGFR-2 from porcine aortic endothelial cell lysates using a polyclonal anti-VEGFR2 antibody, proteolytic digestion, glycan release with PNGase F/18O, and LC-MS/MS analysis on a Q Exactive mass spectrometer (Thermo). Data were processed using Proteome Discoverer 1.4. Glycopeptides were enriched and separated using a HILIC-C18 HPLC-Chip (Agilent) and analyzed via an Agilent 6550 Quadrupole Time-of-Flight MS using collision-induced dissociation.

We detected candidate N-glycosylated peptides of VEGFR-2 after treatment with trypsin followed by PNGase F/18O. Additional proteolytic digestions of VEGFR-2 were performed to search for potential glycosylation sites not observed in the tryptic digests and to assure that each glycopeptide contained only one N-linked site. We found that all seven VEGFR-2 immunoglobulin domains exhibit at least one occupied N-glycosylation site. These results are the first direct evidence that define which potential VEGFR-2 N-glycosylation sites are occupied. Future experiments will determine the detailed structures of the glycoforms present at each site. Such information is essential for determination of the role of glycosylation in VEGFR-2 function and trafficking.

Poster 079: OQ-STrap Technology for using of Large Protein Loads

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We recently introduced the concept of Suspension Trapping (STrap) for bottom-up proteomics sample preparation. The simple method is based on SDS-mediated protein extraction (tolerating SDS concentrations up to 15%) followed by swift detergent removal to yield a fine, detergent free protein suspension. The STrap retains these protein particles in a quartz depth trap where rapid, reactor-type protein digestion can take place within one hour. With an integral C_{18} cleanup feature, the original STrap is suitable for processing microgram and sub-microgram quantities of protein. It has proven to be robust and well-suited to the characterization of all protein types, including membrane proteins.

Success of the STrap led to desire for high-capacity units able to process at the milligram scale: especially in the analysis of post-translational modifications (PTMs), enrichment often requires a several milligrams of input protein. We present a new, large-capacity STrap unit with a quartz trap only – the Only Quartz (OQ) STrap cartridge – which is easily manipulated by syringe or vacuum manifold. Introduction of protein into the trap (SDS depletion, wash and protease addition) requires only 2 minutes. After the one-hour digest at 47°C, peptides are eluted and ready for downstream processing. We have successfully tested STrap OQ performance with 2-mg of protein from PC3 cell lysate.

Large-capacity STrap OQ technology retains the unique advantages STrap – simplicity, speed, robustness and unbiased protein processing, including processing of poorly soluble proteins – qualities not observed jointly in other relevant protein digestion methods. We anticipate STrap OQ units will assist to analyze and discover PTMs.

Poster 080: Phosphoproteomic Comparison of Osteoblasts Stimulated with Forteo or Biased PTH1R Ligand as Determined via SILAC

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The type 1 parathyroid hormone receptor (PTH1R) is a key regulator of calcium homeostasis and bone turnover. The N-terminal fragment of the parathyroid hormone (PTH(1-34)) is the only FDA approved anti-osteoporotic drug that acts anabolically on the bone building osteoblasts. PTH 1-34 is efficacious for approximately 18 months at which time the rate of bone resorption surpasses that of bone formation, necessitating alternative therapeutic strategies.

In vivo studies with the Gs and Gq- coupled receptor ligand, PTH(1-34), and the experimental arrestin-coupled receptor ligand, [D-Trp12,Tyr34]-bovine PTH(7-34), demonstrate both ligands exhibit anabolic effects in murine bone, however, transcriptomic analysis revealed these effects are mediated by different mechanisms. Here, we employed SILAC-based quantitative phosphoproteomic analysis to examine signaling events regulated by acute stimulation of MC3T3-E1 pre-osteoblasts treated with PTH(1-34) or bPTH(7-34) for 5 minutes before or after 10 days of differentiation.

Quantitative proteomic analysis demonstrated before and after 10 days of osteogenic differentiation, PTH(1-34) elicited a robust phosphorylation-mediated response consistent with Gs-coupled PTH1R activation of PKA as expected (day 10 analysis published; doi: 10.1016/j.ymeth.2015.06.022). However, prior to the onset of differentiation, immunoblot analysis revealed acute stimulation of pre-osteoblasts with PTH(7-34) elicits a reproducible effect on phosphorylation events that is distinct from that of PTH(1-34). These differences in ligand response correlate with decreased β-arrestin protein expression. This yields the possibility that ligands with distinct efficacy profiles may exert effects at different points during the differentiation of the cells. SILAC-based phosphoproteomic studies are currently underway to compare the phosphoproteomic profiles and biological effects of these two ligands at the day 0 time point where immunoblot analysis indicates the drug responses diverge.

Poster 081: Understanding DYRK1A Function through Phosphoproteomic Substrate Identification

Zachary Poss; Christopher Ebmeier; William Old
University of Colorado, Boulder, Colorado

Dual-specificity tyrosine phosphorylation regulated kinase 1A (DYRK1A) is a dosage sensitive enzyme located in the Down syndrome critical region (DSCR) of chromosome 21 and has been implicated in phenotypes and pathologies exhibited in Down syndrome. Additionally, DYRK1A appears to have both oncogenic and tumor suppressor roles that are cell type and context dependent. While much effort has been devoted to the study of this kinase, relatively few bona fide substrates and corresponding pathways have been identified. Based on currently known substrates, DYRK1A appears to be localized to both the nucleus and cytoplasm and phosphorylates targets involved in cell division, cell cycle progression and gene expression. To further dissect the cellular functions of DYRK1A, we have conducted phosphoproteomic studies to determine DYRK1A substrates directly from human cells. We have identified several new targets of DYRK1A, some of which are broadly implicated in transcription and chromatin biology, further underscoring the potential importance of this kinase in growth and gene expression.

All posters will be attended by presenting authors on both Monday and Tuesday. All posters should be set up by 10:00 am on Monday morning and removed at 3:00 pm on Tuesday. **Odd**-numbered posters present **1:30 - 2:15 pm** and **even**-numbered posters **2:15 - 3:00 pm**.

Poster 082: Strategies for Global Phosphohistidinomics and the Analysis of Other Labile Phosphospecies

Rob Oslund; Jung-Min Kee; Tom Muir; David Perlman
Princeton University, Princeton, NJ

Protein histidine phosphorylation (pHis) is increasingly recognized as a sparse but critical post-translational modification (PTM) in central metabolism and cell signaling, including cancer cell biology. Although the pHis PTM was discovered more than 50 years ago, the field has advanced little in the last three decades because of the difficulty of detecting this PTM by modern proteomic techniques, due to the labile nature of the pHis moiety and the dearth of tools for selective enrichment and detection.

Recently, we have overcome these challenges, developing a methodology for global proteomic analysis of pHis proteins (*Phosphohistidinomics*) involving the generation of novel pan-specific α-pHis antibodies and their utilization for selective immunoenrichment of pHis-containing peptides. Through MS study of synthetic pHis peptides, we have described that they produce prominent characteristic neutral losses of 98, 80, and 116 Da when subject to collision-induced dissociation (CID). Using stable isotopic labeling, we have demonstrated that the main 98 Da neutral loss typically occurs via gas-phase phosphoryl transfer and dehydration at the peptide C-terminus.

We exploit this predictable fragmentation behavior in a two-step MS strategy where we screen immunoenriched peptides for the pHis CID triplet neutral loss signature, then subject these peptides to further MS/MS analyses using alternative fragmentation techniques. We show that this two-step strategy for peptide identification based on explicit utilization of characteristic neutral loss signatures is effective also when applied to other labile phosphospecies. Using this methodology, we have conducted *Global Phosphohistidinomics* on *E. coli* and have directly characterized known, previously speculative, and novel pHis sites on enzymes of central metabolism, which may have important metabolic regulatory implications. Based on our insights, we propose a universal strategy for Phosphohistidinomics, or more generally, labile-PTM'Omics, including sample prep through upfront peptide-level enrichment and MS data acquisition via a multi-branched decision-tree algorithm to guide the hybrid instrument duty-cycle.

Poster 083: Multiplexed Quantitative Analysis of Mammalian Cell and Tissue Ubiquitylomes using Isobaric Labels

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The ubiquitin-proteasome system (UPS) is responsible for degradation of proteins within the cell ensuring general cell health and survival. Quantitative analysis of ubiquitylated peptides has traditionally employed label free or metabolic label based approaches. However, both of these methods necessitate multiple nLC-MS/MS experiments for measurements of biological replicates, limiting the number of peptides quantified across all samples. Additionally, modified peptides are typically low abundant, requiring large amounts of starting material (5 – 40 mg/sample) and challenging the limits of instrument sensitivity.

Isobaric labels offer many advantages for quantifying post-translation modifications (PTMs) by MS. Isobaric labels facilitate a decrease in starting material used for enrichment and minimize missing values by enabling measurements across all replicates with a single MSn event. However, chemical tags label the primary amine of the di-glycine remnant on ubiquitylated peptides, inhibiting enrichment via immunoprecipitation (IP) after samples are labeled and mixed. The perceived variability of di-glycine enrichment has dissuaded separate enrichment of ubiquitylated samples and by extension, the use of isobaric labels. If the di-glycine remnant IP variability was sufficiently low (e.g., CV<10%), it would be possible to utilize isobaric labeling to enable deep, multiplexed quantitation of ubiquitylomes from cell culture, tissue, and clinical samples.

Here, we describe a method for multiplexed analysis of ubiquitylomes enabling quantitation of 8,000-9,000 ubiquitylation forms in 18 hours beginning with as little as 1 mg/sample. We demonstrate that separate di-glycine remnant antibody IP results in deep, reproducible ubiquitylome quantification of 8,000 ubiquitylation forms for technical replicates of HCT116 cells treated with the proteasome inhibitor bortezomib (Btz). Next we determine the time dependent changes in the ubiquitylome upon Btz treatment, quantifying 9,000 ubiquitylation forms across nine time points. Lastly, we survey 8,000 ubiquitylation events in biological replicates of mouse brain and liver and demonstrate ubiquitylation events on certain proteins are tissue specific.

Poster 084: Quantitative Peptide Assay for Optimized and Reproducible Sample Preparations

Xiaoyue Jiang[1]; Ryan Bomgarden[2]; Ramesh Ganapathy[2]; Sijian Hou[2]; Sergei Snovida[2]; Paul Haney[2]; John Rogers[2]; Julian Saba[3]; Rosa I Viner[1]; Andreas Huhmer[1]
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New advances in mass spectrometry (MS) enable comprehensive characterization and accurate quantitation of complete proteomes. Despite the rapid advances in analytical instrumentation and data processing, quality of the generated data to a large extent depends on the upstream sample preparation techniques. In this study we utilized a peptide quantitation assay to monitor the peptide concentration at each step of a typical bottom-up proteomics workflow, including labeling with Tandem Mass Tag™ (TMT™) reagents and off-line fractionation steps.

A549 human cells grown at different conditions were lysed and digested. Concentrations were normalized before labeling samples with TMT 6plex™ labeling reagents, followed by high PH reverse phase fractionation. The peptide concentrations were measured in between each of above experimental step. The fractionated samples were separated using a 50cm length column followed by the detection on the Thermo ScientificTM Q Exactive™ Plus mass spectrometer.

While the protein concentration was routinely measured by the BCA Protein Assay Kit before the digestion, the true peptide concentration after the digestion could vary substantially due to the different digestion efficiency and separate sample handling. We observed up to 20% difference in protein identifications (assessed in triplicates) based on the initial protein assay when injecting 200ng of samples and analyzing by LCMS. The peptide assay indicated 30% variations in the peptide concentrations, which explained the discrepancy in protein identifications. With the normalization, 200ng of sample load for the triplicates only resulted in 5% variability in protein identifications, a significant improvement in reproducibility.

Similarly, the measurement of peptide concentrations in the cell digests from different conditions allowed the accurate mixing for TMT labeling, and subsequent sample fractionation. The peptide concentration in each fraction was measured and sample load for each MS analysis was adjusted accordingly. As a result, consistency and reproducibility of all MS data improved significantly.

Poster 085: A Comprehensive Temporal Analysis of Differentiating Pancreatic β-Islet Cells from Human Embryonic Stem Cells Provides Insights into Maturation

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Diabetes is a major global health concern. While the differentiation of stem cells into pancreatic β-cells offers tremendous potential as a cure for diabetes, many challenges still exist. One of the most important issues is the fact that end-point differentiation of stem cells produce heterogeneous mixtures of cells, with cells at different stages of differentiation including stem cells that could produce tumors. Another issue is the maturity of the β-cells. These studies show the promise of this approach, but suggest that the endpoint differentiation efficiency and insulin production could be further fine-tuned. The second major impediment is the purification of a homogeneous cell population

consisting exclusively of cells carrying the mature cellular character of interest.

To better understand the maturation process, we quantitatively mapped the proteome and transcriptome dynamics of human stem cells as they differentiate into pancreatic β-islet cells. This dynamic proteome and transcriptome analysis resulted in the identification of stage specific genes and proteins during the maturation to β- islet cell population. Co-regulation analyses of the data identified candidate markers that can be used to target, monitor, and purify an otherwise heterogeneous cell population. This developmental profiling approach identified candidate key factors and changes unique to specific differentiation states as well as specific markers of pancreatic beta cell progenitors including FOXO1, SIRT1, STAT3 and MAPK9 as being involved in the differentiation and maturation of hESC into pancreatic β-islet cells. Human pancreatic cells from postmortem tissue were used to benchmark and characterize the maturity of the hESC derived β-Islet cells. This data suggests that the beta cell differentiation methods could be refined further using the novel identified pathways, factors and methods implicated in our study.

Poster 086: A Proteogenomic Approach to Identify Surface Proteins in Acute Myeloid Leukemia

Hossein Fazelinia[1]; Kian Huat Lim[1]; Tina Glisovic-Aplenc[1]; Lynn A. Spruce[1]; Ian R. Smith[1]; Sarah K. Tasian[1]; Saar Gill[2]; Richard Aplenc[1]; Steven H. Seeholzer[1]

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High-throughput interpretation of MS/MS spectra for mass spectrometry (MS)-based proteomics is performed by searching sequence databases followed by statistical analysis to control for false discovery. This search strategy depends upon completeness of the reference database, but may fail to capture novel peptides arising from genomic variants. Customized protein databases can be built from six-frame translation of the genome through ab initio gene prediction algorithms. This approach generates a more comprehensive database, but its resultant substantially increased data size may detrimentally affect the sensitivity of peptide spectral matching at a given false discovery threshold. To achieve deep understanding of cellular mechanisms at both genomic and proteomic levels, we profiled the human acute myeloid leukemia (AML) MOLM14 cell line via applied RNA sequencing (RNA-seq) and MS proteomic technologies in parallel. In this proteogenomic strategy, the proteomic search database is augmented by the expression of both annotated and novel transcripts discovered from RNA-seq, thereby allowing us to build sample-specific protein sequence databases. The overall goal of these studies is to develop a pipeline for the efficient generation of sample-specific sequence databases for sensitive and specific protein identification from tandem MS data. Our strategy for RNA-seq informatics workflow combines both genome-guided reference mapping and de novo assembly to extract maximal transcriptomic information from RNA-seq data. We use multiple database search engines including X!Tandem, COMET, and MS-GF+ to verify the improvement in peptide spectral matching (PSM) by combining search engine results using the iProphet algorithm. This approach allows us to find complementation for PSMs by using RNA-Seq derived databases in conjunction with UniProt reference database. In the current studies, we identified PSMs unique to the UniProt reference database and new INDEL variants and novel splice junctions found only in the RNA-seq-derived sequence database, thus highlighting the efficient discovery potential of this proteogenomics approach.

Poster 087: Monitoring Signal-Specific Changes in Ribosome Maintenance and Biosynthesis *in vivo*

Andrew Mathis; Bradley Naylor; John Price

Brigham Young University, Provo, Utah

Protein homeostasis within each cell is fundamental to sustained health and fitness for the entire organism. The ribosome is a key control point for maintenance of protein homeostasis. During development, aging, and disease, regulation of ribosomal translation rates and efficiencies shapes the entire proteome. By measuring the *in vivo* turnover of the rRNA backbone and the individual protein components of the ribosome, we observe that exchange of protein subunits occurs rapidly in relation to the synthesis and degradation of the assembled ribosome. We show that modulation of the *in vivo* demand for cellular protein synthesis changes the exchange rate for many of the ribosomal proteins. This work suggests mechanisms for the observed connection between slower protein synthesis and improved protein homeostasis. Identifying cellular strategies for maintenance of the ribosome may lead to therapeutic routes for preventing the progressive loss of protein homeostasis that leads to diabetes, neurodegeneration, and aging.

Poster 088: The Signature Exosome Map for Bone Marrow Stromal Cells: The Development of an Optimal Sample Preparation Approach

Qin (Stefanie) Liang; Michail A. Alterman

FDA, Silver Spring, MD

The emergence of Human Multipotent Stromal Cells (hMSCs) as potential therapeutics in diverse range of diseases is due to three major properties: potential for cell replacement and multipotent differentiation, immune and inflammatory modulation, and tissue repair. More recent studies on MSC's biodistribution and engraftment led to the proposal that MSC's therapeutic effect is linked to the secreted extracellular vesicles (EVs). Exosomes are now considered as the specifically secreted EVs that enable intercellular communication. There is exponentially increasing interest to study their functions and to use them in minimally invasive diagnostics. Our laboratory has previously performed a comprehensive proteomic analysis of hMSC and compiled and comparatively assessed the largest to date proteomic dataset of culture-expanded MSC from various human donors with a total of 7753 protein groups (FDR≤3.4).

Our aim is to explore and document the influence of in vitro cell passaging on dynamic changes of the exosome proteome. A crucial part of any proteomic study is designing an optimal sample preparation approach; in the case of exosomes there is no established approach. Here we describe the development and comparison of various methods of cell culturing, exosome isolation, and exosome protein extraction to obtain high-quality exosomes with good proteins yield. MSC cultured in serum-free medium produced much less exosome particles compared to that grown in cell culture medium with exosome-depleted FBS. We compared three commercial exosome isolation kits, which are Total Exosome Isolation (Invitrogen), ExoQuick (SBI) and PureExo (101Bio). Our data showed that exosomes isolated by Total Exosome Isolation and ExoQuick were with comparable purity and protein yields; while protein extraction of PureExo-isolated exosomes had lower protein abundance. We provide a potential standardized approach to obtain high yields of pure MSC exosomes and believe the method will benefit the field of MSC exosome research.

Poster 089: Quantitative Proteomics & Network Analysis Identifies Retinoic Acid Therapy to Bypass Impaired Vitamin A Metabolism in Heart Failure.

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Background: Given the paucity of drugs for the treatment of heart failure (HF) and prevention of sudden cardiac death, it behooves us seek new models and methods that may more faithfully inform us about human disease. Recently, we have leveraged a pressure-overload/chronic catecholamine guinea pig model of cardiac hypertrophy (HYP) and HF with the unique features of acquired long QT syndrome and sudden cardiac death. Specifically, we have conducted global-scale quantitative proteomics of HF progression by iTRAQ, quantified by with a robust "median-sweep" algorithm and statistically assessed with an empirical Bayesion modified t-Test.

Results and Implications : Proteomic network analysis predicted that guinea pig HF is characterized by early and sustained impairment of Vitamin A metabolism, yielding a deficit in the bioactive metabolite, retinoic acid (RA, $p=1.88x10^{-11,}$ z-score<-1.2). RA deficit, in turn, is predicted to attenuate transcriptional programs for which activation of the Retinoid X Receptor (RXR) or Retinoic Acid Receptor (RAR) is required. These include genes that control fatty acid oxidation, excitation/contraction-coupling, contraction and antioxidant defense —

all of which are downregulated in HF. Metabolomic profiling of resident cardiac retinoids confirmed an acquired in situ deficiency in RA early in HF progression (Down 32% $p<0.05$) . Moreover, the combined data suggest a bottleneck in Vitamin A metabolism at the level of retinaldehyde dehydrogenase 1 (RALDH1). Treatment with RA mitigates hypertrophy and improves fractional shortening in guinea pig HF. Studies are underway to determine, whether the RA deficit is recapitulated in human HF. If so, the the prospects for rapid translation are enticing as RA is already FDA-approved for the treatment of promyelocytic leukemia.

Poster 090: RgpB - Arginine Specific Proteas with Applications in Proteomics

Malin Mejare; Magdalena Widgren Sandberg; Stephan Bjork; Maria Nordgren; Frederick Olsson
Genovis AB, Lund, Sweden

Biological drugs are complex molecules and require careful characterization during development and production. To obtain full amino acid sequence information on a therapeutic protein, mass spectrometry is often applied in combination with complete or limited proteolysis. In this work we set out to characterize the enzymatic activity of the cysteine protease, RgpB, from Porphorymonas gingivalis as a potential tool for proteomic applications.

RgpB from P. gingivalis specifically digests peptides and proteins C-terminally of arginine residues. The specific Arg-X activity of RgpB was confirmed on both peptides and proteins with LC-MS. Arg-Pro is known to be difficult to digest by Trypsin. Using a peptide, MOG (35-55) containing 3 arg residues, one of which is preceding a proline residue, RgpB was shown to efficiently digest Arg-Pro. Additionally, the activity of RgpB was compared to that of Arg-C (clostripain), an arginine specific endopeptidase used for protein sequencing. RgpB is specific for cleavage of Arg-X peptide bonds whereas Arg-C has unwanted additional activities on lysine residues besides the Arg-X activity. RgpB was shown to be active on proteins in a broad pH range (pH 5.0-9.0), in presence of denaturing agents i.e. urea concentrations up to 6M and 0.1% SDS.

Taken together, these results suggest that the arginine specific RgpB is a novel protease in the toolbox of very specific digestion enzymes for proteomics and mass spectrometry applications.

Poster 091: A Biomimetic, Synthetic RNA Platform for *in vivo*, co-Translational Labeling of Proteins

Randi Turner; Daniel Dwyer
University of Maryland, College Park, Maryland

Integrative '-omics' research relies on broad-spectrum assessment of biochemical steady states to provide insight into biological behaviors. Techniques like ribosome profiling and shotgun proteomics offer comprehensive transcriptome and proteome analysis methods but lack the ability to accurately resolve existent from conditionally remodeled states. To address this challenge, we are developing an RNA synthetic biology platform that provides a biomimetic mechanism for in vivo, co-translational labeling of nascent polypeptides at the ribosome. We are leveraging naturally evolved features of the transfer messenger RNA (tmRNA), a tRNA-like RNA found in all sequenced bacteria, in our forward-engineered system. tmRNAs naturally serve as ribosome rescue systems that alleviate ribosome stalling, preventing toxic ribosome titration and aberrant polypeptide aggregation. A unique, short open reading frame (ORF) encoded on tmRNAs initiates the addition of a short degradation tag to the nascent, stalled polypeptide, which targets it for proteolysis. With respect to our synthetic tmRNA platform, the ORF offers an attractive modular domain for introduction of synthetic polypeptide tags, provided that requisite structural and thermodynamic properties are maintained. For our initial phase, we are using 6xHis isolation tags in Escherichia coli. We have successfully isolated 6xHis-tagged proteins using affinity purification. We anticipate an inducible system with which synthetic tags will only be introduced into actively translated proteins on-demand, and the platform scaled for use in theoretically any bacteria. Ultimately we expect for our synthetic tmRNA system to improve upon studies using ribosomal profiling and systems-level proteomics in bacterial models by offering a means for isolating conditionally modeled proteomes.

Poster 092: Rapid Changes in the Proteome of Gut Microbiota in Response to Short-Term Dietary Changes in Baboons

Prahlad Rao; Kimberly Spradling-Reeves; Vicki Mattern; Laura Cox; Anthony Comuzzie; Michael Olivier
Texas Biomed Res Inst., San Antonio, TX

Background: Obesity is a complex multifactorial disease, and recent studies have shown that changes in gut microbiota composition are correlated with obesity-related traits in humans and mouse models. It is unclear, however, how quickly the abundance of the different bacterial species varies in response to diet changes. We explored whether a comprehensive genomic and proteomic analysis from fecal samples can reveal functional and metabolic changes in gut microbes of non-human primates in response to an altered diet.

Methods: Baboons normally fed a chow diet were given a high-saturated fat, high-simple carbohydrate diet (HFHS) for seven weeks. At weeks 0 and 7, fecal samples were collected and the microbiota was analyzed using shotgun proteomics on a Q Exactive instrument (Thermo). Data analysis was carried out using database from Human Microbiome Project (HMP). Differences in protein abundance were quantified using label-free quantitation, and only species with at least 2 significantly altered proteins were included.

Results: Our metaproteomic analysis using the HMP database shows the most detected proteins from phylum *Bacteroidetes*, from phylum *Firmicutes* and from *Actinobacteria*. Correlation of protein abundances between week 0 and week 7 was high ($r^2 = 0.73$). Comparison of genus data between week 0 and week 7 indicated a decrease in lactobacillus after week 7 of HFHS diet.

Proteomic data also revealed that protein abundance does not necessarily correlate with abundances of certain phyla. From the proteomic data, we recorded differences in the protein abundance data in different taxa in the same phylum in response to HFHS diet. We also recorded differences in protein abundance in the same species in certain pathways such as the glycolytic pathway. Both these data highlight the dynamic responses of microbiota to changes in diet which can be dissected using a metaproteomic approach.

Poster 093: Proteome and Secretome Profiling Reveals Temporal Differences in the Toll-like Receptor Responses to Different Ligands

Marijke Koppenol-Raab; Virginie Sjoelund; Bhaskar Dutta; Zachary Benet; Iain Fraser; Aleksandra Nita-Lazar
NIH/NIAID, Bethesda, Maryland

Toll-like receptors (TLRs) are essential sensors in innate immunity and among the first to detect invading pathogens. Many studies of TLR responses have focused on the intracellular signaling events that occur upon receptor stimulation. However, proteins released from the cell play a key role in cell-cell communication during an immune response. Targeted studies have identified various cytokines among the factors released in response to TLR signaling, but comprehensive studies of the secretome are limited. We are using mass spectrometry-based proteomic methods to perform complementary analyses of both the intracellular and extracellular responses of activated macrophages.

RAW264.7 macrophages were stimulated with ligands to TLR4, TLR2 and TLR7 for up to 24 hours. TLR2 and TLR7 signal from the cell surface and endosome, respectively, and both use the adaptor MyD88, whereas TLR4 can signal through MyD88-dependent and MyD88-independent pathways. Cell lysates (proteome) and conditioned media (secretome) were collected and processed for proteomic analysis. We observe some overlap in the proteins identified in the different treatments, indicating that certain signaling components are shared, however the TLR4 response is more distinct in both datasets. The 24h TLR4 secretome also exhibits significant down-regulation, suggesting that this pathway is shut off faster than the TLR2 and TLR7 responses. Transcriptome data from cells treated with TLR ligands for up to 4 hours show the strongest correlation with later secretome time-points consistent with the lag needed for protein production and export. Only 18% of the proteins identified in the secretome are predicted to have a signal peptide, suggesting that proteins released via other mechanisms are important for cell-cell

communication. We identified proteins exhibiting differential regulation across treatments to target in a follow-up study of the secretome response to S.aureus, P.aeruginosa, or B.cenocepacia treatment. This research was supported by the Intramural Research Program of the NIH, NIAID.

Poster 094: The Characterization of IFIX as an Anti-Viral Factor during Infection with DNA Viruses

Marni S. Crow; Michelle-Ann R. Tan; Tuo Li; Benjamin A. Diner; Ileana M. Cristea
Princeton Univeristy, Princeton, NJ - New Jersey

Intracellular recognition of pathogens is critical for cellular defense. Among these important defense factors are DNA sensors, proteins that bind to foreign DNA and trigger immune responses. Recently, we identified a new viral DNA sensor, the interferon inducible protein IFIX. However, its mechanisms of action remained unknown. We integrated proteomics with virology, molecular biology, and microscopy to characterize this antiviral factor. We show that IFIX is capable of binding viral DNA both in the nucleus and cytoplasm of cells, in response to herpes simplex virus 1 (HSV-1) or vaccinia virus, respectively. As IFIX is predominantly nuclear, we characterized its localization-dependent sensing by defining nuclear localization and export signals, and demonstrate its modulation by acetylation. The location of viral DNA is dependent on the type of virus and host cell, highlighting the importance of mechanistic studies in understanding innate immune responses. To discover how IFIX exerts its antiviral functions, we used different HSV-1 strains that are able or not able to trigger an interferon response, and measured the relative expression of resulting antiviral cytokines. We next performed the first characterization of IFIX interactions with human and viral proteins during infection. We established IFIX associations with transcriptional regulatory proteins and PML nuclear body components. The PML interaction was lost during infection with wild-type HSV-1, as the viral E3 ubiquitin ligase ICP0 target PML for degradation. This interaction was present during infection with an ICP0 mutant virus, and validated by microscopy. This is important, as, unlike wild-type HSV-1, the interferon response is not inhibited by the mutant virus. Therefore, we can distinguish IFIX interactions occurring in an active immune signaling environment. Importantly, IFIX overexpression led to decreased virus titers for both wild type and mutant HSV-1, and modulated viral gene transcription. Altogether, our studies indicate IFIX provides a possible target for anti-viral therapies.

Poster 095: Identifying Organisms by MALDI starting from Genomic Databases

Kenneth Parker
SimulTOF Systems, Marlborough, MA

In 2003, it was first recognized by Pineda et al. that bacteria could be identified starting from the theoretical masses of proteins deduced whole genome sequencing experiments. At that time, only a few hundred sequenced genomes were available. Since then, whole organism MALDI has been widely accepted by clinical laboratories for identification of organisms of pathological interest, based on spectral matching of profiles, but little attention has been paid to genomic matching. We have developed a fingerprinting program that can match MALDI spectra to a database containing > 10000 strains and >3000 genera downloaded from TrEMBL or NCBI. We desribe the advantages and disadvantages of using this technique for identifying bacteria from environmental isolates. We have developed an interface that places the strains that are identified into an evolutionary context. Species identification can be accomplished by focusing attention on matching to ribosomal proteins only. We also discuss strain discrimination upon matching to all encoded proteins.

Poster 096: Understanding the Network Signaling Capacity of HBx in HBV Host Infection

Emanuela Milani; Charlotte Nicod; Bernd Wollscheid
ETH Zurich, Zurich, Switzerland

Viruses are obligatory intracellular organisms. Their survival and propagation rely on the interplay with host proteins and the hijacking of host signaling machineries. A detailed knowledge of viral-host protein-protein interactions, and virus perturbed host signaling protein network nodes is informative for the understanding of key signaling mechanisms of viral infection and propagation as well as for the identification of novel cellular targets for potential antiviral therapies.

To gain a comprehensive and quantitative understanding of viral-host interaction networks we used two orthogonal quantitative mass spectrometry-based proteomic approaches: affinity purification mass spectrometry (APMS) and proximity-dependent biotinylation coupled to affinity purification and mass spectrometry (BioID). The combination of these two complementary strategies coupled to functional genetic approaches enabled the elucidation of the viral-host protein interaction map of the hepatitis B virus (HBV), in particular of the pleiotropic HBV X protein (HBx) which plays an essential role in HBV replication. The obtained high confident HBx interaction network confirm and extend current knowledge on the pleiotropic activities of HBx, pointing towards an involvement in multiple distinct cellular signaling processes. Identified interactors show HBx involvement and potentially modulation of cellular proliferation, mRNA processing and proteasome-dependent degradation.

We further dissected the dynamic protein expression changes induced by HBx signaling at the total proteome level. Among the differentially expressed proteins we observed specifically nuclear import/export proteins, mitochondrial proteins and splicing factors. These results indicate that HBx achieve its pleiotropic effects not only through direct interactions with a set of distinct proteins but also indirectly through the regulation of expression and/or turnover of proteins involved in distinct secondary signaling processes.

Together, probing signaling network structure of HBx-host interactions by the combination of APMS, BioID and whole proteome analysis revealed a defined subnetwork structure moonlighting the pleiotropic signaling capacities of HBx, which provides in turn new leads for pharmacological intervention.

Poster 097: Proteomic Level Identification of Degradation Resistant Proteins, Complexes & Aggregates in Human Plasma

Hannah Trasatti; Ke Xia; Wilfredo Colon, *RPI, Troy, NY*

Protein half-life is an integral component of protein function and the homeostasis of any biological system. A protein's biological half-life is a combination of its intrinsic stability and catabolism rate. Some proteins, complexes and aggregates have intrinsic hyperstability, demonstrated by their resistance to proteolysis and detergents. This hyperstability, also known as "kinetic stability", is a result of a high energy barrier toward unfolding or dissociation, resulting in a conformational trap. Kinetic stability may be an evolutionary mechanism to protect against premature degradation or protein aggregation, the latter being implicated in human diseases such as senile systemic amyloidosis and amyloid A amyloidosis. To investigate the sub-proteome of kinetically stable protein species in human plasma we applied immunodepletion, differential proteolysis, and Diagonal-2-Dimensional SDS PAGE to pooled human plasma. Identified hyperstable proteins, complexes and aggregates may have biological or pathological implications in the general population. This study may be further extended to understand how the plasma profile of degradation-resistant proteins species change as a result of normal aging and aging-related diseases, and has implications for biomarker discovery.

Poster 098: Analysis of the Effects of Dietary Signals on Protein Homeostasis

Bradley Naylor; Richard Carson; Monique Speirs; John Price
Brigham Young University, Provo, 0

Dietary interventions such as calorie restriction (CR) have been shown to significantly alter protein homeostasis, the control of protein concentration and flux. This change in protein homeostasis is hypothesized to be responsible for the significant increase in longevity and other beneficial effects experienced by organisms on a CR diet. We have observed that a change in specific dietary signals to mice significantly changes how CR affects liver protein homeostasis. We are exploring the mechanism of this signal-specific proteostatic control by monitoring changes mRNA concentrations and protein synthesis. This work will aid in understanding regulation of the proteome on a global level as well as direct effect of specific dietary signals.

POSTER ABSTRACTS

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Poster 099: High Purity Myonuclear Isolation from Skeletal Muscle

Alicia Cutler; Grace Pavlath
Emory University, Atlanta, Georgia

Skeletal muscle is essential for mobility and survival. Skeletal muscle cells called myofibers are one of only a few multinucleated cell types in the body. Myofibers can contain thousands of nuclei and these nuclei exhibit heterogeneous protein localization and transcriptional activity. However, detailed analyses of changes to the myonuclear proteome in altered physiological conditions like disease, regeneration, and aging have been difficult because of technical challenges associated with isolating pure myonuclei. Isolated nuclei are essential because nuclear proteins comprise the minority of proteins in muscle tissue due to highly abundant cytoplasmic proteins, especially contractile proteins. Two major challenges to purifying nuclei from skeletal muscle are: 1) muscle structural components are difficult to break up without damaging myonuclei, and these components co-sediment with nuclei, leading to contamination and 2) skeletal muscle is composed of multiple cell types leading to impure populations of isolated nuclei. To address these challenges, we have optimized a technique by which intact myonuclei can be isolated with high purity from mouse muscles. Purified nuclei are ~90% myonuclei, which are depleted of cytoplasmic and mitochondrial contamination. The nuclei are intact and suitable for use in a variety of downstream applications including flow cytometry, biochemistry, molecular biology, and proteomics. Immunofluorescence labeling can be used to sort purified myonuclei into subsets using flow cytometry. Proteomic analysis of isolated myonuclei versus whole muscle tissue illustrates the increased depth of information available from isolated nuclei. This myonuclear isolation technique opens possibilities of examining proteomic changes of myonuclei and subsets of myonuclei in aging, regeneration, and disease states without genetic myonuclear markers.

Poster 100: Investigation into the Mechanism of AGE-Mediated Cancellation of Calorie Restriction Benefits

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

Calorie restriction (CR) is the gold standard method to increase lifespan and overall health in laboratory animals, and provides one of the best models for studying aging processes. The incidence of age-related diseases in calorie-restricted animals has been shown to be dramatically lower than in normal-fed animals; however, the addition of advanced glycation end products (AGEs), which are also present at high levels in the Western diet, to the diet of calorie-restricted animals has been reported to cancel the lifespan and health benefits of calorie restriction. Our overall objective is to investigate the mechanism by which dietary AGE-modified proteins cancel the benefits of CR. We hypothesize that AGE-mediated cancellation of CR is closely tied to the triggering of inflammatory responses. Using a mouse model, we have explored the correlation between dietary AGEs, inflammation, and resulting perturbations in the proteome. Our lab uses a novel kinetic proteomics method to measure not only the concentrations of thousands of proteins simultaneously, but also protein turnover rates in vivo, giving an unprecedented glimpse of protein homeostatic maintenance within the cell. Understanding how AGE-related signaling decreases cellular fitness and lifespan at the level of protein regulation is expected to eventually lead to identification of potential therapies for age-related diseases such as diabetes, kidney and cardiovascular disease, and dementia.

Poster 101: Confident Identification of Chemical Crosslinks in Nonspecifically-Digested LC-MS/MS Samples by Locus-centric Aggregate Scoring

Mark Adamo[1]; Scott Gerber[2]; Andrew Grassetti[3]
[1]Norris Cotton Cancer Center, Lebanon, New Hampshire; [2]Geisel School of Medicine at Dartmouth, Lebanon, New Hampshire; [3]Dartmouth College, Lebanon, NH

Chemical crosslinking provides a means of assessing the structural conformations and connectivity of proteins and protein complexes by inducing bonds between specific amino acids in spatial proximity. After a protein sample is treated with crosslinker and proteolytically digested, LC-MS/MS analysis can be used to localize both intra- and inter-protein crosslinks.

As previously established in non-crosslinked samples, digestion with nonspecific proteases allows access to areas of proteins via LC-MS/MS that are inaccessible using conventional trypsin digests. In order to develop a method for localizing crosslinks in nonspecifically-digested samples, we used disuccinimidyl suberate (DSS) to individually crosslink several samples of purified proteins with known crystal structures: bovine serum albumin, serotransferrin, and the PP2A heterotrimer. Each protein sample was alternately digested using trypsin (specific) and proteinase K (nonspecific), and subjected to LC-MS/MS analysis. The spectrum data was analyzed using Kojak, a software application that identifies crosslinked peptides through database search.

After searching against a target-decoy database, conventional validation of peptide-spectral matches uses orthogonal scoring criteria to filter identifications to target a given false-discovery rate (FDR). Validation of crosslink searches in this way is challenged by the combinatorial expansion of the decoy space, a problem that becomes especially pronounced with nonspecific digests. However, the nonspecific digest can increase confidence in a given crosslink identification when we observe a crosslink locus embedded in several flanking amino acid sequences of varying length. By grouping all crosslink identifications that share a pair of linked loci, we can generate a probabalistic model for the existence of crosslinks based on their identification frequency. Using this model with our test samples, we recover a number of new crosslink sites with a high selectivity for crosslinks approximately 11.4 Å in distance (the length of the DSS spacer arm).

Poster 102: Proteomic Visualization of Cellular Entry and Trafficking

Linna Wang; Li Yang; Li Pan; W. Andy Tao
Purdue University, West Lafayette, Indiana

Our understanding of the complex cell entry pathways would greatly benefit from a comprehensive characteriza-tion of key proteins involved in this dynamic process. Here we devise a novel proteomic strategy named TITAN (Tracing Internalization and TrAfficking of Nanomaterials) to reveal real-time protein-dendrimer interactions using a systems biology approach. Dendrimers functionalized with photore-active crosslinkers were internalized by HeLa cells and irra-diated at set time intervals, then isolated and subjected to quantitative proteomics. In total, 809 interacting proteins crosslinked with dendrimers were determined by TITAN in a detailed temporal manner during dendrimer internalization, traceable to at least two major endocytic mechanisms, clath-rin-mediated and caveolar/raft-mediated endocytosis. The direct involvement of the two pathways was further estab-lished by the inhibitory effect of dynasore on dendrimer uptake and changes in temporal profiles of key proteins.

Poster 103: SOMAmer® Reagents and the SOMAscan® Assay: Tools for Targeted Proteomic Measurements

Sheri Wilcox; Stephan Kraemer; Dominic Zichi; Nebojsa Janjic
SomaLogic, Inc., Boulder, Colorado

SOMAmer® reagents are a novel class of affinity binding reagents made from single-stranded DNA engineered with amino-acid like side chains. By modifying the 5-position of uracil, SomaLogic has greatly expanded the physicochemical diversity of the large combinatorial SELEX (Systematic Evolution of Ligands by EXponential enrichment) libraries from which reagents are selected, resulting in binding to more proteins, and with generally higher affinity (median Kd < 1 nM), than observed with traditional aptamers. In particular, the hydrophobic nature of these binding interactions results in exquisite shape complementarity between the reagents and their protein targets. SOMAmer reagents have been proven effective in biomarker discovery, diagnostic products, and research tools. SomaLogic has developed a proteomic assay called the SOMAscan® assay for biomarker discovery that transforms protein concentrations in a biological sample into a corresponding DNA signature that can be measured using any DNA quantification technology. The commercially available SOMAscan assay was recently updated to measure over

1,300 human proteins simultaneously in biological samples and we are continuing to expand the assay to measure additional proteins. Recent SOMAscan data have shown that certain SOMAmer reagents are able to distinguish between proteins resulting from single-nucleotide polymorphisms (SNPs) and wild-type proteins in human plasma. Applications of the SOMAscan assay range from broad proteomic profiling of thousands of proteins to exquisite specificity measurements for certain analytes. In addition, hundreds of the same reagents are available as individual life science tools for direct translation of biomarker discovery results to targeted assays.

Poster 104: Offline Pentafluorophenyl (PFP)-RP Pre-fractionation for Comprehensive LC-MS/MS Proteomics and Phosphoproteomics

Andrew Grassetti[1]; Rufus Hards[1]; Scott Gerber[2]
[1]Dartmouth College, Lebanon, NH; [2]Geisel School of Medicine at Dartmouth, Lebanon, New Hampshire

Mass spectrometers are powerful tools that enable in-depth analysis of proteins and post-translational modifications on a proteome-wide scale. Although new instrumentation has greatly improved the speed and accuracy of online LC-MS/MS based peptide identification, analyses of complex proteomes and post-translational modifications requires sample pre-fractionation prior to online LC-MS/MS.

In order to reduce individual online LC-MS/MS injection complexity and generate more complete datasets, most laboratories utilize offline pre-fractionation methods such as strong cation exchange (SCX) or high-pH reversed-phase chromatography. These methods provide a means of separation that is orthogonal to the online low-pH (ion-paired) reversed-phase chromatographic platform to which mass spectrometers are typically attached.

In the present work, we have developed alternative methods of proteomic and phosphoproteomic sample complexity reduction through the utilization of off-line pentafluorophenyl (PFP)-reversed phase chromatography. Similar to SCX and high-pH reversed phase approaches, these methods are orthogonal to online reversed-phase separation. A benefit of PFP pre-fractionation is that samples are separated using TFA as an ion-pairing agent; thus, these fractions do not need to be desalted prior to injection on a mass spectrometer. Furthermore, direct comparisons between high-pH reverse phase and PFP suggest that PFP chromatography performs as well or better than high-pH reverse phase with respect to complexity reduction as measured by fractional peptide overlap. Finally, we developed PFP methods for the separation of TMT labeled peptides and unlabeled phosphopeptides, wherein methanol as opposed to acetonitrile is used as an organic mobile phase. Methanol is a less expensive solvent and actually outperformed acetonitrile with respect to total phosphopeptide identifications from an identical, single-stage TiO2-enriched starting sample. We assert that offline PFP fractionation of complex peptide and phosphopeptide samples reduces cost, sample handing, and time while maintaining or exceeding current expectations for sample complexity reduction by other offline chromatographic methods.

Poster 105: Demystifying Proteolytic Digestion under High Pressure Cycling: Enzyme Specificity, Efficiency and Synergy of Pressure with Acid-Labile Detergents

Vera Gross[1]; John Wilson[2]; Alexander Lazarev[1]; Darryl Pappin[2]
[1]Pressure BioSciences, Inc, Medford, MA; [2]Protifi, LLC, Huntington, NY

Incomplete proteolytic digestion is a significant obstacle for quantitative proteomics. Attempts to improve the efficiency of enzymatic digestion have prompted the development of mass spec-compatible detergents, immobilized enzymes, sequential or concurrent digestion by more than one enzyme, as well as thermodynamic enhancements such as ultrasonic agitation, microwave heating or pressure cycling (PCT). Additionally, thermostable enzymes have been engineered to perform digestion at elevated temperatures where proteins denature.

Here we present a systematic study investigating the impact of hydrostatic pressure on the specificity and activity of several enzymes including trypsin, chymotrypsin, Lys-C and the new enzyme Tryp-N (ProtiFi, LLC), which cleaves N-terminally to Lys and Arg. Using defined protein samples and tissue lysates, we examine the effect of pressure cycling alone or in combination with various reagents. We further examine the activity and specificity of trypsin and Tryp-N with an emphasis on quantitative recovery of peptides, maximal sequence coverage and minimal rates of miscleavage. Digests performed for 60-90 minutes were run on Orbitrap class instruments and analyzed using Mascot and a suite of home-build informatics tools.

Our data suggest that pressure cycling leads to a general increase in total and average peptide intensity, thus improving the sensitivity and accuracy of quantitative proteome analysis. Simultaneously, proteolysis at increased pressure or temperature significantly reduces sample preparation time. The effects of increased pressure and temperature on proteolysis are in part substrate specific. Such changes can result in significant improvements in analysis of difficult-to-digest proteins that may be missed or underrepresented in traditional sample prep workflows. Moreover, pressure cycling works in synergy with MS-compatible detergents such as Rapigest (Waters Corp.). In digestion of complex rat liver proteome with and without high pressure denaturation, rates of miscleavage, non-specific cleavage (semi-cleaved peptides) or and protein modifications (oxidation and deamidation) were statistically unchanged.

Poster 106: Improving the HPLC Workflow with Vacuum Driven Samplicity Gen 2 Filtration System

Jun Young Park; Vivek Josh; Chris Scott
EMD Millipore, Danvers, Massachusetts

Sample filtration represents the most commonly used step during the HPLC sample preparation by enabling the removal of unwanted particles and extractables prior to the downstream analysis. However, despite its near ubiquitous use, commonly used manual filtration is time consuming, tedious, and labor intensive, particularly with increased sample size and hard to filter samples. Here we describe vacuum driven Samplicity Gen 2 filtration system aimed at addressing these issues in compact easy to use bench top format suitable for mid throughput (10 ~ 99 samples) workflow. The system is designed to work with any existing Millex 33mm syringe filters as well as proprietary filters, offering much flexibility in choice of membrane types. The system is capable of rapid filtration with any vacuum source greater than 18 inches Hg and up to 8 samples at a given time can be directly filtered into standard HPLC vials for downstream analysis. Overall the system offers convenient and simple alternative to manual syringe filters with significant time saving and ergonomic benefits.

Poster 107: Novel Means for Coupleing Protein Separations with MALDI-TOF Mass Spectrometry for Top-Down Proteomics

Kenneth Parker; Marvin Vestal; Stephen Hattan
SimulTOF Systems, Marlborough, MA

This presentation demonstrates innovative means for coupling protein separations to MADLI-TOF mass spectrometry for “top-down” proteomic investigations. Top-down proteomic approaches are targeted because they are more adept at dealing with the wide dynamic range in concentration characteristic of biological samples and because the maintenance intact protein structure helps preserve iso-form and glyco-form information; characteristics that may imply disease.

We demonstrate novel substrates that capture and concentrate proteins after separation, preserving the resolution gained, while acting a stationary phase for further sample processing (e.g., protein digestion, chemical modification). We overview substrate construction, characterization and demonstrate their application for coupling HPLC[1] and SDS-PAGE[2] separations of complex protein mixtures to mass spectrometry.

We present a membrane of bi-functional design that allows proteins separated by gel electrophoresis (SDS-PAGE) to be detected as peptides by mass spectrometry. The membrane provides an efficient coupling between the two technologies were currently one does not exist. Protein identification by peptide mass fingerprinting of yeast whole cell lysate separated by 1D SDS-PAGE demonstrates 75% of identified proteins isolated to a single fraction with an average of 6 peptides / identification.

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We demonstrate a novel 3-dimensional MALDI target that uses in-bedded monolithic chromatography media to effectively capture and concentrate proteins after HPLC separations. The unique design of these plates allows the use of larger capacity columns and higher flow rates to enable increases sample load for the detection of lower abundant protein species.

1: Hattan SJ, Du J, Parker KC. Bifunctional glass membrane designed to interface SDS-PAGE separations of proteins with the detection of peptides by mass spectrometry. *Anal Chem.* 2015 Apr 7;87(7):3685-93.

2: Hattan SJ, Vestal ML. Novel three-dimensional MALDI plate for interfacing high-capacity LC separations with MALDI-TOF. *Anal Chem.* 2008 Dec1;80(23):9115-23.

Poster 108: Biosensor Development for Time-Resolved FRET Kinase Assay and Fluorescence Lifetime Imaging

Wei Cui; Laurie L. Parker
University of Minnesota Twin Cities, Minneapolis, Minnesota

Peptide-based biosensor is an ideal platform that has broad application in drug discovery and cell imaging. Fluorescent inhibitor screening assays are critical for drug discovery. By using the combination of Tb^{3+} sensitizing peptides and quantum dots (QD), here we demonstrate an antibody-free, time-resolved FRET kinase assay strategy that exploited the spectral features of Tb^{3+} and QDs. This flexible, high-throughput screening compatible assay is adaptable to rapidly changing workflows and targets. By incorporating cell penetrating peptide and organic fluorophore to the same biosensors, we were also able to develop kinase biosensors for fluorescence lifetime imaging (FLIM) in living cells. The change of kinase activity and the variation of single cell response to inhibitor treatment were reported by FLIM. The applications of peptide-based biosensors demonstrated in this research are ideal for further development that could help drug screening efforts as well as kinase biology study.

Poster 109: Industrializing SWATH Proteomics with Microflow LC

Christie Hunter[1]; Ken Hamill[2]
[1]SCIEX, Redwood City, CA; [2]SCIEX, Framingham, MA

Data independent acquisition (DIA) strategies have been used to increase the comprehensiveness of data collection while maintaining high quantitative reproducibility. In DIA, larger fixed-width Q1 windows are stepped across the mass range in an LC timescale, transmitting populations of peptides for fragmentation, and high resolution MS and MS/MS spectra are acquired. Previous work has shown that using more narrow variable width Q1 windows can improve peptide detection and increase sample coverage. Many labs are now using DIA to perform larger scale quantitative proteomic experiments with solid reproducibility on 1000s of proteins in complex matrices. As this technique increasingly proves to be a solid tool for biomarker research, larger sample sets are being analyzed, driving the need for further investigation of workflow improvements for throughput and robustness.

Here microflow LC was investigated in combination with SWATH® acquisition on a number of complex matrices, to assess depth of coverage and robustness relative to current nanoflow strategies. The MS analysis was performed on a TripleTOF® System equipped with an nanoLC™ 425 system with microflow modules. A number of column diameter and lengths were explored, along with different gradient times and sample loads, to understand the workflow options in this flow regime. Optimization of SWATH acquisition settings for each chromatographic condition was done, to fully understand impact on results. Key workflow recommendations have been established to provide researchers additional strategies for performing large scale, higher throughput SWATH acquisition studies.

SWATH acquisition coupled with microflow chromatography provides an additional workflow options to researchers with higher throughput and robustness needs. When more sample is available to move to the higher flow rate regime, very high reproducibility is achievable with much faster run times, while still achieving reasonable depth of coverage.

Poster 110: Rapid Protein Extraction and Digestion for Mass Spectrometric Analysis

Brendan Redler; Rohan Varma; Natalie Hong; Jonathan Minden
Carnegie Mellon University, Pittsburgh, PA

Protein identification from one- and two-dimensional electrophoresis gels requires in-gel digestion with an enzyme (typically trypsin), physical extraction of the resultant peptides from the gel, and analysis by mass spectrometer. Current sample preparation methods are time consuming, prone to sample loss, and are plagued by sample contamination from auto-digested trypsin peptides and many physical handling steps throughout the procedure. We are developing a robotic method of electrophoretic transport of proteins & peptides, and protein digestion, which greatly reduces protein and peptide loss and contamination. This method reduces the processing time by 50-fold, relative to current methods. In addition to preparation of trypsin-digested samples, our method shows promise for use with other modes of mass spectrometry to identify proteins and their post-translational modifications.

Poster 111: Rigorous MRM Quantitation of a Multiplexed Panel of Salivary Proteins for Biomarker Assessment Studies

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Under the umbrella of absolute quantitative techniques for protein biomarker screening lies the increasingly popular MRM approach with stable isotope-labeled standards (SIS, commonly peptides). Using that general approach, much effort in biomedical and clinical research has been devoted to blood plasma. Considering that non-invasive biofluids represent attractive alternatives for disease biomarker evaluations and diagnosis, we aimed here to develop a robust panel of multiplexed assays for the deep quantitative analyses of candidate disease protein biomarkers in human saliva. The method utilized upfront protein precipitation (with TCA-acetone-DTT) prior to a bottom-up LC-MRM/MS workflow with SIS peptides spiked post-digestion. Protein quantitation was performed by linear regression analysis of peptide standard curves (standard addition also performed), with interference-free peptides serving as protein surrogates. Development encompassed the systematic evaluation of various preliminary cleanup/concentration procedures, optimization of the LC-MRM platform and target protein/peptide panel, and assessment of the quantitative repeatability, reproducibility, and accuracy. Using a broad set of qualification criteria, the resulting curves in the final method exhibited excellent linearity (average R2 of 0.981) and a broad dynamic range (2-3 orders, on average), with stable chromatographic characteristics (intra-assay CVs: <0.1% for retention time and <9% in relative response). This enabled the reproducible quantitation (12% CV, on average, over analytical triplicate) of 168 proteins (from 256 interference-free peptides) in unstimulated whole saliva with a concentration range spanning from 113 µg/mL (for mucin-5B) to 88 pg/mL (for apolipoprotein A-IV). Overall, the panel comprises proteins that are both cytosolic and membrane-associated, with putative linkages to several oral and systemic diseases that require verification/validation to assess their true biomarker utility. To be presented here is an overview of the development, the quantitative results, the pilot application, and the biological implications for future applications.

Poster 112: Protein Profiling Comparison of Modified DNA Aptamer Screening to Data Dependent Mass Spectrometry across Cancer Cell Lines

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Proteomic profiling of biological fluids and cell lines by mass spectrometry has traditionally been limited by depth of coverage due to sample complexity. Techniques such as the use of antibody depletion columns or fractionation can improve proteome coverage by removing highly abundant species and reducing sample complexity, however this is at the expense of sample throughput. A modified DNA-aptamer

technology (SOMAscan® assay and SOMAmer® reagents) developed for profiling thousands of proteins in a parallel and multiplexed high-throughput manner is being applied more commonly in the biomedical industry. We have screened ten cancer cell lines by the SOMAscan assay and compared the results to data dependent mass spectrometry profiling using TMT10 on the Q Exactive Plus. Although the total number of proteins measured by the SOMAscan assay is smaller than the number detected in the data dependent discovery experiment, proteome coverage increases with use of the two complementary technologies. Twenty proteins were selected and analyzed in cell lysate by multiple reaction monitoring (MRM) after pull-down using the respective SOMAmer reagent. When compared to traditional fractionation approaches prior to MRM analysis, SOMAmer reagent pull-down increases sample throughput 10-fold. Incorporation of SOMAmer reagents into mass spectrometry workflows enables fast, multiplexed analysis of target proteins.

Poster 113: Two Product Extensions Combine Albumin and Immunoglobulin Depletion in Consumable Formats – Called AlbuSorb™ PLUS and AlbuVoid™ PLUS

Swapan Roy; Amenah Soherwardy; Ravish Amin; Matthew Kuruc
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From a foundational NuGel™ platform chemistry, a library of bead architectures has been created to support proteomic enrichment use. These beads are general non-specific protein adsorbents, or stated another way - beads with weak affinity or imperfect fit interactions. So their binding behavior is very different from classical high affinity binding which demands near perfect fits. When conditions support protein binding saturation, progressive displacement allows the beads to bias for or against certain proteins. So in this manner, all derivative NuGel™ products were empirically characterized to meet the needs of the application. Two of our products support Albumin Removal: **AlbuSorb™** for selective binding of Albumin & **AlbuVoid™** for negative selection or voidance of Albumin with consequent enrichment of the remaining serum proteome on the bead. We now report on adding Immunoglobulin depletion as an extension to both of these products. A LC-MS/MS analysis on human serum revealed between 500-600 total proteins, many of which are qualitatively and quantitatively biased to sub-proteomes either depleted of Immunoglobulins, or not depleted of Immunoglobulins. A **PLUS** designation now distinguishes these new products that include Immunoglobulin depletion. So **AlbuSorb™ PLUS** and **AlbuVoid™ PLUS** feature the advantages of: *1) Consumable Use:* not derived from tissue sourced biologicals, no regeneration, cost-effective, no specialized instruments or HPLC, *2)Functional Integrity:* retains enzymatic and biological activity for functional and chemical proteomics, 3) *Enrichment or Depletion:* strategies for both enrichment of low abundance proteomes, or depletion of high abundance proteins, and 4) *On-Bead Digestion:* improves performance and workflow, with unique proteolytic efficiencies.

Poster 114: The Nuclear Proteome of a Vertebrate

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The composition of the nucleoplasm determines the behavior of key processes such as transcription, yet there is still no reliable and quantitative resource of nuclear proteins. Furthermore, it is still unclear how the distinct nuclear and cytoplasmic compositions are maintained. To describe the nuclear proteome quantitatively, we isolated the large nuclei of frog oocytes via microdissection and measured the nucleocytoplasmic partitioning of ~9,000 proteins by mass spectrometry. Most proteins localize entirely to either nucleus or cytoplasm; only ~17% partition equally. A protein's native size in a complex, but not polypeptide molecular weight, is predictive of localization: partitioned proteins exhibit native sizes larger than ~100 kDa, whereas natively smaller proteins are equidistributed. To evaluate the role of nuclear export in maintaining localization, we inhibited Exportin 1. This resulted in the expected re-localization of proteins toward the nucleus, but only 3% of the proteome was affected. Thus, complex assembly and passive retention, rather than continuous active transport, is the dominant mechanism for the maintenance of nuclear and cytoplasmic proteomes.

Poster 115: Quantitative Proteomic Profiling of PANDER Transgenic Mice Reveals Increased Lipogensis and Fatty Acid Synthesis Modulated by the Liver X Receptor

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PANcreatic DERived factor is a member of a superfamily of FAM3 proteins that are uniquely structured and strongly expressed from the endocrine pancreas. Animal models indicate that PANDER can induce a selective hepatic insulin resistant phenotype whereby insulin signaling is blunted yet lipogenesis is increased. We employed quantitative mass spectrometry based proteomic analysis using Stable Isotope Labeling by Amino Acids in Cell Culture, identifying the global hepatic proteome differences between PANDER transgenic overexpressing mice to matched wild-type mice under three metabolic states (fasted, fed, insulin stimulated). Phosphoproteomic analysis was also performed revealing quantitative changes in the phosphorylation state of proteins involved in hepatic signaling networks affected by PANDER overexpression. Upon acquisition of the dataset by use of liquid chromatography tandem mass spectrometry (LTQ Orbitrap), Ingenuity Pathway Analysis was utilized to reveal the effect of PANDER on hepatic signaling. IPA identified lipid metabolism and fatty acid synthesis as top cellular functions. Several molecules with a role in lipid metabolism were identified including FASN, ApoA1, ApoA4, SCD1, CD36, CYP7A1 and ACC. Glycogen phosphorylase kinase (PHK) as well as Glycogen phosphorylase were upregulated; concordant with increased phosphorylation of Glycogen Synthase demonstrated by phoshphoproteomic analysis. Central to the differentially expressed proteins involved in lipid metabolism was activation of the liver X receptor (LXR) pathway. Western blotting confirmed FASN, CYP7A1 and LXRα in all metabolic states. Luciferase assays were performed utilizing an LXRα reporter construct where activation was statistically significantly with PANDER treatment at 1nM. Our SILAC approach revealed numerous PANDER induced molecules and pathways resulting in increased hepatic lipogenesis. Our phosphoproteomic analysis allowed for mechanistic insight pertaining to decreased hepatic glycogen content with PANDER overexpression. We have demonstrated strong utility of this approach in comprehensively phenotyping animal models. Taken together, PANDER strongly impacts hepatic lipid metabolism inducing a SHIR phenotype via LXR modulation.

Poster 116: Characterization of Ubiquitin Trimers by Top-down Mass Spectrometry

Catherine Fenselau; Amanda E. Lee; Lucia Geis-Asteggiante; Emma K. Dixon; Yeji Kim; Tanuja R. Kashyap; Yan Wang; David Fushman
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The profound effects of ubiquitination on the movement and processing of cellular proteins depend exquisitely on the structures of mono and polyubiquitin modifications. Unconjugated polyubiquitins also have a variety of intracellular functions. Structures and functions are not well correlated yet, because the structures of polyubiquitins and polyubiquitin modifications on proteins are difficult to decipher. We are moving towards a robust strategy to provide that structural information. In this report electron transfer dissociation mass spectra (with supplemental “high” energy collisional activation) of six synthetic branched and chain ubiquitin trimers (multiply branched proteins with molecular masses exceeding 25,600 Da) are recorded using an orbitrap fusion lumos instrument and examined to determine how top-down mass spectrometry can characterize linkage topology and linkage sites in a single, facile workflow. The efficacy of this method relies on the formation, detection, and interpretation of extensive fragmentation. Our interpretation is presented in five steps, using ProSight Lite to map the product ions onto various ubiquitin-based templates.

All posters will be attended by presenting authors on both Monday and Tuesday. All posters should be set up by 10:00 am on Monday morning and removed at 3:00 pm on Tuesday. **Odd**-numbered posters present **1:30 - 2:15 pm** and **even**-numbered posters **2:15 - 3:00 pm**.

Poster 117: Use of a Digest-Free Profiling Approach for Neurological Disorder Biomarker Discovery Operations

Jerome Vialaret[1]; Sylvain Lehrmann[1]; Audrey Gabelle[1, 2]; Pierre-Olivier Schmit[3]; Christophe Hirtz[1]
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Measuring the intact mass of proteins in tissue samples or biofluids has the advantage over bottom-up approaches to be able to reveal much more easily the results of major biological processes like alternative splicing, proteolytic processing or modification of PTM pattern distribution, as the information relative to the different proteoforms distribution is encoded in the intact mass of proteins.

In this study we have used a last-generation UHRQ-Tof (Impact II, Bruker Daltonics) to perform a protein profiling approach with the objective of detecting and afterwards identifying proteoforms which are specific are discriminating for neurological disorders

CSF extracts have been prepared from a pool of 30 patient representatives of three different pathological statuses. Each sample has been submitted to an LC-MS analysis after either pre-concentration or separation on either a 50cm monolithic column, working in nanoflow, or separation on a 15cm C4 analytical column. Corresponding methods duration was 240 and 45min, respectively. Data have undergone an automated processing workflow (Calibration, Compound extraction, charge assessment, peak area determination, peak data export) prior to statistical analysis. Statistical analyses have been performed on more than 5000(nanoFlow) or 1500 (normal Flow) compounds reproducibly detected in every patient sample. Interestingly, there was few overlap for the compounds detected with the two chromatographic setups.

Distinctive up and Down-regulation have been shown with both separations, and a Scheduled Precursor List (SPL has been established to perform CID MS/MS on the regulated proteoforms for identification and characterization purposes. Proteoforms from several proteins known to be markers of synaptic losses, like Chromogranin A or secretogranin-2 have been identified.

While the identification and characterization processes are still ongoing, these early results are underlining the high potential of this digest-free approach for straightforward biomarker candidate discovery operations on real-life clinical samples.

Poster 118: IEF-SPLC-MS for generalized high resolution intact glyco-proteoform analysis and Top-Down Proteomics

Steven Patrie
UT Southwestern Medical Center, Dallas, Texas

N-glycans derive from competing biosynthesis reactions where mannosidases (Man), and N-acetylglucosaminyl- (GnT), fucosyl- (FucT), galactosyl- (GalT), and sialyl- (SiaT) transferases sequentially add (or remove) monosaccharides from specific glycosidic bonds. The order of the enzymes, substrate availability, and reaction rate, kcat/Km, create a number of reaction paths that lead to unique terminal glycan products (e.g., simple, hybrid, or complex glycans). If incomplete, the secretory pathway can create 100s of intermediate glycans, giving rise to high glycan microheterogeneity. Conventional glycoproteomics approaches are well established for glycan location, composition or structure interrogation in high-throughput environments. However, the selectivity and complexity of sample preparation often limits discovery to small sets of glycopeptides or glycans and challenges proteoform-level quantitation. Plus, glycan removal from glycoproteins removes protein-specific information that may provide insight into dysfunction occurring within specific cell types. To address these limitations, we propose a top-down mass spectrometry (TDMS) procedure to efficiently monitor glycoprotein microheterogeneity on intact proteins, avoiding tedious peptide digestion and glycan removal steps.

This work addresses the well-recognized paucity of research resources in the glycoscience field that, if overcome, provides non-expert scientists in the research community with assessable glycoproteomics tools. We will show that off-gel isoelectric focusing, superficially porous liquid chromatography, and Fourier transform MS (IEF-SPLC-MS) is a robust method for the sensitive interrogation of glycoproteoforms from mono- to tetra-glycosylated proteins found in biofluids. IEF-SPLC-MS emphasizes high resolution analysis for the three physiochemical properties (mass, pI, and hydrophobicity) monitored which is critical because it alleviates MS spectral congestion and enhances dynamic range by separation of glycoproteoforms by their isoelectric point (pI). The IEF-SPLC-MS workflow yields data conceptually analogous with 2D gel electrophoresis (2DGE) but has the resolution to observe 10s-100s of proteoforms per “spot”. When the tools are applied on the proteome level, 100s-1000s of “spots” are resolved into 10,000s of proteoforms.

Poster 119: Investigation of N-terminal sequence heterogeneity and comprehensive glycosylation modification from a therapeutic recombinant enzyme

Bao Quoc Tran; David R. Goodlett; Young Ah Goo
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Protein top-down analysis by mass spectrometry (MS) has provided efficient characterization of proteins in their intact forms including any “proteoforms”. Analysis of intact proteins provides additional capabilities to identify posttranslational modifications (PTMs) such as glycosylation that are commonly introduced during synthesis and manufacturing process of therapeutic molecules. A therapeutic recombinant enzyme was characterized by top-down analyses with multiple fragmentation techniques including electron transfer dissociation (ETD) and matrix-assisted laser desorption ionization in-source decay (MALDI-ISD) to investigate glycosylation and N-terminal heterogeneity. Fourier transform ion cyclotron resonance (FT-ICR) and high performance liquid chromatography electrospray ionization (HPLC-ESI) on an Orbitrap was employed. These experiments provided a comprehensive view on the protein proteoforms for different glycosylation level with high mass accuracy. In specific we observed proteoforms with high level of sialic acid and galactose+GlcNAc. Incorporating de-glycosylaton with an enzyme, PNGase F, enhanced top-down fragmentation efficiency with MALDI-ISD analysis and enabled extended terminal sequence coverage without sample pre-fractionation. We detected two major forms of N-terminal truncation variants together with deamidation of asparagines in the protein sample.

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